SPRINGER NATURE
Reference

Joaquim Miguel Oliveira
Hajer Radhouani
Rui L. Reis
Editors

Polysaccharides of Microbial Origin

Biomedical Applications

Springer

Polysaccharides of Microbial Origin

Joaquim Miguel Oliveira •
Hajer Radhouani • Rui L. Reis
Editors

Polysaccharides of Microbial Origin

Biomedical Applications

With 257 Figures and 85 Tables

Springer

Editors
Joaquim Miguel Oliveira
3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics
University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Zona Industrial da Gandra
Barco, Guimarães, Portugal

ICVS/3B's – PT Government Associate Laboratory
Braga/Guimarães, Portugal

Rui L. Reis
3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics
University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Zona Industrial da Gandra
Barco, Guimarães, Portugal

ICVS/3B's – PT Government Associate Laboratory
Braga/Guimarães, Portugal

Hajer Radhouani
3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics
University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Zona Industrial da Gandra
Barco, Guimarães, Portugal

ICVS/3B's – PT Government Associate Laboratory
Braga/Guimarães, Portugal

ISBN 978-3-030-42214-1 ISBN 978-3-030-42215-8 (eBook)
ISBN 978-3-030-42216-5 (print and electronic bundle)
https://doi.org/10.1007/978-3-030-42215-8

This Springer imprint is published by the registered company Springer Nature Switzerland AG.
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Preface

This book overviews and discusses the polysaccharides produced and secreted by microorganisms, used in the most recent developments in biomedical applications. In fact, the authoritative reference work revises the sources, properties, characteristics, and biotechnology and biomedical applications of important microbial polysaccharides.

The main goal of this book is to provide a comprehensive analysis of microbial polysaccharides and their current uses and highlight biomedical opportunities. Thus, the topics comprise the following: (a) the overview of the main bacterial, fungal, and microalgal polysaccharides; (b) their extraction, isolation, purification, and advanced production processes; (c) the characterization of their structural, physicochemical, and biological properties, among others, by several techniques; (d) the description of the advanced functionalization and modification methods for the polysaccharide-based material; and (e) their applications and uses in pharmaceutical and tissue engineering fields. Each chapter is written by world-renowned academics and practitioners on their field, being a reference in the area of biomedical and material engineering.

Guimarães, Portugal
March 2022

Joaquim Miguel Oliveira
Hajer Radhouani
Rui L. Reis

Contents

Volume 1

About the Editors

Dr. Joaquim Miguel Oliveira, BSc, PhD, is a Portuguese Principal Investigator with Habilitation who has focused his work on the field of biomaterials for tissue engineering, nanomedicine, stem cells, and cell/drug delivery (http://www.3bs.uminho.pt/users/migueloliveira). Since December 2018, he is Vice-President of I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics of the University of Minho. He is also Director of Pre-clinical Research at the FIFA Medical Center, Estádio do Dragão, Porto, PT, since February 2013. Currently, he is a lecturer in Doctoral Program in Tissue Engineering, Regenerative Medicine and Stem Cells (TERM&SC) at UMinho, PT (since December 2013). He is also an invited lecturer at the Faculty of Medicine, U. Porto (since September 2013) and Department of Polymer Engineering, UM, PT (2009-present). Recently, he has been nominated for integrating the National Ethics Committee for Clinical Research (CEIC), Serviço Nacional de Saúde (SNS), Portugal, for the period of 2020–2023. He has been awarded several prizes including the prestigious Jean Leray Award 2015 from the European Society for Biomaterials for Young Scientists for Outstanding Contributions within the field of Biomaterials. In addition, he is member of the advisory board of the *Journal of Materials Science: Materials in Medicine*, *International Journal of Tissue Engineering*, *Journal ISRN Biomaterials*, the *Journal of Experimental Orthopaedics*, and one of the Editors-in-chief of In vitro Models (Springer), and is referee in more than 40 international journals. As result of his proficiency (as of November 2021), Dr. Oliveira has published more than 470 scientific contributions in scientific journals with referee (some in

high impact factor journals), 16 of those papers produced under invitation. Dr. Miguel Oliveira is inventor of 22 patents and published 10 books (+3 in preparation), 9 special issues/topical collections in scientific journals, 124 book chapters in books with international circulation, in international encyclopaedias, and science dissemination. He has great experience in intellectual property rights and patent exploitation. He has participated in more than 630 communications in national/international conferences. Due to his expertise, he participated as invited/keynote speaker in more than 77 plenary sessions. He made great contributions in the osteochondral field, namely by proposing bilayered scaffolds, work that has been highly cited by its peers. Dr. Miguel Oliveira (as of November 2021) has an h-index of 54, i10 of 183, and received more than 10,700 citations (Google Scholar), or an h-index of 45 and ~7710 total citations (Scopus). He has an RG46.09 (ResearchGate).

Dr. Hajer Radhouani, Eng, MSc, PhD, is a postdoctoral researcher at the Portuguese Government Associate Laboratory ICVS/3B's, University of Minho (https://3bs.uminho.pt/users/hradhouani). She graduated in Industrial Engineering in Agronomy (Agro-Industries and Biotechnologies) at Institut Supérieur Industriel Agronomique de Huy/Gembloux (Huy, Belgium) and obtained her European PhD in Technologic, Comparative and Molecular Genetics from the Universidade de Trás-os-Montes e Alto Douro (Vila Real, Portugal) in a collaboration with the Universidad de La Rioja (Logroño, Spain) and the Universidad de Vigo (Ourense, Spain). Since 2013, she has been working with biomaterials based on natural polysaccharides to treat different articular cartilage pathologies. Currently, she has been working on Kefiran polymer as a novel therapy for osteoarthritis treatment. She has published 54 publications listed in ISI Web of Knowledge, 3 international granted patents, and 8 book chapters, among others. She has a Scopus h-index of 22 and more than 1279 citations. She also presented more than 100 works (posters and oral presentations) in conferences and meetings. She has several scientific profiles such as E-1837-2011 (Researcher ID); 25724661600 (Scopus

Author ID); orcid.org/0000-0003-1007-4073 (ORCID); FE1C-88E7-0A8A (Ciência ID).

Prof. Rui L. Reis, PhD, DSc, Hon. Causa MD, Hon Causa PhD, FBSE, FTERM, member of NAE, FAIMBE, FEAMBES, was born in 1967 in Porto, Portugal. He is a Full Professor of Tissue Engineering, Regenerative Medicine, Biomaterials and Stem Cells at the University of Minho (UMinho), Director of the 3B's Research Group – member of the I3Bs – Institute for Biomaterials, Biodegradables and Biomimetics, and Director of the ICVS/3B's Associate Laboratory, both of UMinho. He is also the CEO of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, the Global Past-President of the Tissue Engineering and Regenerative Medicine International Society (TERMIS), and the founding Editor-in-Chief of the *Journal of Tissue Engineering and Regenerative Medicine*. He is a recognized World expert in the TERM and biomaterials fields who has edited several books and has more than 1375 published works listed on ISI Web of Knowledge with an h index of 95 (1260 works and h = 100 in Scopus, and 2135 and h = 119 in Google Scholar), being also an inventor of around 115 patents. Based on those, he co-founded several companies that raised important private investments. According to Google Scholar his work has been cited more than 63,000 times. He has been awarded many important international prizes, including among several others different innovation awards, the Jean Leray and George Winter Awards (ESB), the Clemson Award (SFB), the TERMIS-EU contributions to the literature Award, the TERMIS-EU Career Achievement Award, and in 2018 the UNESCO – International Life Sciences Award and the IET A. F. Harvey Engineering Research Prize. He is one of the most successful scientists working in Europe in the field of biomaterials, tissue engineering, and regenerative medicine and is the PI of projects with a budget totalizing more than 100 MEuros of which around 50 MEuros are UMinho funding. Researcher ID (A-8938-2008); Scopus Author ID 56861715700; ORCID 0000-0002-4295-6129.

Contributors

Yousra Abid ENIS, Laboratoire Analyse, Valorisation et Sécurité des Aliments, Université de Sfax, Sfax, Tunisia

A. G. Abraham Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA); Facultad de Ciencias Exactas, Universidad Nacional de La Plata – CONICET CCT La Plata – CIC, La Plata, Argentina

Área Bioquímica y Control de Alimentos – Facultad de Ciencias Exactas, Universidad Nacional de La Plata, La Plata, Argentina

Dinesh Chand Agrawal Department of Biotechnology, Faculty of Engineering and Technology, Rama University, Kanpur, Uttar Pradesh, India

Garima Agrawal School of Basic Sciences, Indian Institute of Technology Mandi, Kamand, India

Carolina Patricia Aires Department of BioMolecular Sciences, School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo (USP), Ribeirão Preto, São Paulo, Brazil

Gizem Akan IBSB Industrial Biotechnology and Systems Biology Research Group, Department of Bioengineering, Marmara University, Istanbul, Turkey

Priscilla Barbosa Sales Albuquerque Departamento de Medicina, Universidade de Pernambuco, Garanhuns, PE, Brazil

Ana Gabrielle Pires Alvarenga Laboratory of Microbiology and Biochemistry, College of Chemistry and Food Engineering, Federal University of Rio Grande, Rio Grande, RS, Brazil

Ana Alves CBQF – Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Porto, Portugal

K. R. Anand Fungal Genetics and Mycotoxicology Laboratory, Department of Microbiology, School of Life Sciences, Pondicherry University, Pondicherry, India

P. Andhare PDPIAS, CHARUSAT University, Anand, India

Waqas Asghar School of Food and Agricultural Sciences, University of Management and Technology, Lahore, Pakistan

Samia Azabou ENIS, Laboratoire Analyse, Valorisation et Sécurité des Aliments, Université de Sfax, Sfax, Tunisia

Cristina Balas National Institute for Chemical Pharmaceutical Research and Development, Bucharest, Romania

Indranil Banerjee Department of Bioscience and Bioengineering, IIT, Jodhpur, India

Sílvia Baptista UCIBIO-REQUIMTE, Chemistry Department, Faculty of Sciences and Technology, NOVA School of Science and Technology, Caparica, Portugal

73100 Lda., Lisboa, Portugal

Rafael Maza Barbosa Department of Engineering of Materials and of Bioprocesses, School of Chemical Engineering, University of Campinas, Campinas, Brazil

R-Crio Cryogenics S.A., Campinas, Brazil

Aneli M. Barbosa-Dekker Beta-Glucan Produtos Farmoquímicos - EIRELI, Lote 24A, Bloco Zircônia, Universidade Tecnológica Federal do Paraná, Avenida João Miguel Caram, Londrina, Paraná, Brazil

N. Benbasat Department of Pharmacognosy and Pharmaceutical Chemistry, Medical University-Plovdiv, Plovdiv, Bulgaria

Department of Pharmacognosy and Pharmaceutical Botany, Medical University Sofia, Sofia, Bulgaria

Deepti Bharti Department of Biotechnology and Medical Engineering, NIT, Rourkela, India

Neha Bhilare Department of Pharmaceutical Chemistry, Arvind Gavali College of Pharmacy, Satara, India

Karlin Blackwell Department of Molecular Biosciences, The University of Texas at Austin, Austin, TX, USA

Fernanda Carla Bombaldi de Souza R-Crio Cryogenics S.A., Campinas, Brazil

Renata Francielle Bombaldi de Souza Department of Engineering of Materials and of Bioprocesses, School of Chemical Engineering, University of Campinas, Campinas, Brazil

Mariana Caldas Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, I3B's Research Institute on Biomaterials Biodegradables and Biomimetics, Universidade do Minho, Barco, Guimarães, Portugal

ICVS/3B's – PT Government Associated Laboratory, Braga/Guimarães, Portugal

Carla Maísa Camelini Sole Vitae® Cosméticos Brazil and Chile, Palmital, SP, Brazil

M. S. Carranza Graduate School of Engineering Science, Osaka University, Osaka, Japan

College of Science, De La Salle University, Manila, Philippines

Ana C. Carvalho 3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Zona Industrial da Gandra, Barco, Guimarães, Portugal

ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães, Portugal

Arturo Casadevall Department of Molecular Microbiology and Immunology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA

Luana Cassandra Breitenbach Barroso Coelho Departamento de Bioquímica, Centro de Biociências, Universidade Federal de Pernambuco, Recife, PE, Brazil

Maurice N. Collins School of Engineering, University of Limerick, Limerick, Ireland

Stokes Laboratories, Bernal Institute, University of Limerick, Limerick, Ireland

Health Research Institute, University of Limeric, Limerick, Ireland

Joan Combie Montana Biopolymers Inc., Winnsboro, SC, USA

Susana Correia 3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Zona Industrial da Gandra, Barco, Guimarães, Portugal

ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães, Portugal

Vitor Correlo Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, I3B's Research Institute on Biomaterials Biodegradables and Biomimetics, Universidade do Minho, Barco, Guimarães, Portugal

ICVS/3B's – PT Government Associated Laboratory, Braga/Guimarães, Portugal

Jorge Alberto Vieira Costa Laboratory of Biochemical Engineering, College of Chemistry and Food Engineering, Federal University of Rio Grande, Rio Grande, RS, Brazil

Bárbara Emanoele Costa Oliveira Graduate Program in Dentistry, University Ceuma, São Luís, Maranhão, Brazil

Karina Cruz-Aldaco Department of Food Research (DIPA), School of Chemistry, Universidad Autónoma de Querétaro, Querétaro, Mexico

Mario Culebras School of Engineering, University of Limerick, Limerick, Ireland

Stokes Laboratories, Bernal Institute, University of Limerick, Limerick, Ireland

Jacinta Lutécia Vitorino da Silva Laboratory of Biochemical Engineering, College of Chemistry and Food Engineering, Federal University of Rio Grande, Rio Grande, RS, Brazil

Miriam Desiree Dávila-Medina School of Chemistry, Universidad Autónoma de Coahuila, Saltillo, Mexico

Regina Sharmila Dass Fungal Genetics and Mycotoxicology Laboratory, Department of Microbiology, School of Life Sciences, Pondicherry University, Pondicherry, India

Anabelle Camarotti de Lima Batista Agriculture Department, CCHSA, Federal University of Paraiba, Bananeiras, PB, Brazil

PROFBIO, CCEN, Federal University of Paraiba, João Pessoa, PB, Brazil

Weslley Felix de Oliveira Departamento de Bioquímica, Centro de Biociências, Universidade Federal de Pernambuco, Recife, PE, Brazil

Francielle Tramontini Gomes de Sousa Division of Infectious Diseases and Vaccinology, School of Public Health, University of California-Berkeley, Berkeley, CA, USA

Paula Cristina de Sousa Faria-Tischer Glucom Biotech Ltda., Londrina, Brazil

Francisco Ernesto de Souza Neto Nova Esperança, College of Mossoro, Mossoró, RN, Brazil

Weslley de Souza Paiva RENORBIO, Federal University of Rio Grande do Norte, Natal, RN, Brazil

Guy Decante 3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Zona Industrial da Gandra, Barco, GMR, Portugal

ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães, Portugal

Robert F. H. Dekker Universidade Tecnológica Federal do Paraná (UTFPR), Programa de Pós-Graduação em Engenharia Ambiental, Câmpus Londrina, Londrina, Paraná, Brazil

C. Delattre Université Clermont Auvergne, Clermont Auvergne INP, CNRS, Institut Pascal, Clermont-Ferrand, France

Institut Universitaire de France (IUF), Paris, France

Suneela Dhaneshwar Department of Pharmaceutical Chemistry, Amity Institute of Pharmacy, Lucknow, Amity University Uttar Pradesh, Noida, India

Joy Elvin Dhinakar Department of Studies in Biochemistry, University of Mysore, Mysore, India

Rajendra Sukhjadeorao Dongre Department of Chemistry, RTM Nagpur University, Nagpur, India

Maria Tereza dos Santos Correia Departamento de Bioquímica, Centro de Biociências, Universidade Federal de Pernambuco, Recife, PE, Brazil

Ana Carolina dos Santos Ré Department of BioMolecular Sciences, School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo (USP), Ribeirão Preto, São Paulo, Brazil

Priscila Marcelino dos Santos Silva Departamento de Bioquímica, Centro de Biociências, Universidade Federal de Pernambuco, Recife, PE, Brazil

P. Dubessay Université Clermont Auvergne, CNRS, SIGMA Clermont, Institut Pascal, Clermont-Ferrand, France

Benjamin Obukowho Emikpe Departments of Veterinary Pathology, University of Ibadan, Ibadan, Nigeria

P. J. P. Espitia Nutrition and Dietetics School, Universidad del Atlántico, Puerto Colombia, Atlántico, Colombia

Carlos Flores i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal

IBMC – Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal

Centre for Urological Biology, Department of Renal Medicine, University College London, London, UK

Filomena Freitas UCIBIO-REQUIMTE, Chemistry Department, Faculty of Sciences and Technology, NOVA School of Science and Technology, Caparica, Portugal

73100 Lda., Lisboa, Portugal

C. A. Fuenmayor Institute of Food Science and Technology (ICTA), Universidad Nacional de Colombia, Bogotá, DC, Colombia

N. Gagliarini Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA); Facultad de Ciencias Exactas, Universidad Nacional de La Plata – CONICET CCT La Plata – CIC, La Plata, Argentina

Admir Giachini Ressacada de Pesquisas em Meio Ambiente, Universidade Federal de Santa Catarina, Florianopolis, SC, Brazil

J. M. Gomes 3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Zona Industrial da Gandra, Barco, Guimarães, Portugal

ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães, Portugal

Rafael Gomes-Araújo School of Chemistry, Universidad Autónoma de Coahuila, Saltillo, Mexico

Tecnologico de Monterrey, School of Engineering and Sciences, Monterrey, Mexico

Cristiana Gonçalves 3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Zona Industrial da Gandra, Barco, Guimarães, Portugal

ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães, Portugal

Mayela Govea-Salas School of Chemistry, Universidad Autónoma de Coahuila, Saltillo, Mexico

Ana Rita Inácio 3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Zona Industrial da Gandra, Barco, Guimarães, Portugal

ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães, Portugal

Amit Kumar Jaiswal Centre for Biomaterials, Cellular and Molecular Theranostics, Vellore Institute of Technology, Vellore, Tamil Nadu, India

Namita Jindal Department of Botany, Punjabi University, Patiala, India

Onur Kırtel Bioengineering Department, Industrial Biotechnology and Systems Biology Research Group – IBSB, Marmara University, İstanbul, Turkey

Mayuree Kanlayavattanakul School of Cosmetic Science, Mae Fah Luang University, Chiang Rai, Thailand

Phytocosmetics and Cosmeceuticals Research Group, Mae Fah Luang University, Chiang Rai, Thailand

P. Katsarov Department of Pharmaceutical Sciences, Medical University, Plovdiv, Bulgaria

Research Institute at Medical University-Plovdiv, Medical University – Plovdiv, Plovdiv, Bulgaria

D. Kavitake Department of Food Science and Technology, Pondicherry University, Pondicherry, India

Johanna J. Kenyon Centre for Immunolgy and Infection Control University of Minho, (CIIC), School of Biomedical Sciences, Queensland University of Technology, Brisbane, QLD, Australia

Nauman Khalid School of Food and Agricultural Sciences, University of Management and Technology, Lahore, Pakistan

Jasvirinder Singh Khattar Department of Botany, Punjabi University, Patiala, India

Pritisha S. Khillar Centre for Biomaterials, Cellular and Molecular Theranostics, Vellore Institute of Technology, Vellore, Tamil Nadu, India

Yuriy A. Knirel N. D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, Moscow, Russia

Chandana Kruthiventi Department of Biotechnology, Chaitanya Bharathi Institute of Technology, Hyderabad, Telangana, India

Anuj Kumar School of Chemical Engineering, Yeungnam University, Gyeongsan, South Korea

Decha Kumla CBQF – Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Porto, Portugal

Banani Kundu 3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Zona Industrial da Gandra, Barco, Guimarães, Portugal

ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães, Portugal

Subhas C. Kundu 3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Zona Industrial da Gandra, Barco, Guimarães, Portugal

ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães, Portugal

Dwi Liliek Kusindarta Department of Anatomy, Universitas Gadjah Mada, Yogyakarta, Indonesia

Araceli Loredo-Treviño School of Chemistry, Universidad Autónoma de Coahuila, Saltillo, Mexico

Nattaya Lourith School of Cosmetic Science, Mae Fah Luang University, Chiang Rai, Thailand

Phytocosmetics and Cosmeceuticals Research Group, Mae Fah Luang University, Chiang Rai, Thailand

P. Lukova Department of Pharmacognosy and Pharmaceutical Chemistry, Medical University-Plovdiv, Plovdiv, Bulgaria

Salih Maçin Department of Medical Microbiology, Selçuk University, Konya, Turkey

Sanjeeb Kumar Mandal Department of Biotechnology, Chaitanya Bharathi Institute of Technology, Hyderabad, Telangana, India

Schonna R. Manning Department of Molecular Biosciences, The University of Texas at Austin, Austin, TX, USA

Albino Martins 3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Zona Industrial da Gandra, Barco, Guimarães, Portugal

ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães, Portugal

M. Medrano Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA); Facultad de Ciencias Exactas, Universidad Nacional de La Plata – CONICET CCT La Plata – CIC, La Plata, Argentina

P. Michaud Université Clermont Auvergne, Clermont Auvergne INP, CNRS, Institut Pascal, Clermont-Ferrand, France

Bishwambhar Mishra Department of Biotechnology, Chaitanya Bharathi Institute of Technology, Hyderabad, Telangana, India

S. Chandra Mohan Division of Phytochemistry, Shanmuga Centre for Medicinal Plants Research, Thanjavur, Tamil Nadu, India

Luiza Moraes Laboratory of Biochemical Engineering, College of Chemistry and Food Engineering, Federal University of Rio Grande, Rio Grande, RS, Brazil

Ângela Maria Moraes Department of Engineering of Materials and of Bioprocesses, School of Chemical Engineering, University of Campinas, Campinas, Brazil

Alcina M. M. B. Morais CBQF – Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Porto, Portugal

Michele Greque Morais Laboratory of Microbiology and Biochemistry, College of Chemistry and Food Engineering, Federal University of Rio Grande, Rio Grande, RS, Brazil

Rui M. S. C. Morais CBQF – Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Porto, Portugal

Misu Moscovici National Institute for Chemical Pharmaceutical Research and Development, Bucharest, Romania

Rita Mota i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal

IBMC – Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal

V. Nalbantova Department of Pharmacognosy and Pharmaceutical Chemistry, Medical University-Plovdiv, Plovdiv, Bulgaria

A. Nesic Department of Chemical Dynamics and Permanent Education, Vinca Institute for Nuclear Sciences, University of Belgrade, Belgrade, Serbia

Miguel A. D. Neves Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, I3B's Research Institute on Biomaterials Biodegradables and Biomimetics, Universidade do Minho, Barco, Guimarães, Portugal

ICVS/3B's – PT Government Associated Laboratory, Braga/Guimarães, Portugal

Nuno M. Neves 3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Zona Industrial da Gandra, Barco, Guimarães, Portugal

ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães, Portugal

Michael Ayodele Odeniyi Department of Pharmaceutics and Industrial Pharmacy, University of Ibadan, Ibadan, Nigeria

Catarina Oliveira 3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Zona Industrial da Gandra, Barco, Guimarães, Portugal

ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães, Portugal

Joaquim Miguel Oliveira 3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Zona Industrial da Gandra, Barco, Guimarães, Portugal

ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães, Portugal

Ebru Toksoy Oner IBSB Industrial Biotechnology and Systems Biology Research Group, Department of Bioengineering, Marmara University, Istanbul, Turkey

Kunal Pal Department of Biotechnology and Medical Engineering, NIT, Rourkela, India

Katherine A. Perri Department of Molecular Biosciences, The University of Texas at Austin, Austin, TX, USA

J. A. Piermaria Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA); Facultad de Ciencias Exactas, Universidad Nacional de La Plata – CONICET CCT La Plata – CIC, La Plata, Argentina

Área Bioquímica y Control de Alimentos – Facultad de Ciencias Exactas, Universidad Nacional de La Plata, La Plata, Argentina

G. Pierre Université Clermont Auvergne, Clermont Auvergne INP, CNRS, Institut Pascal, Clermont-Ferrand, France

Bikash Pradhan Department of Biotechnology and Medical Engineering, NIT, Rourkela, India

Hajer Radhouani 3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Zona Industrial da Gandra, Barco, Guimarães, Portugal

ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães, Portugal

Hafiz Ubaid ur Rahman School of Food and Agricultural Sciences, University of Management and Technology, Lahore, Pakistan

M. Ramesh Department of Mechanical Engineering, KIT-Kalaignarkarunanidhi Institute of Technology, Coimbatore, Tamil Nadu, India

Rita Rebelo 3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Zona Industrial da Gandra, Barco, Guimarães, Portugal

ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães, Portugal

C. Nagendranatha Reddy Department of Biotechnology, Chaitanya Bharathi Institute of Technology, Hyderabad, Telangana, India

Rui L. Reis 3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Zona Industrial da Gandra, Barco, Guimarães, Portugal

ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães, Portugal

Guang Ren School of Engineering, University of Limerick, Limerick, Ireland

Stokes Laboratories, Bernal Institute, University of Limerick, Limerick, Ireland

Lara Reys 3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Zona Industrial da Gandra, Barco, Guimarães, Portugal

ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães, Portugal

Antônio Pedro Ricomini Filho Department of Biosciences, Piracicaba Dental School, University of Campinas (UNICAMP), Piracicaba, São Paulo, Brazil

L. C. Rodrigues 3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Zona Industrial da Gandra, Barco, Guimarães, Portugal

ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães, Portugal

Gabriel Martins Rosa Laboratory of Biochemical Engineering, College of Chemistry and Food Engineering, Federal University of Rio Grande, Rio Grande, RS, Brazil

Márcio José Rossi Departamento de Microbiologia, Imunologia e Parasitologia, Universidade Federal de Santa Catarina, Florianopolis, SC, Brazil

Supriya Roy Department of Pharmacology, Amity Institute of Pharmacy, Lucknow, Amity University Uttar Pradesh, Noida, India

Wael Sabra Faculty of Life Sciences, Rhine-Waal University of Applied Sciences, Kleve, Germany

Damini Saha Fungal Genetics and Mycotoxicology Laboratory, Department of Microbiology, School of Life Sciences, Pondicherry University, Pondicherry, India

K. Sakthishobana Department of Biotechnology, KIT-Kalaignarkarunanidhi Institute of Technology, Coimbatore, Tamil Nadu, India

Aleksandra Serafin School of Engineering, University of Limerick, Limerick, Ireland

Stokes Laboratories, Bernal Institute, University of Limerick, Limerick, Ireland

P. H. Shetty Department of Food Science and Technology, Pondicherry University, Pondicherry, India

Simone S. Silva 3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Zona Industrial da Gandra, Barco, Guimarães, Portugal

ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães, Portugal

Tiago H. Silva 3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Zona Industrial da Gandra, Barco, Guimarães, Portugal

ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães, Portugal

Joana Silva-Correia 3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Zona Industrial da Gandra, Barco, GMR, Portugal

N. Simonelli Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA); Facultad de Ciencias Exactas, Universidad Nacional de La Plata – CONICET CCT La Plata – CIC, La Plata, Argentina

Davinder Pal Singh Department of Botany, Punjabi University, Patiala, India

S. B. Suriya Department of Biotechnology, KIT-Kalaignarkarunanidhi Institute of Technology, Coimbatore, Tamil Nadu, India

Paula Tamagnini i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal

IBMC – Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal

Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Porto, Portugal

A. V. Thanusha School of Engineering, University of Limerick, Limerick, Ireland

Stokes Laboratories, Bernal Institute, University of Limerick, Limerick, Ireland

Anand Thirupathi Faculty of Sports Science, Ningbo University, Ningbo, China

Pooja Thorat Fungal Genetics and Mycotoxicology Laboratory, Department of Microbiology, School of Life Sciences, Pondicherry University, Pondicherry, India

Cesar A. Tischer Biotechnology and Glicoconjugate Laboratory, Department of Biochemistry and Biotechnology, State University of Londrina – UEL, Londrina, Brazil

Antonio Trincone Institute of Biomolecular Chemistry, Consiglio Nazionale delle Ricerche, Pozzuoli (Naples), Italy

Evrim Umut Department of Medical Imaging Techniques, Dokuz Eylul University, School of Healthcare, Izmir, Turkey

A. V. Ursu Université Clermont Auvergne, CNRS, SIGMA Clermont, Institut Pascal, Clermont-Ferrand, France

Sarika Verma Council of Scientific and Industrial Research Advanced Materials and Processes Research Institute (CSIR-AMPRI), Bhopal, India

Maggie P. Wear Department of Molecular Microbiology and Immunology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA

Cecília Buzatto Westin Department of Engineering of Materials and of Bioprocesses, School of Chemical Engineering, University of Campinas, Campinas, Brazil

Hevi Wihadmadyatami Department of Anatomy, Universitas Gadjah Mada, Yogyakarta, Indonesia

Fernanda Zamboni School of Engineering, University of Limerick, Limerick, Ireland

Stokes Laboratories, Bernal Institute, University of Limerick, Limerick, Ireland

Health Research Institute, University of Limeric, Limerick, Ireland

Introduction

1

Joaquim Miguel Oliveira, Hajer Radhouani, and Rui L. Reis

Contents

Abstract

Microbial polysaccharides are a renewable resource that has gained increasing interest for biomedical applications due to their outstanding properties. The abundance of these polysaccharides allows their sustainability and provides monetary value for new functional biomaterials. Furthermore, the increasing worldwide demand for active substances offering several health benefits could be the future source of innovation for microbial polysaccharides. The latest developments involving polysaccharides of microbial origin and their biomedical applications are overviewed herein.

Keywords

Microbial · Polysaccharides · Biomedical applications

J. M. Oliveira (✉) · H. Radhouani · R. L. Reis
3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Zona Industrial da Gandra, Barco, Guimarães, Portugal

ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães, Portugal
e-mail: miguel.oliveira@i3bs.uminho.pt; hajer.radhouani@i3bs.uminho.pt; rgreis@i3bs.uminho.pt

J. M. Oliveira et al. (eds.), *Polysaccharides of Microbial Origin*,
https://doi.org/10.1007/978-3-030-42215-8_1

1 Context

Nature comprises a wide diversity of microorganisms, and in consequence it is predictable that microorganisms produce new materials with different structures and properties. This unlimited structural diversity results in an extensive range of applications for microbial polysaccharides that can be obtained from microorganisms such as bacteria, yeast, fungi, and microalgae (Smelcerovic et al. 2008). Polysaccharides, in many forms, play an essential function in all living organisms for storage and supply of energy and/or protection and structural integrity of cells (Shanmugam and Abirami 2019). Microbial polysaccharides, one of the most diverse families of biopolymers, are high molecular weight polymers being able to reach until several millions of Dalton, sharing a substantial component of the cellular carbohydrates found in and surrounding microbial cells (Delattre et al. 2007). Thus, microbial polysaccharides can serve as renewable resources and are now widely used in different industry sectors, principally due to their amazing rheological properties, where they are used as coagulants, binders, gelling agents, lubricants, film formers, emulsifiers, thickeners, and stabilizers, among others (Jindal and Singh Khattar 2018).

In the last few years, polysaccharide-based materials have been receiving an increasing interest in the pharmaceutical and materials engineering fields because of their biocompatibility, bio-absorbability, versatility, and nontoxic nature, among others (Dave 2016). In fact, xanthan, xylinan, gellan, curdlan, dextran, pullulan, scleroglucan, schizophyllan, and alginate are, among others, common microbial polysaccharides in current use (Ahmad et al. 2015; Lei and Edmund 2019). These polysaccharides can also offer not only the advantages of a well-controlled production process with consistent and reproducible yield that can be continued throughout the year but also constant structural, chemical, and physical characteristics (Giavasis 2013). Commonly, microbial polysaccharides are being used in food industries (Giavasis 2013; Ramalingam et al. 2014; Jindal and Singh Khattar 2018; Nešić et al. 2020) but recently other successfully developed microbial polysaccharides have found their applications in cosmetic, pharmaceutical, and medical fields (Morris and Harding 2009; Ahmad et al. 2015). In fact, there are significant advances focused on the use of polysaccharides of microbial origin for biomedical applications including but not limited to drug delivery (Miao et al. 2018), imaging (Moscovici 2015), wound healing and dressing (Azuma et al. 2015; Zhu et al. 2019), surgery (Gupta et al. 2019), and tissue engineering (Delattre et al. 2007; Silva et al. 2017). Developments in the applications of polysaccharides from microorganisms are thoroughly related to the capability of the scientific community to entirely understand the complexity of these biopolymers. Hence, translating this knowledge to practical applications is necessary to fully characterize their structural, biological, and physicochemical properties.

Herein, the "Polysaccharides of Microbial Origin: Biomedical Applications" book presents the microbial polysaccharides status, from their classifications to their wide range of biomedical applications. The book is presented in six major sections, conceptualized in Fig. 1.

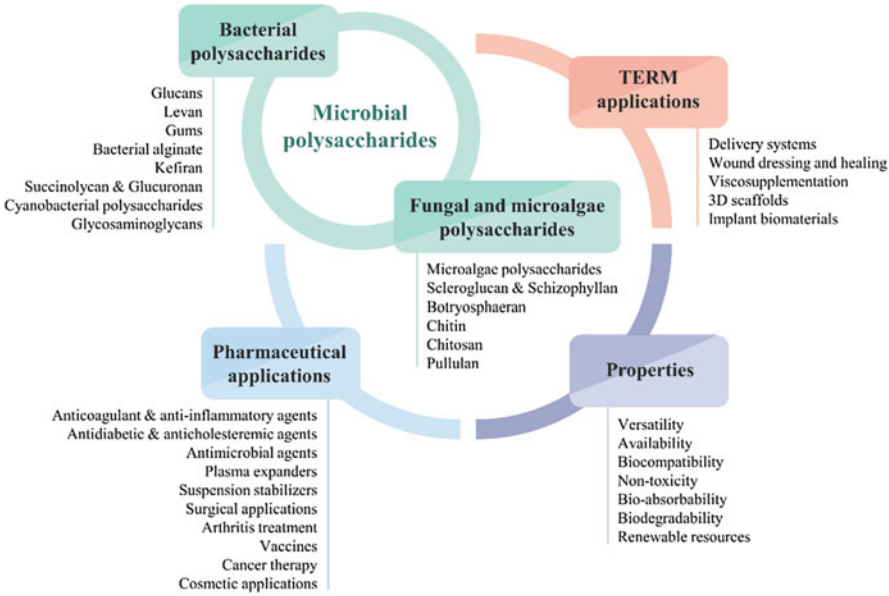


Fig. 1 Polysaccharides of microbial origin in biomedical applications

Section 1 provides an overview of some of the major bacterial polysaccharides such as cellulose, dextran, levan, gums, curdlan, bacterial alginate, bacterial hyaluronic acid, kefiran, and cyanobacterial polysaccharides, among others. Essentially, they have been identified both in Gram-positive and Gram-negative bacteria, having in general an exocellular location (Delattre et al. 2007).

Section 2 comprises the main fungal and microalgal polysaccharides which include chitin, chitosan, pullulan, scleroglucan, and schizophyllan, among others, with successful examples of their biomedical exploitation. Moreover, new bioactive polysaccharides from marine bacteria are highlighted in this section as a valid alternative to traditional polysaccharides.

Section 3 describes the most recent isolation, extraction, purification, and production processes used to obtain microbial polysaccharides of high purity and yields, together with an economic efficiency. These production processes are currently a matter of intense research, and appropriate techniques need to be implemented to enhance process optimization. There are several methods, described in this book, that are used to isolate and purify microbial polysaccharides; the main criterion to select a certain method is to maintain the intrinsic properties of these polymers unchanged during the whole process.

Section 4 presents a comprehensive understanding on the structural, physicochemical, and biological properties of microbial polysaccharides that could guide researchers in the development of target therapeutic products meeting desired characteristics and profiles. Furthermore, this section provides a number of approaches that can be followed to modify these polymers in order to enhance

their functional and technological properties through either physical or chemical cross-linking reactions. One of the greatest biotechnological approaches is the transfer of specific gene constructs by several methods (Ahmed 2003). In this section, genetic engineering technologies are described to increase the production yield of biopolymers in a short span of time. Genetically modified organisms are continually being explored, and these techniques certainly offer promising results as well as pose both technical and scientific challenges.

Sections 5 and 6 provide a review on the pharmaceutical and tissue engineering applications of microbial polysaccharides according to current and outstanding research works in biomedical science. The probable future trends for the utilization of these bioactive compounds in biomedical fields are also discussed. It has been previously shown that these polysaccharides have huge potential owing to their unique rheological properties, and great antitumor, antioxidant, anti-inflammatory, anticoagulant, antiallergic, and antidiabetic properties, among others (Ahmad et al. 2015; Jenab et al. 2020). Promising applications of these polysaccharides have been reported in different therapeutic fields such as cancer, diabetes, vaccines, wound healing, and surgery, among many others (Hasnain and Nayak 2019). During the last decades, the use of microbial polysaccharides, in tissue engineering and regenerative medicine, has noticeably changed from a simple three-dimensional scaffold for cell and drug incorporation, to be used as new functional biomaterials with excellent physicochemical and biological properties. Due to their unique properties, microbial polysaccharides are considered potential candidates for natural product drug discovery and for the delivery of new derived products such as genes, drugs, vaccines, peptides, and proteins, particularly in form of matrix tablets, microspheres, nanoparticles, hydrogels, and capsules (Tiwari et al. 2012), resulting in a powerful synergism of treatment options for biomedical applications. The development of controlled drug delivery systems opens a new opportunity in the biomedical use of these biopolymers being their vast area of application supported by the multifarious series of derivatives available whose valuable properties can be controlled. The potential of these bioactive polysaccharides is being constantly studied, and a new generation of therapeutics will be accomplished by combining their biological activity with natural biodiversity. Nonetheless, the growing worldwide demand for active ingredients providing health benefits will expectedly act as a springboard for future innovation regarding microbial polysaccharides.

References

Ahmad NH, Mustafa S, Man YBC. Microbial polysaccharides and their modification approaches: a review. Int J Food Prop. 2015;18:332–47.

Ahmed FE. Genetically modified probiotics in foods. Trends Biotechnol. 2003;21:491–7.

Azuma K, Izumi R, Osaki T, et al. Chitin, chitosan, and its derivatives for wound healing: old and new materials. J Funct Biomater. 2015. https://doi.org/10.3390/jfb6010104.

Dave SR. Microbial exopolysaccharide – an inevitable product for living beings and environment. J Bacteriol Mycol Open Access. 2016. https://doi.org/10.15406/jbmoa.2016.02.00034.

Delattre C, Laroche C, Michaud P. Production of microbial and fungal polysaccharides. In: Advances in fermentation technology. New Delhi: Asiatech Publisher Inc.; 2007. p. 483–522.
Giavasis I. Production of microbial polysaccharides for use in food. In: Microbial production of food ingredients, enzymes and nutraceuticals. Oxford: Woodhead Publishing; 2013.
Gupta RC, Lall R, Srivastava A, Sinha A. Hyaluronic acid: molecular mechanisms and therapeutic trajectory. Front Vet Sci. 2019. https://doi.org/10.3389/fvets.2019.00192.
Hasnain MS, Nayak AK. Natural polysaccharides in drug delivery and biomedical applications. San Diego: Elsevier Science & Technology; 2019.
Jenab A, Roghanian R, Emtiazi G. Bacterial natural compounds with anti-inflammatory and immunomodulatory properties (mini review). Drug Des Devel Ther. 2020;14:3787–801.
Jindal N, Singh Khattar J. Microbial polysaccharides in food industry. In: Biopolymers for food design. London: Academic; 2018.
Lei S, Edmund TF. Polysaccharides, microbial. In: Encyclopedia of microbiology. San Diego: Academic; 2019.
Miao T, Wang J, Zeng Y, et al. Polysaccharide-based controlled release systems for therapeutics delivery and tissue engineering: from bench to bedside. Adv Sci. 2018;5:1700513.
Morris G, Harding S. Polysaccharides, microbial. In: Encyclopedia of microbiology. Academic Press, London, UK. 2009;482–494.
Moscovici M. Present and future medical applications of microbial exopolysaccharides. Front Microbiol. 2015;6:1012.
Nešić A, Cabrera-Barjas G, Dimitrijević-Branković S, et al. Prospect of polysaccharide-based materials as advanced food packaging. Molecules. 2020;25:135.
Ramalingam C, Priya J, Mundra S. Applications of microbial polysaccharides in food industry. Int J Pharm Sci Rev Res. 2014;27:322–4.
Shanmugam M, Abirami RG. Microbial polysaccharides – chemistry and applications. J Biol Act Prod Nat. 2019. https://doi.org/10.1080/22311866.2019.1571944.
Silva SS, Fernandes EM, Pina S, et al. Natural-origin materials for tissue engineering and regenerative medicine Simone. In Comprehensive Biomaterial II. Ducheyne P, Healy KE, Hutmacher DE., Grainger DW, Kirkpatrick CJ (Eds), Elsevier, Amsterdam, Netherlands. 2017;228–252.
Smelcerovic A, Knezevic-Jugovic Z, Petronijevic Z. Microbial polysaccharides and their derivatives as current and prospective pharmaceuticals. Curr Pharm Des. 2008. https://doi.org/10.2174/138161208786404254.
Tiwari G, Tiwari R, Bannerjee S, et al. Drug delivery systems: an updated review. Int J Pharm Investig. 2012. https://doi.org/10.4103/2230-973x.96920.
Zhu T, Mao J, Cheng Y, et al. Recent progress of polysaccharide-based hydrogel interfaces for wound healing and tissue engineering. Adv Mater Interfaces. 2019.

Part I

Bacterial Polysaccharides

Glucans

2

Cesar A. Tischer

Contents

Abstract

Glucans are a group of polysaccharides that are isolated from plants and microorganisms that have interesting specificities on physicochemical properties that make each one useful polymers for food and other industrial applications, but that regard some key resemblance, the β-1➔3-glucopyranose presence and the modulation of the immune system. Versatile, accept a wide range of conjugable groups, and its being receiving more and more attention as investigations reveals the mechanisms and the relation between structure and function that move specific events in response to antigens. This chapter review a short period of time, mostly the last 3 years on the research of this immunomodulators polysaccharides as therapeutic agents and vaccine adjuvants.

Keywords

Exopolysaccharide · Immune system · Vaccine adjuvant · Dectin-1 · Structural motif

C. A. Tischer (✉)
Biotechnology and Glicoconjugate Laboratory, Department of Biochemistry and Biotechnology, State University of Londrina – UEL, Londrina, Brazil
e-mail: cesar.tischer@uel.br

J. M. Oliveira et al. (eds.), *Polysaccharides of Microbial Origin*,
https://doi.org/10.1007/978-3-030-42215-8_2

1 Introduction

Linear glucans are produced by a variety of organisms as plants, bacteria, and fungus but cannot be found on mammals. Great reviews about structure, production, industrial uses of glucans were written that give us a wide spectrum of potentialities of these biopolymers:

- A critical review on production and industrial applications of beta-glucans (Zhu et al. 2016)
- Bacterial glucans: production, properties, and applications (Xu and Zhang 2016)
- A critical review on the impacts of β-glucans on gut microbiota and human health (Jayachandran et al. 2018)
- A concise review on the molecular structure and function relationship of β-glucan (Du et al. 2019)
- Effect of the modifications on the physicochemical and biological properties of β-glucan – a critical review (Yuan et al. 2020)
- Cell wall glucans of fungi. A review (Ruiz-Herrera and Ortiz-Castellanos 2019)
- β-glucan, a dietary fiber in effective prevention of lifestyle diseases – an insight (Maheshwari et al. 2019)
- Fermentative production of beta-glucan: properties and potential applications (Philippini et al. 2019)
- Industrial production and applications of α/β linear and branched glucans (Venkatachalam et al. 2020a)

The attention that it has been receiving is an indicator of the value of these materials, how versatile on its production as renewable, secure to use, and liable to be chemically modified.

The uses to tune the rheological behavior of aqueous systems, on the scope of the science of colloids (Dickinson 2006), are interesting with regard to its applications for the food industry but also in health. Many interesting properties as curdlan, i.e., that once heated in an aqueous suspension can form two types of gels depending on the heating temperature, one of which is a high-set thermal nonreversible gel (∼80 °C) (Yan et al. 2020), that can be a tunable excipient for antibacterial formulations (Tong et al. 2020), or dextran that generates fluids with Newtonian characteristics and it is used as anticoagulant and blood volume expander by the pharmaceutical industry (Schött et al. 2018).

These interesting properties are by themselves a huge field of exploration and are given by its tridimensional arrangement, but most of time the iteration with other molecules and particularly with proteins (Zielke et al. 2018). Nilson and cols. (Zielke et al. 2019) summarize data that correlate the serine, threonine, and tyrosine phosphorylation to the hydrogen bond that aggregates proteins to phosphorylated β-glucans, and that these interactions are due by not only charge distribution but through specific amino acids.

Dectin-1 is a membrane protein that activate immune responses depending on oligomerization to act in response to β-glucans; Dectin-1 will be detailed later in this

chapter. Dulal et al. observed that if tryptophan, histidine, tyrosine, respectively, W221, H223, and Y228 lacks on Dectin-1, the dimers and trimers does not aggregate and no immune response happened even in the presence of the polysaccharide (Dulal et al. 2018).

The structural motif arginylglycylaspartic acid (RGD), a tripeptide constituted of the arginine-glycine-aspartic acid sequence related as key to aggregation between polysaccharides and proteins like fibrinogen (Alipour et al. 2020; Tchobanian et al. 2019), also show that play a role on the linkage of β-glucans with β-1,3-glucan binding proteins (LGBPs). The LGBPs are present on the surface of the hemocytes and is involved in the pattern recognition mechanism in invertebrates (Huang and Ren 2020), that recognizes the pathogen cell wall and act against these PAMPs (pathogen-associated molecular patterns: β-glucan, laminarin, lipopolysaccharide, lipotechoic acid, peptidoglycan). Sivakamavalli et al. found that changing Asp in RGD motif results in complete loss of binding of *Pm*-LGBP of Asian tiger shrimp (*Penaeus monodon*) to βG and pathogen recognition introducing a specific mutation D134K, a central area of the sugar-binding (βG) site (Sivakamavalli et al. 2019). (Fig. 1).

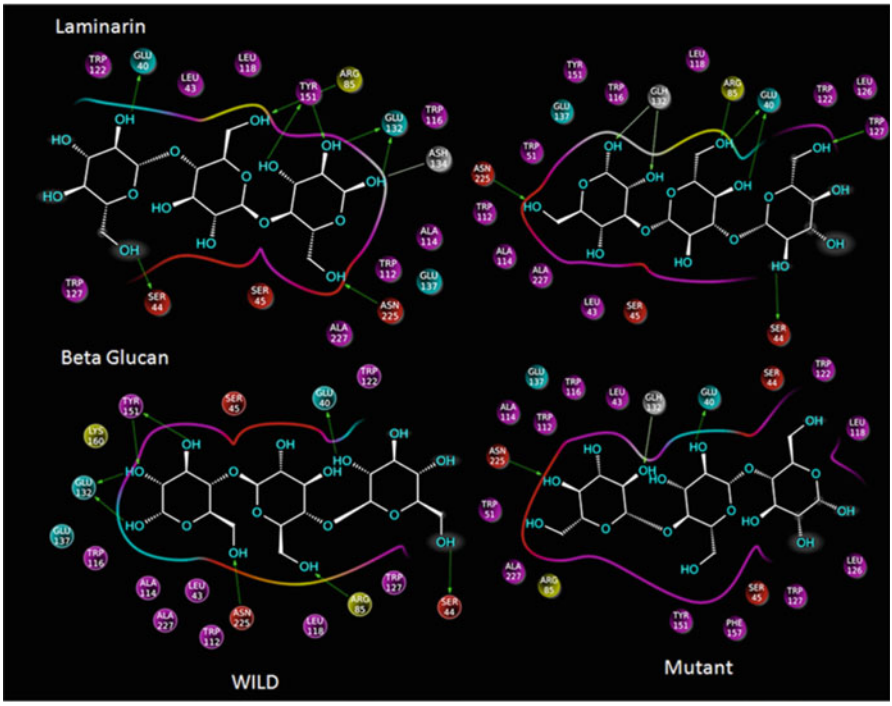


Fig. 1 β-glucan and laminarin are pathogen-associated molecular patterns, PAMPs, that are recognized by immune system and led the interactions with specific amino acids. Due to the change of Asp (mutant D134K), laminarin and glucan could not interact with protein of LGBP. (From Sivakamavalli et al. 2019, with permission)

2 Glucans and the Immune System: Firsts Experiments

Immune system discerns what is and what is not proper, and clean tissues from cells or molecules potentially deleterious. This system also recognizes abnormal cells engendered inside the tissues. Molecules that are recognized by the immune system are considered antigens, Ag.

It was near to 1980s when the firsts experiments shown that glucans plays a role on immune system, stimulating the cell and humoral immunity. The β-(1➔3) glucan from the *Saccharomyces cerevisiae* cell wall received attention and its influence on lymphoreticular system resulting in a hypertrophy of the major reticuloendothelial organs and intense macrophage proliferation was studied (Di Luzio and Williams 1978), as well induced septicemia has regressed (Di Luzio and Williams 1979).

At this time, a variety of glucans was tested. Reynolds et al. show that *S. cerevisiae* glucan alter the course of several lethal experimental infections in mice and rats (Reynolds et al. 1980). They inoculate Rift Valley fever virus and Venezuelan equine encephalomyelitis (VEE) virus on the animals 0.5 g to 2 mg/100 g, via intraperitoneal or inhalation.

The uses against a variety of pathogens with the immune system tools knew at those time, for example, stimulating the cell-mediated and humoral immunity against *Plasmodium berghei* showing that 1 mg/mouse of β-1,3 glucan provides complete protection through development of a well-defined cellular and humoral immunity (Kumar and Ahmad 1985).

3 Immune Responses

3.1 Basis of the Immune Response to Polysaccharides

The pattern recognition receptors (PRRs) induces immune defense on virtually in all live kingdoms, from plants, invertebrates, and vertebrates, recognizing molecular structures, the pathogen-associated molecular patterns or PAMPs (Wang et al. 2018).

Some microbial carbohydrates are PAMPs that could induce adaptative and the training immunity that depends on receptors at the functional cells surface (Netea et al. 2020).

The C-type lectins are proteins that binds to carbohydrate at the "*C-type carbohydrate recognition domain*" (CTLD), a compact region, and the major β-glucan receptors in mammals is Dectin-1, encoded by *CLEC7A* (Adachi et al. 2004). The T helper cells Th1, Th17, cytotoxic T lymphocyte responds to carbohydrates as β-glucan, mannan, and monophosphoryl lipid A (MPLA) (Lang and Huang 2020).

The Th1 cells, Th17 have the Dectin-1 as receptor, and the integrin CR3 (complement receptor 3, also called CD11b/CD18) is the main response activator for macrophages (Brown et al. 2002; Li et al. 2019).

The concise Brown and Gordon article hold out a glimpse of what expect about Dectin-1 and opens an entirely field of studies that covers from glucan structure,

phenotypic biochemical changes, and physiological interactions (Brown and Gordon 2001).

Dectin-1 receptor plays an important role for training immunity, that develops long-term memory in response to some PAMPs, as bacilli Calmette-Guerin (BCG) (Arts et al. 2016) and β-Glucans, developing nonspecific cross protection (Cheng et al. 2014). The trained immunity led to the new field of trained immunity-based vaccines (TIbV), defined as "*vaccine formulations that induce training in innate immune cells*" (Sánchez-Ramón et al. 2018).

Metabolic changes are the key to phenotypic reprogramming inducing immune response, driving to the Warburg effect (DeBerardinis and Chandel 2020), from oxidative phosphorylation to aerobic glycolysis. Under β-glucan PAMPs exposure, Dectin-1 primes serine/threonine protein kinase AKT, mechanistic target of rapamycin (mTOR), and hypoxia-inducible factor 1α (HIF1α), that increases glucose consumption and higher the lactate production (Mulder et al. 2019; Schmitz et al. 2008). These changes affects glutaminolysis and cholesterol synthesis with epigenetic rewiring in monocytes and macrophages inducing the innate immunity (Sánchez-Ramón et al. 2018).

3.2 Dectin-1 Receptor

Dectin-1 is a 28-kDa type II membrane protein, expressed on a variety of cells as monocytes, macrophages, neutrophils, Kupffer cells, Langerhans cells, CD1c+DC, pDC, CD141+DC, B cells, basophils, and eosinophils (Tone et al. 2019). The mast cells release some of its immuno-regulatory and anti-inflammatory molecules in dependence of Dectin-1 (Żelechowska et al. 2020). Is a C-type lectin, it means that have carbohydrate binding properties, within a compact protein region with a unique structural fold that became known as the C-type carbohydrate recognition domain (CTLD) (Weis and Drickamer 1996).

More than 1000 C-type lectin receptors, 17 groups, affects immunity against pathogens and autoimmune diseases, cancer, also homeostasis and physiological regulation (Brown et al. 2018). The Dectin-1 is one on the cluster with seven structurally related receptors (group V), all with a single carbohydrate recognition domain, encoded in human genome (Tone et al. 2019).

The three parts of Dectin-1 are (Effendi et al. 2020; Goodridge et al. 2012):

- Single extracellular C-type lectin-like domain (CTLD)
- A transmembrane region
- A cytoplasmic tail that contains a single tyrosine-based activation motif

Analytical tools such as x-ray diffraction, solid state cross polarized-magical angle spinning nuclear magnetic resonance (^{13}C CP-MAS NMR), ^{1}H-NMR titration and saturation transfer difference (STD)-NMR, diffusion ordered spectroscopy (DOSY)-NMR help to define the role of interaction groove where the glucan like molecules could link to the CTLD. The higher lengths of the β-glucan chains

apparently increases the recognition, that could change the arrangement from unstructured to helically, or triple-helically complexes, to the activation of the innate immune response by mouse Dectin-1 (Hanashima et al. 2014). The exclusion chromatography and multi-angle light scattering experiments shown that induces the oligomerization of the Dectin-1 receptor leading to an active tetramer (Dulal et al. 2018). Studies with mutant proteins varying key amino acids shown that not only the presence of the hydrophobic Trp221/His223 groove CTLD is fundamental to accommodate a β-glucan chain, but also interaction loci are present and acts cooperatively to elicit a response in order to promote the tetramer aggregation.

The Förster resonance energy transfer (FRET) measurements carried out by Anaya et al. revealed that molecular aggregation of Dectin-1 occurs dependently upon glucan higher order structure, forming very small (<15 nm) receptor aggregates revealed by super-resolution microscopy (Anaya et al. 2020).

The antifungal immune response starts with β-glucans interaction with Dectin-1 that phosphorylates spleen tyrosine kinase (Syk) and recruits the CARD9 adaptor protein (Strasser et al. 2012; Wagener et al. 2018). The tyrosine phosphorylation switch is essential to active or on inhibitory immune responses against pathogens (Effendi et al. 2020). The immunoreceptor tyrosine-based activation motif (ITAM) and immunoreceptor tyrosine-based inhibition motif (ITIM)-mediated participates on signal transduction responses for the innate immunity responses, cell activation, as well release of the various antimicrobial molecular species (Fei et al. 2016) (Fig. 2).

The Dectin-1 expression could be influenced by factors such cytokines that causes significant upregulation, as IL-4, IL-13, GM-CSF (granulocyte–macrophage colony-stimulating factor) (Serezani et al. 2012), and microbial stimuli. In other way, IL-10, LPS, and dexamethasone, as well microbial meta trigger downregulation (Gudmundsdottir et al. 2019).

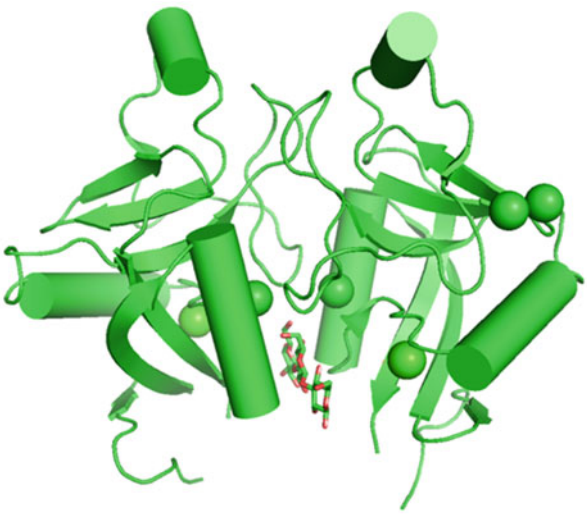


Fig. 2 Dectin-1 dimer with glucan on its binding locus. (From the data of Protein Data Bank, PDB ID 2CL8 added by Brown et al. 2007, processed with PyMol molecular graphics program)

3.2.1 Other Dectin-1 Triggers

The zymosan is cell walls yeast extract that contains proteins but mainly glucans (1–3)-β-D-linked polymer backbones with (1–6)-β-linked side chains of varying length and distribution that are generically called β-glucans (Venkatachalam et al. 2020b). The zymosan β-glucans are the reference to experimental studies on Dectin-1, but many other compounds are recognized. Following are some PAMPs molecules that are recognized by Dectin-1:

- β-glucan (Hossain and Wall 2019)
- Tropomyosin (Gour et al. 2018)
- Mycobacterium (Ishikawa et al. 2017; Wagener et al. 2018)
- Leishmania (Lefèvre et al. 2013)
- Galectin (Daley et al. 2017)
- Galactosylated immunoglobulin (Karsten et al. 2012)

Galactein-9 (Gal9) is an endogenous molecule that is recognized by Dectin-1, that play an important role on adaptive immune response but on the other hand promotes a immunosuppressive microenvironment that causes an peritumoral immune tolerance (Elola et al. 2018). The blockage of the Gal9-Dectin-1 complex increases CD4+ and CD8+ T-cell responses contributing to eradicate pancreatic tumors in different models (Daley et al. 2017).

3.3 Glucan as Immunoadjuvant

Vaccines are the most effective medical intervention to prevent diseases in human and animals (Sun et al. 2018). The impact on millions of people directly as a healthy issue, the pressure over the healthcare system efficiency and the food quality and availability are tangible and intangible gains provided by vaccination (Cebadera Miranda et al. 2020; Rémy et al. 2015).

Vaccines are produced to obtain immune response and keep this effectiveness through the time, and the composition could present generally, in addition to the immunogenic compounds as (CDC 2019):

- **Adjuvants,** help boost the body's response to vaccine
- **Stabilizers,** to help keep vaccine effective after manufactured
- **Inactivating ingredients,** to inactivate the bacteria or virus associated to disease on the vaccine manufacturing process
- **Preservatives,** to prevent contamination, like thimerosal

The mechanism of action of adjuvants depends mainly on their physicochemical properties and/or molecular characteristics. Polysaccharides are promising compounds to act as adjuvants (Son et al. 2020; Sun et al. 2018), and many approaches could be tested, glucan itself, glucan-based conjugates to a glucan-based delivery and adjuvant platform, as particles that carries the antigen co-administrated with

antigen, cross-linked with or encapsulating it (Abraham et al. 2019; Junter and Karakasyan 2020; Vetvicka et al. 2020).

Glucans alone can be used to treat illnesses, which may raise doubts for some whether it is a food supplement or a drug (Vetvicka et al. 2019). This polysaccharide by itself induces trained immunity against *Mycobacterium tuberculosis* through the ways discussed above, and activates the cascade of genes evolved with cytokines (Moorlag et al. 2020).

However, as particle that its activities are potentialized. Particles of β-Glucan from *S. cerevisiae* was tested as adjuvant aggregating hepatitis B surface antigen (HBsAg) in the subcutaneous vaccination of mice showing 2500-fold increased IgG compared to HBsAg alone (Soares et al. 2019b). Other remarkable aspect that Soares et al. reinforces is the fact that glucan particle induces strong Th1 and Th17 immune responses, associated with high HBsAg-specific IFN-γ and IL-17 splenic production.

As adjuvant glucan particles can carry not only antigens but help to get more response in techniques based on DNA vaccines, incorporated on matrixes of poly [2-(dimethylamino)ethyl methacrylate] and poly(β-amino ester) polyplexes (Soares et al. 2019a). This approach show a gain of 40% on the seroconversion, on in vivo vaccination proved to be excellent to modulate a balanced Th1/Th2 immune response, already tested in vaccination studies with HBsAg (Borges et al. 2008).

The glucan-antigen chemical conjugation shows positive results against Zika virus, (Qi et al. 2020), live attenuated, viruses, inactivated viruses, DNA and mRNA vaccines, and the protein-based vaccine was tested against ZIKV, and from these, the ones based on E protein demonstrate being promising, active, and safe. Qi et al. primarily conjugated E protein with the pristine Glucan, in an oxidized form, after all, hydrazone or/and disulfide linkers as E protein scissors was added and its role was observed. This engineered molecule was able to release E protein that rearrange on its active conformer, expressing high E protein-specific IgG titers and low β-glucan-specific IgG titers and high levels of IFN-γ, TNF-α, and IL-2.

Great efforts are due to find new antigens against pathogens, and glucans are used as carrier due to its known effectivity as adjuvant. Hester et al. worked to express 16 new antigens that lead protection against *Cryptococcus neoformans* meningitis, that was incorporated to glucan particles, and 7 where able to protect BALB/c and/or C57BL/6 mice against an otherwise lethal challenge with *C. neoformans* strain KN99 (Hester et al. 2020).

3.3.1 Oral Administration

The particles from *S. cerevisiae* glucan sizes 3–4 μm are hollow and porous microspheres, and are transported across the intestinal epithelium by the Peyer's patch (PP) M-cells, can be absorbed and can carry antigens inside, and that it is a feasible way to deliver vaccines via oral administration (De Smet et al. 2013). New glucans are emerging as PAMPs, like Schizophyllan, an β-1➔3Glucan from basidiomycete *Schizophyllum commune* (Zhang et al. 2013), that is recognized by the intestinal epithelium and shows that it can carry oligonucleotides (Miyamoto et al. 2019).

The affinity of the PP M-cells can be increased or modulated by grafting the glucan with compounds that are recognized by cell membrane proteins. For example, coating glucan with dopamine generates adhesion and increase the uptake of the particle in the blood (Soto et al. 2019).

4 Final Considerations

The interaction between glucans and its recognizing sites is being deeply known, its uses as immunomodulator and the feasibility to produce are reasons that – with a large margin of assurance – have made this material a real option to be used as an adjuvant. Many other options are slightly explored, i.e., mimic its structural motif to be recognized by Dectin-1, and the multitude of grafting and possible cross-linkages.

Otherwise what is clear is the use of glucans as real functional food, absorbable by gut and effective to training immunity from early ages. The overall features of Glucans, reported in this chapter reinforce that this polysaccharide could play a key role as a rich complement for a healthy nutrition on the defense against pathogens.

References

Abraham A, Ostroff G, Levitz SM, Oyston PCF. A novel vaccine platform using glucan particles for induction of protective responses against Francisella tularensis and other pathogens. Clin Exp Immunol. 2019;198(2):143–52. https://doi.org/10.1111/cei.13356.

Adachi Y, Ishii T, Ikeda Y, Hoshino A, Tamura H, Aketagawa J, et al. Characterization of beta-glucan recognition site on C-type lectin, Dectin 1. Infect Immun. 2004;72(7):4159–71. https://doi.org/10.1128/IAI.72.7.4159-4171.2004.

Alipour M, Baneshi M, Hosseinkhani S, Mahmoudi R, Jabari Arabzadeh A, Akrami M, et al. Recent progress in biomedical applications of RGD-based ligand: from precise cancer theranostics to biomaterial engineering: a systematic review. J Biomed Mater Res A. 2020;108(4):839–50. https://doi.org/10.1002/jbm.a.36862.

Anaya EU, Amin AE, Wester MJ, Danielson ME, Michel KS, Neumann AK.. Dectin-1 molecular aggregation and signaling is sensitive to β-glucan structure and glucan exposure on Candida albicans cell walls. BioRxiv. 2020;824995. https://doi.org/10.1101/824995.

Arts RJW, Carvalho A, La Rocca C, Palma C, Rodrigues F, Silvestre R, et al. Immunometabolic pathways in BCG-induced trained immunity. Cell Rep. 2016;17(10):2562–71. https://doi.org/10.1016/j.celrep.2016.11.011.

Borges O, Silva M, de Sousa A, Borchard G, Junginger HE, Cordeiro-da-Silva A. Alginate coated chitosan nanoparticles are an effective subcutaneous adjuvant for hepatitis B surface antigen. Int Immunopharmacol. 2008;8(13–14):1773–80. https://doi.org/10.1016/j.intimp.2008.08.013.

Brown GD, Gordon S. A new receptor for β-glucans. Nature. 2001;413(6851):36–7. https://doi.org/10.1038/35092620.

Brown GD, Taylor PR, Reid DM, Willment JA, Williams DL, Martinez-Pomares L, et al. Dectin-1 is a major β-glucan receptor on macrophages. J Exp Med. 2002;196(3):407–12. https://doi.org/10.1084/jem.20020470.

Brown J, O'Callaghan CA, Marshall ASJ, Gilbert RJC, Siebold C, Gordon S, et al. Structure of the fungal β-glucan-binding immune receptor Dectin-1: implications for function. Protein Sci. 2007;16(6):1042–52. https://doi.org/10.1110/ps.072791207.

Brown GD, Willment JA, Whitehead L. C-type lectins in immunity and homeostasis. Nat Rev Immunol. 2018;18(6):374–89. https://doi.org/10.1038/s41577-018-0004-8.

CDC. Vaccine excipient summary. Johns Hopkins University. 2019. Retrieved from http://www.fda.gov/BiologicsBloodVaccines/Vaccines/ApprovedProducts/ucm093833.htm

Cebadera Miranda E, Castillo Ruiz-Cabello MV, Cámara Hurtado M. Food biopharmaceuticals as part of a sustainable bioeconomy: edible vaccines case study. New Biotechnol. 2020;59:74–9. https://doi.org/10.1016/J.NBT.2020.06.005.

Cheng SC, Quintin J, Cramer RA, Shepardson KM, Saeed S, Kumar V, et al. MTOR- and HIF-1-α-mediated aerobic glycolysis as metabolic basis for trained immunity. Science. 2014;345 (6204):1250684. https://doi.org/10.1126/science.1250684.

Daley D, Mani VR, Mohan N, Akkad N, Ochi A, Heindel DW, et al. Dectin 1 activation on macrophages by galectin 9 promotes pancreatic carcinoma and peritumoral immune tolerance. Nat Med. 2017;23(5):556–67. https://doi.org/10.1038/nm.4314.

De Smet R, Demoor T, Verschuere S, Dullaers M, Ostroff GR, Leclercq G, et al. β-Glucan microparticles are good candidates for mucosal antigen delivery in oral vaccination. J Control Release. 2013;172(3):671–8. https://doi.org/10.1016/j.jconrel.2013.09.007.

DeBerardinis RJ, Chandel NS. We need to talk about the Warburg effect. Nat Metab. 2020;2(2): 127–9. https://doi.org/10.1038/s42255-020-0172-2.

Di Luzio NR, Williams DL. Protective effect of glucan against systemic Staphylococcus aureus septicemia in normal and leukemic mice. Infect Immun. 1978;20(3):804–10. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/352959

Di Luzio NR, Williams DL. Glucan-induced modification of the increased susceptibility of cyclophosphamide-treated mice to Staphylococcus aureus infection. Cancer Immunol Immunother. 1979;6(2):73–9. https://doi.org/10.1007/BF00200135.

Dickinson E. Colloid science of mixed ingredients. Soft Matter. 2006;2(8):642–52. https://doi.org/10.1039/b605670a.

Du B, Meenu M, Liu H, Xu B. A concise review on the molecular structure and function relationship of β-glucan. Int J Mol Sci. Multidisciplinary Digital Publishing Institute. 2019;20 (16):4032. https://doi.org/10.3390/ijms20164032.

Dulal HP, Adachi Y, Ohno N, Yamaguchi Y. β-Glucan-induced cooperative oligomerization of Dectin-1 C-type lectin-like domain. Glycobiology. 2018;28(8):612–23. https://doi.org/10.1093/glycob/cwy039.

Effendi WI, Nagano T, Hasan H, Yudhawati R. Immunoregulatory property of C-type Lectin-like receptors in fibrosing interstitial lung diseases. Int J Mol Sci. 2020;21(10):3665. https://doi.org/10.3390/ijms21103665.

Elola MT, Ferragut F, Méndez-Huergo SP, Croci DO, Bracalente C, Rabinovich GA. Galectins: multitask signaling molecules linking fibroblast, endothelial and immune cell programs in the tumor microenvironment; 2018. https://doi.org/10.1016/j.cellimm.2018.03.008.

Fei C, Pemberton JG, Lillico DME, Zwozdesky MA, Stafford JL. Biochemical and functional insights into the integrated regulation of innate immune cell responses by teleost leukocyte immune-type receptors. Biology. Multidisciplinary Digital Publishing Institute. 2016;5(1):13. https://doi.org/10.3390/biology5010013.

Goodridge HS, Underhill DM, Touret N. Mechanisms of Fc receptor and Dectin-1 activation for phagocytosis. Traffic. Wiley. 2012;13(8):1062–71. https://doi.org/10.1111/j.1600-0854.2012.01382.x.

Gour N, Lajoie S, Smole U, White M, Hu D, Goddard P, et al. Dysregulated invertebrate tropomyosin-Dectin-1 interaction confers susceptibility to allergic diseases. Sci Immunol. 2018;3(20):eaam9841. https://doi.org/10.1126/sciimmunol.aam9841.

Gudmundsdottir AB, Brynjolfsdottir A, Olafsdottir ES, Hardardottir I, Freysdottir J. Exopolysaccharides from Cyanobacterium aponinum induce a regulatory dendritic cell phenotype and inhibit SYK and CLEC7A expression in dendritic cells, T cells and keratinocytes. Int Immunopharmacol. 2019;69:328–36. https://doi.org/10.1016/J.INTIMP.2019.01.044.

Hanashima S, Ikeda A, Tanaka H, Adachi Y, Ohno N, Takahashi T, Yamaguchi Y. NMR study of short β(1–3)-glucans provides insights into the structure and interaction with Dectin-1. Glycoconj J. 2014;31(3):199–207. https://doi.org/10.1007/s10719-013-9510-x.

Hester MM, Lee CK, Abraham A, Khoshkenar P, Ostroff GR, Levitz SM, Specht CA. Protection of mice against experimental cryptococcosis using glucan particle-based vaccines containing novel recombinant antigens. Vaccine. 2020;38(3):620–6. https://doi.org/10.1016/j.vaccine.2019.10.051.

Hossain M, Wall K. Use of dendritic cell receptors as targets for enhancing anti-cancer immune responses. Cancers. 2019;11(3):418. https://doi.org/10.3390/cancers11030418.

Huang Y, Ren Q. Research progress in innate immunity of freshwater crustaceans. Dev Comp Immunol. 2020;104:103569. https://doi.org/10.1016/j.dci.2019.103569.

Ishikawa E, Mori D, Yamasaki S. Recognition of mycobacterial lipids by immune receptors. Trends Immunol. 2017;38(1):66–76. https://doi.org/10.1016/J.IT.2016.10.009.

Jayachandran M, Chen J, Chung SSM, Xu B. A critical review on the impacts of β-glucans on gut microbiota and human health. J Nutr Biochem. Elsevier. 2018;61:101–10. https://doi.org/10.1016/j.jnutbio.2018.06.010.

Junter GA, Karakasyan C. Polysaccharides against viruses: Immunostimulatory properties and the delivery of antiviral vaccines and drugs. Crit Rev Ther Drug Carrier Syst. 2020;37(1):1–64. https://doi.org/10.1615/CritRevTherDrugCarrierSyst.2019027229.

Karsten CM, Pandey MK, Figge J, Kilchenstein R, Taylor PR, Rosas M, et al. Anti-inflammatory activity of IgG1 mediated by Fc galactosylation and association of FcγRIIB and Dectin-1. Nat Med. 2012;18(9):1401–6. https://doi.org/10.1038/nm.2862.

Kumar P, Ahmad S. Glucan-induced immunity in mice against Plasmodium berghei. Ann Trop Med Parasitol. 1985;79(2):211–3. https://doi.org/10.1080/00034983.1985.11811908.

Lang S, Huang X. Carbohydrate conjugates in vaccine developments. Front Chem. 2020;8:284. https://doi.org/10.3389/fchem.2020.00284.

Lefèvre L, Lugo-Villarino G, Meunier E, Valentin A, Olagnier D, Authier H, et al. The C-type Lectin receptors Dectin-1, MR, and SIGNR3 contribute both positively and negatively to the macrophage response to Leishmania infantum. Immunity. 2013;38(5):1038–49. https://doi.org/10.1016/J.IMMUNI.2013.04.010.

Li D, Bai C, Zhang Q, Li Z, Shao D, Li X. β-1,3-Glucan/CR3/SYK pathway-dependent LC3B-II accumulation enhanced the fungicidal activity in human neutrophils. J Microbiol. 2019;57(4):263–70. https://doi.org/10.1007/s12275-019-8298-1.

Maheshwari G, Sowrirajan S, Joseph B. β-Glucan, a dietary fiber in effective prevention of lifestyle diseases – an insight. Bioact Carbohydr Diet Fibre. Elsevier. 2019;19:100187. https://doi.org/10.1016/j.bcdf.2019.100187.

Miyamoto N, Mochizuki S, Sakurai K. A novel polysaccharide carrier for functional oligonucleotides: immunocyte targeting through β-glucan receptors. ACS Symp Ser. 2019;1309:79–92. https://doi.org/10.1021/bk-2019-1309.ch004.

Moorlag SJCFM, Khan N, Novakovic B, Kaufmann E, Jansen T, van Crevel R, et al. β-glucan induces protective trained immunity against Mycobacterium tuberculosis infection: a key role for IL-1. Cell Rep. 2020;31(7):107634. https://doi.org/10.1016/j.celrep.2020.107634.

Mulder WJM, Ochando J, Joosten LAB, Fayad ZA, Netea MG. Therapeutic targeting of trained immunity. Nat Rev Drug Discov. 2019;18(7):553–66. https://doi.org/10.1038/s41573-019-0025-4.

Netea MG, Domínguez-Andrés J, Barreiro LB, Chavakis T, Divangahi M, Fuchs E, et al. Defining trained immunity and its role in health and disease. Nat Rev Immunol. 2020;20(6):375–88. https://doi.org/10.1038/s41577-020-0285-6.

Philippini RR, Martiniano SE, dos Santos JC, da Silva SS, Chandel AK. Fermentative production of beta-glucan: properties and potential applications. In: Molina G, Gupta V, Singh B, Gathergood N, editors. Bioprocessing for biomolecules production. Chichester: Wiley; 2019. p. 303–20. https://doi.org/10.1002/9781119434436.ch15.

Qi J, Qi J, Yin Y, Yu W, Shen L, Xu J, Hu T. Conjugation of β-glucan with the hydrazone and disulfide linkers markedly improves the immunogenicity of Zika virus e protein. Mol Pharm. 2020;17(6):1933–44. https://doi.org/10.1021/acs.molpharmaceut.0c00010.

Rémy V, Largeron N, Quilici S, Carroll S. The economic value of vaccination: why prevention is wealth. J Mark Access Health Policy. 2015;3(1):29284. https://doi.org/10.3402/jmahp.v3.29284.

Reynolds JA, Kastello MD, Harrington DG, Crabbs CL, Peters CJ, Jemski JV, et al. Glucan-induced enhancement of host resistance to selected infectious diseases. Infect Immun. 1980;30(1):51–7. https://doi.org/10.1128/iai.30.1.51-57.1980.

Ruiz-Herrera J, Ortiz-Castellanos L. Cell wall glucans of fungi. A review. Cell Surface. Elsevier. 2019;5:100022. https://doi.org/10.1016/j.tcsw.2019.100022.

Sánchez-Ramón S, Conejero L, Netea MG, Sancho D, Palomares Ó, Subiza JL. Trained immunity-based vaccines: a new paradigm for the development of broad-spectrum anti-infectious formulations. Front Immunol. Frontiers. 2018;9:2936. https://doi.org/10.3389/fimmu.2018.02936.

Schmitz F, Heit A, Dreher S, Eisenächer K, Mages J, Haas T, et al. Mammalian target of rapamycin (mTOR) orchestrates the defense program of innate immune cells. Eur J Immunol. 2008;38(11):2981–92. https://doi.org/10.1002/eji.200838761.

Schött U, Kander T, Bentzer P. Effects of dextran-70 and albumin on coagulation in experimental hemorrhage in the Guinea pig. Shock. 2018;50(3):366–72. https://doi.org/10.1097/SHK.0000000000001025.

Serezani CH, Kane S, Collins L, Morato-Marques M, Osterholzer JJ, Peters-Golden M. Macrophage Dectin-1 expression is controlled by leukotriene B4 via a GM-CSF/PU.1 axis. J Immunol. 2012;189(2):906–15. https://doi.org/10.4049/jimmunol.1200257.

Sivakamavalli J, Selvaraj C, Singh SK, Park K, Kwak IS, Vaseeharan B. Effect of amino acid substitution in the Penaeus monodon LGBP and specificity through mutational analysis. Int J Pept Res Ther. 2019:1–13. https://doi.org/10.1007/s10989-019-09960-x.

Soares E, Cordeiro R, Faneca H, Borges O. Polymeric nanoengineered HBsAg DNA vaccine designed in combination with β-glucan. Int J Biol Macromol. 2019a;122:930–9. https://doi.org/10.1016/j.ijbiomac.2018.11.024.

Soares E, Groothuismink ZMA, Boonstra A, Borges O. Glucan particles are a powerful adjuvant for the HBsAg, favoring antiviral immunity. Mol Pharm. 2019b;16(5):1971–81. https://doi.org/10.1021/acs.molpharmaceut.8b01322.

Son S, Nam J, Zenkov I, Ochyl LJ, Xu Y, Scheetz L, et al. Sugar-nanocapsules imprinted with microbial molecular patterns for mRNA vaccination. Nano Lett. 2020;20(3):1499–509. https://doi.org/10.1021/acs.nanolett.9b03483.

Soto ER, Kim HC, Yagita H, De Jesus M, Ostroff GR. Polydopamine coating of glucan particles increases uptake into Peyer's patches. ACS Appl Bio Mater. 2019;2(9):3748–54. https://doi.org/10.1021/acsabm.9b00379.

Strasser D, Neumann K, Bergmann H, Marakalala MJ, Guler R, Rojowska A, et al. Syk kinase-coupled C-type lectin receptors engage protein kinase C-δ to elicit card9 adaptor-mediated innate immunity. Immunity. 2012;36(1):32–42. https://doi.org/10.1016/J.IMMUNI.2011.11.015.

Sun B, Yu S, Zhao D, Guo S, Wang X, Zhao K. Polysaccharides as vaccine adjuvants; 2018. https://doi.org/10.1016/j.vaccine.2018.07.040.

Tchobanian A, Van Oosterwyck H, Fardim P. Polysaccharides for tissue engineering: current landscape and future prospects. Carbohydr Polym. 2019;205:601–25. https://doi.org/10.1016/j.carbpol.2018.10.039.

Tone K, Stappers MHT, Willment JA, Brown GD. C-type lectin receptors of the Dectin-1 cluster: physiological roles and involvement in disease. Eur J Immunol. Wiley. 2019;49(12):2127–33. https://doi.org/10.1002/eji.201847536.

Tong X, Qi X, Mao R, Pan W, Zhang M, Wu X, et al. Construction of functional curdlan hydrogels with bio-inspired polydopamine for synergistic periodontal antibacterial therapeutics. Carbohydr Polym. 2020;245:116585. https://doi.org/10.1016/j.carbpol.2020.116585.

Venkatachalam G, Arumugam S, Doble M. Industrial production and applications of α/β linear and branched glucans. Indian Chem Eng. 2020a:1–15. https://doi.org/10.1080/00194506.2020.1798820.

Venkatachalam G, Arumugam S, Doble M. Synthesis, characterization, and biological activity of aminated zymosan. ACS Omega. 2020b;5(26):15973–82. https://doi.org/10.1021/acsomega.0c01243.

Vetvicka V, Vannucci L, Sima P, Richter J. Beta glucan: supplement or drug? From laboratory to clinical trials. Molecules. 2019;24(7):1251. https://doi.org/10.3390/molecules24071251.

Vetvicka V, Vannucci L, Sima P. β-glucan as a new tool in vaccine development. Scand J Immunol. Wiley. 2020;91(2):e12833. https://doi.org/10.1111/sji.12833.

Wagener M, Hoving JC, Ndlovu H, Marakalala MJ. Dectin-1-Syk-CARD9 signaling pathway in TB immunity. Front Immunol. 2018;9:225. https://doi.org/10.3389/fimmu.2018.00225.

Wang W, Song X, Wang L, Song L. Pathogen-derived carbohydrate recognition in molluscs immune defense. Int J Mol Sci. Multidisciplinary Digital Publishing Institute. 2018;19(3):721. https://doi.org/10.3390/ijms19030721.

Weis WI, Drickamer K. Structural basis of lectin-carbohydrate recognition. Annu Rev Biochem. 1996;65:441–73. https://doi.org/10.1146/annurev.bi.65.070196.002301.

Xu L, Zhang J. Bacterial glucans: production, properties, and applications. Appl Microbiol Biotechnol. 2016;100(21):9023–36. https://doi.org/10.1007/s00253-016-7836-6.

Yan JK, Cai WD, Wang C, Yu YB, Zhang HN, Yang Y, Wang WH. Macromolecular behavior, structural characteristics and rheological properties of alkali-neutralization curdlan at different concentrations. Food Hydrocoll. 2020;105:105785. https://doi.org/10.1016/j.foodhyd.2020.105785.

Yuan H, Lan P, He Y, Li C, Ma X. Effect of the modifications on the physicochemical and biological properties of β-glucan-a critical review. Molecules. Multidisciplinary Digital Publishing Institute. 2020;25(1):57. https://doi.org/10.3390/molecules25010057.

Żelechowska P, Różalska S, Wiktorska M, Brzezińska-Błaszczyk E, Agier J. Curdlan stimulates tissue mast cells to synthesize pro-inflammatory mediators, generate ROS, and migrate via Dectin-1 receptor. Cell Immunol. 2020;351:104079. https://doi.org/10.1016/J.CELLIMM.2020.104079.

Zhang Y, Kong H, Fang Y, Nishinari K, Phillips GO. Schizophyllan: a review on its structure, properties, bioactivities and recent developments. Bioact Carbohydr Diet Fibre. 2013;1(1):53–71. https://doi.org/10.1016/j.bcdf.2013.01.002.

Zhu F, Du B, Xu B. A critical review on production and industrial applications of beta-glucans. Food Hydrocoll. Elsevier. 2016;52:275–88. https://doi.org/10.1016/j.foodhyd.2015.07.003.

Zielke C, Lu Y, Poinsot R, Nilsson L. Interaction between cereal β-glucan and proteins in solution and at interfaces. Colloids Surf B: Biointerfaces. 2018;162:256–64. https://doi.org/10.1016/j.colsurfb.2017.11.059.

Zielke C, Lu Y, Nilsson L. Aggregation and microstructure of cereal β-glucan and its association with other biomolecules. Colloids Surf A Physicochem Eng Asp. 2019;560:402–9. https://doi.org/10.1016/j.colsurfa.2018.10.042.

Levan

3

Onur Kırtel and Joan Combie

Contents

O. Kırtel
Bioengineering Department, Industrial Biotechnology and Systems Biology Research Group – IBSB, Marmara University, İstanbul, Turkey

J. Combie (✉)
Montana Biopolymers Inc., Winnsboro, SC, USA
e-mail: joan@polysaccharides.us

J. M. Oliveira et al. (eds.), *Polysaccharides of Microbial Origin*,
https://doi.org/10.1007/978-3-030-42215-8_3

Abstract

Polysaccharides generated by microorganisms display structural diversity in terms of monosaccharide structure and organization, molecular weight, and branch design. This diversity facilitates their use in a plethora of food, health, biomedical, pharmaceutical, and cosmeceutical applications. Besides some of the well-known and industrially popular microbial polysaccharides like xanthan and dextrans, the effort to discover novel polymers with distinct functionalities has been on the rise in recent decades. Among these polymers, fructans are a class of homopolysaccharides made up of fructose residues. Though the β-2,1 linked fructan inulin has been extensively studied and widely used in the food industry for a long time, its β-2,6 linked counterpart, levan, has now started to spark interest. Already levan has proven to be an incredibly versatile polymer thanks to its unique combination of physicochemical properties and potent biological activities. Health-related opportunities reported on in recent publications include levan as an antitumor, antioxidant, immunostimulant, antimicrobial, and a wound healing substance. The polymer has been tested for a potential role in the treatment of ulcers, diabetes, elevated cholesterol, and low calcium absorption. This overview summarizes recent findings on prospective uses of levan for the health and well-being of mankind.

Keywords

Polysaccharide · Fructan · Levan · Tissue engineering · Wound healing

1 Introduction

The levan narrative dates back hundreds of years to the development of a traditional Japanese food that became associated with long life and good health. That food, natto, is made by the fermentation of soybeans by *Bacillus subtilis* natto. We now know that levan is among natto components, knowledge that has rekindled interest in the health benefits of levan.

Levan is a homopolymer of fructose (i.e., fructan), containing β-2,6 linked fructose units on its polymer backbone. Varying degrees of branching points on β-2,1 positions are encountered in most levans. For decades, inulin (a fructan that is β-2,1 linked) has attracted much greater attention than levan, in industry and scientific areas perhaps because of its ready availability and low cost. Inulin and its short chain derivatives are among the most widely used functional food ingredients in the world thanks to their prebiotic activity. However, over the last decade, levan has received increased attention due to the discovery of its exceptional physicochemical properties and biological activities (Öner et al. 2016).

Levan-type fructans are produced by many different genera of both Gram-positive and Gram-negative bacteria, mainly *Bacillus, Erwinia, Gluconacetobacter, Lactobacillus, Leuconostoc, Paenibacillus, Pseudomonas, Streptococcus, Zymomonas,* etc.

(Öner et al. 2016). Furthermore, recently it was shown that some halophilic Archaea are able to synthesize levan as well (Kırtel et al. 2019). Some plants also accumulate levan-type fructans (Joaquim et al. 2018). However, their molecular weights are much lower compared to their microbial counterparts. Molecular weights of microbial levans are usually expressed in million Da values while plants produce much smaller molecules. The molecular weight and branching pattern of levans affect their properties.

This chapter summarizes recent reports on levan with special emphasis on its biomedical applications. Production, functionalization, and properties of levans are also discussed.

2 Extraction, Isolation, Purification and Production Advanced Processes

2.1 Production by Fermentation

Levan is being made in dozens of research labs around the world. There are two approaches: (1) long-established fermentation and (2) more recent innovations for enzymatic production.

The feedstock for both methods is commonly commercial sucrose. Use of various waste products has been tested in an effort to decrease costs. Molasses were suitable but some preprocessing of the molasses was needed for optimal yield (Küçükaşik et al. 2011). Sugarcane syrup, tomato juice, and maple syrup have been successfully tested. Raffinose is found in wastewater from soybean processing and has been proven as an alternative to sucrose for making levan (Yamamoto et al. 1985).

In addition to 50–500 g/L sucrose, media for fermentation must include elements required by the microorganism such as sources of nitrogen and phosphorus. Many researchers add a complex component such as yeast extract or peptone to serve as a source of trace elements and vitamins. Trace elements vary with the microorganism but commonly include iron, magnesium, and calcium (Esawy et al. 2013). Sarilmiser et al. (2015) found boric acid was an effective stimulator of levan production in *Halomonas smyrnensis* AAD6. Boron occurs at high levels in some salt lakes and is part of the interspecies quorum sensing structure AI-2, demonstrating the necessity of understanding the habitat of isolated species when selecting trace elements. As researchers have begun to scale up from shake flasks to stirred fermenters, many have included pH control in the protocol. For some organisms, a steady pH resulted in an increase in the amount of levan generated but pH control was detrimental to production by other microbes. For fermentations, temperature is largely determined by the optimum for the microorganism, allowing little influence on the balance between transfructosylation and hydrolysis. This is in contrast to enzymatic production where temperature is a consequential parameter.

Glucose is a potent inhibitor of levansucrase (Euzenat et al. 1997). As sucrose is hydrolyzed to free the fructose residue for polymerization into levan, the other half of the sucrose molecule, glucose, begins to accumulate in the medium. On a lab scale, using glucose oxidase to convert the inhibitory glucose to gluconolactone,

adding yeast or utilization of a membrane reactor to remove the glucose allows the reaction to continue longer. All of those methods add to the cost of production so the usual approach at a larger scale is to end the fermentation when it becomes evident no further high molecular weight levan will be formed.

There is a question about the optimum volume of the inoculation. As much as 10% inoculum can be used although 1–5% is more common. A very high inoculation rate will start the fermentation faster, but the need for a larger seed tank and the transfer of more waste products to the production fermenter may offset the time advantage.

Sucrose concentration impacts the fermentation efficiency and the molecular weight of the levan generated. When the number of sucrose molecules far exceeds the number of levansucrase molecules, production of 6-kestose trisaccharide precursors that bind the enzyme is favored. A lot of levan is produced but the chains are short (Öner et al. 2016). Some microorganisms inherently produce higher molecular weight levans than others. For example, *Kozakia baliensis* produces levans with a molecular weight in excess of one billion Daltons (Jakob et al. 2013), while levans from *B. subtilis* tend to fall in the range of a few million Daltons. Other factors must be considered when selecting the optimum sucrose concentration. Although a high concentration of sucrose (300–400 g/L) produces the largest amount of levan, a medium with less sucrose (50–100 g/L) is more efficient, converting a larger portion of the feedstock to levan in a shorter period of time.

As production is scaled up, all of these factors must be balanced against the cost of the fermenter space. What is the total cost of the feedstock, production medium, and fermenter volume per unit of time for each kg of levan? Is it cost effective to run at a lower efficiency but in a smaller fermenter (facilitated by a higher sucrose concentration)? Or does the cost of the sucrose outweigh the cost of the larger size fermenter? When the sucrose level drops low enough, levansucrase changes from a polymerization force to an exolevanase, cleaving off the terminal fructose unit of levan in a consecutive manner (Hernández et al. 1995), thus lowering the molecular weight of the levan that has just been produced. The ideal time to end fermentation will depend on the microorganism and the production parameters so must be established for each situation.

At the end of the fermentation, cells are promptly removed to avoid losing the higher molecular weight levan to hydrolysis by levansucrase. This is typically accomplished by centrifugation or filtration. Levan is accumulated in the extracellular space, greatly reducing production costs compared with most biopolymers which are synthesized intracellularly or remain bound to the cell membrane. Levan is usually recovered from the clarified fermentation medium by solvent precipitation. Ethanol is the most commonly used solvent. Isopropyl alcohol can be used but requires a larger volume. Again, costs must be considered. Does the greater yield offset the cost of a much larger volume of isopropanol? Further, will the end use permit processing with isopropanol or will toxicity issues eliminate this solvent for use in certain applications? To remove low molecular weight sugars, a water wash followed by a second ethanol precipitation or dialysis is often used as a final purification step.

2.2 Enzymatic Production

Levan is synthesized by the enzyme levansucrase (EC 2.4.1.10). It is a member of the Glycoside Hydrolase Family 68 (GH68) enzymes and is usually secreted into the extracellular space. The second enzyme in the GH68 family is inulosucrase (EC 2.4.1.9), which produces the other main type of fructan, inulin. All levansucrases harbor a five-bladed β-propeller domain, wherein the active site of the enzyme is located in the deep central cavity. Sucrose is the preferred substrate for all levansucrases. The enzyme hydrolyzes sucrose and transfers the resulting fructosyl moiety to an acceptor molecule, which is usually another sucrose in the case of levan production. Consequently, a trisaccharide called 6-kestose is formed, and the continuous transfer of fructosyl moieties to this elongating chain of oligosaccharide results in the formation of levan polymer (Lammens et al. 2009).

Depending on the reaction conditions such as temperature, initial sucrose concentration, and enzyme origin, levansucrases may synthesize both short-chain fructans (fructooligosaccharides, FOSs) or levan polymers with molecular weights of millions of Daltons. Higher temperatures usually impede the polymerization process and favor sucrose hydrolysis, since water molecules become better fructosyl acceptors compared to an elongating oligosaccharide chain. Similarly, increasing initial sucrose concentrations usually favor FOS formation instead of long-chain fructan synthesis. In that case, excess amounts of sucrose molecules act as better fructosyl acceptors, thus resulting in enhanced FOS yields (Öner et al. 2016). Depending on the enzyme origin, some levansucrases are able to produce significant amounts of both FOSs and levan under the same reaction conditions, while others predominantly synthesize one or the other.

Enzymatic production of levan has distinct advantages compared to its microbial production such as shorter production times, less by-product formation, higher flexibility in terms of reaction conditions, and a wide range of immobilization techniques. Table 1 shows some enzymatic levan production studies from the literature.

3 Structural, Physicochemical, and Biological Properties

Levan is distinguished from other polysaccharides by its unusual combination of properties. Particularly rare is a material with an exceptionally low intrinsic viscosity combined with a very high adhesive strength but that is a good characterization of levan.

No two levans produced by different microorganisms or different sources of isolated enzyme are identical. The structural properties are largely determined by the production organism or enzyme, but the cultivation conditions will also affect molecular weight, particle size, degree of branching, and to a lesser extent, viscosity, bioactivity, and stability of the biopolymer.

Table 1 Enzymatic production of some levans via levansucrase

Microorganism	Sucrose (g/L)	Levan (g/L)	Production conditions	Production method	References
Bacillus methylotrophicus SK 21.002	250	100	pH 6, 40 °C, 24 h	Native enzyme	Zhang et al. (2014)
Bacillus subtilis (Natto)	350	63.6	pH 7, 35 °C, 36 h	Native enzyme	Bersaneti et al. (2018)
Brenneria goodwinii	500	185	pH 6, 35 °C, 12 h	Recombinant enzyme	Liu et al. (2017)
Clostridium acetobutylicum	100	60	pH 6, 60 °C	Recombinant enzyme	Gao et al. (2017)
Lactobacillus reuteri LTH5448	500	183.2	pH 6, 35 °C, 12 h	Recombinant enzyme	Ni et al. (2018)
Zymomonas mobilis ATCC 10988	200	83	pH 5, 15 °C	Immobilized recombinant enzyme	Chiang et al. (2009)

3.1 Structural Properties

Levan is soluble in water and in DMSO (dimethyl sulfoxide). Levan does not dissolve in most organic solvents. Rather than forming a large, spread out, multi-branched structure like most polysaccharides, levan is packed into a spheroidal structure, roughly 25–250 nm in diameter (Jakob et al. 2013). It is this compressed arrangement that is responsible for the low intrinsic viscosity. The spheroidal shape does not lend itself to making a flexible film although additives do facilitate film formation. For example, when blended with 10% montmorillonite, the clay platelets are bridged by uncoiled levan molecules producing a tough, flexible, transparent film (Chen et al. 2014).

3.2 Rheology

As noted earlier, the compact form of levan is reflected in its low intrinsic viscosity. Different microorganisms produce levans with different intrinsic viscosities but they are generally under 0.5 dL/g (Benigar et al. 2014; Gojgić-Cvijović et al. 2019). In comparison, polymers used as thickeners such as carboxymethyl cellulose have an intrinsic viscosity values that approach 100 dL/g.

Plotting zero-shear-specific viscosity for levan against concentration multiplied by the intrinsic viscosity on a log-log scale illustrates how the levan spheroids are impacted as the amount of levan in a solution is increased. The most dilute region shows no interaction between the tiny spheres. As the concentration rises, individual molecules begin to make contact. The swept-out volume occupied by the spheres and the branches sticking out of the surfaces approaches the total volume of the solution. Eventually the spheres are pushed close enough together for molecular interconnections and for branches to become entangled at which point the specific

viscosity rises dramatically. A study by Benigar et al. (2014) illustrates sharp differences in rheological behavior of levans produced by different bacteria. One percent solutions of levan from *B. subtilis*, *Erwinia herbicola*, and *Zymomonas mobilis* had similar intrinsic and specific viscosities. However, raising the levan concentration to 8% showed large differences in the resistance to flow of the solutions. The specific viscosity for levan from *B. subtilis* was very low, for levan from *E. herbicola* the specific viscosity increased ten-fold, and for levan from *Z. mobilis* the specific viscosity rose 1000-fold. The obvious assumption is that some levans in this group had far fewer branches than others, though this is not true. All three levans had 10–11% branching (Benigar et al. 2014). Nothing is known about the length or structure of those branches and this may be where the specific viscosity disparities are explained. Are some branches only a single residue long (Djurić et al. 2017) while others carry a dozen fructose moieties? Detailed characterization of the branching patterns of different levans is essential to better understand the various characteristics of this biopolymer.

3.3 Degree of Branching

Two levans are known to have no branches (*Brenneria* sp. and *Halomonas smyrnensis* AAD6) and at least two have 6% or less branching (*B. subtilis* AF17 and *Pseudomonas*). Most others described in the literature have between 7% and 20% branching (Djurić et al. 2017; Gojgić-Cvijović et al. 2019). Branch length of few levans is known. The branches on *Brachybacterium paraconglomeratum* have only one residue (Djurić et al. 2017) and *Streptococcus salivarius* levan branches are made up of four residues each. Nothing is known about the branch design – straight chains, branching, subbranching – structures that could explain diverse biological activities. It should be noted that the production conditions of levan may greatly affect its branching pattern. While levan obtained from cultures of *H. smyrnensis* AAD6 carries almost no branches, levan synthesized via the recombinant levansucrase enzyme of the same bacterium shows more than 10% branching (Kırtel et al. 2019).

3.4 Molecular Weight

The molecular weights of levans cover a broad range. A *Zymomonas mobilis* levan reportedly has a molecular weight of 700,000 Da. On the other end of the scale is *Klebsiella baliensis* levan with 95% of the polymer coming in at 576–2069 million Daltons and 5% topping out at nearly 620 billion Da (Jakob et al. 2013). The next largest levan known at the moment comes from *Brenneria goodwinii* with a molecular weight of 1.3×10^8 Da (Liu et al. 2017). More typical are levans in the molecular weight range of $4–25 \times 10^6$ (Öner et al. 2016). Higher molecular weight levans are more compact due to increased intramolecular interactions, resulting in smaller differences in hydrodynamic diameters than might be anticipated (Jakob et al.

2013). As with most other properties, the molecular weight is largely determined by the producing microorganism but production parameters can have a considerable impact. For example, at 400 g/L of sucrose, almost all of the levan made by *B. subtilis* had a molecular weight around 8000 Da but in medium containing only 20 g/L sucrose the molecular weight was 2 million Da. Quite often there is a bimodal distribution of the molecular weight with the apportionment shifting as the sucrose level is altered.

3.5 Adhesive

One of the distinguishing properties of levan is its high adhesive strength. Adhesion is the result of hydrogen bond formation between the levan hydroxyl groups and the substrate and to Van der Waal forces. The fact that this can be accomplished at a low intrinsic viscosity is an advantage for spray-on applications. A thin solution that does not clog the sprayer orifice is the winning edge. Curing is by water removal allowing modification of the open time to suit a range of applications. As water is eliminated, the levan molecules are pushed closer together. It is possible that entanglement of the levan branches further enhance the adhesive strength, but with so little known about the length of the branches and the fact that one levan with no branches has been shown to have good adhesive strength leaves this possibility an open question.

3.6 Anti-Inflammatory and Antioxidant Activity

Oxidative stress and inflammation are interrelated. The inflammation triggered by oxidative stress is the cause of many chronic diseases. Anti-inflammatory and antioxidant activity of levan has been demonstrated in a number of laboratories. The ability of levan to act as an antioxidant is attributed to its ability to interrupt free radical chain reactions. This activity is modified by structural parameters, such as molecular weight, functional groups, and branching.

It has been found that levan and sulfated levan are potent free radical scavengers when tested against 1,1-diphenyl-2-pycryl-hydrazyl (DPPH) radicals. In some cases, the sulfated levan has higher antioxidant activity than the parent polymer. Such is the case for derivatized *Paenibacillus polymyxa* and *B. subtilis* levans. The SC_{50} was 1.5 micrograms per ml for a sulfated levan and 3.5 micrograms per ml for the native levan (Abdel-Fattah et al. 2012) comparing favorably with the SC_{50} of 8.0 micrograms per ml for ascorbic acid. When levan from *Acetobacter xylinum* NCIM2526 was assessed for its antioxidant potential via DPPH free radical scavenging assay, it exhibited 81.26% of the antioxidant activity shown by ascorbic acid at equivalent concentration (1 mg/ml, w/v) (Srikanth et al. 2015). In another study, Kim and Chung (2016) developed levan-coated cerium oxide nanoparticles. Cerium oxide is used in biomedical applications due to its redox activity. However, naked cerium oxide particles have poor water solubility; thus, coating this material with water-soluble polysaccharides is a promising method to increase its bioavailability. The authors also used other polymers for coating cerium oxide nanoparticles, namely,

inulin, dextran, starch, and hyaluronic acid. The DPPH free radical scavenging assay revealed that levan was superior in terms of antioxidant activity. The inhibition rate of DPPH activity was around 85% for levan, followed by dextran at around 65% inhibition. Levan from the moderately halophilic bacterium *Halomonas smyrnensis* AAD6 was used to treat pancreatic β-cell line INS-1E under high glucose conditions and H_2O_2 stress. Results showed that *Halomonas* levan was remarkably efficient in decreasing both oxidative stress and apoptosis (Kazak et al. 2014). Antioxidant activity of *B. subtilis* levan has also been demonstrated by ABTS decolorization and the phosphomolybdate method. Fullerene C_{60} is a powerful antioxidant but its poor water solubility has limited its use. Noncovalent modification of fullerene C_{60} by levan decreased the hydrophobicity, facilitating biological use of the antioxidant activity. The ability of levan to increase superoxide dismutase, catalase, and cytokine IL-4 has been established.

Potential real-world uses of levan antioxidant activity have been described. Systemically administered levan inhibited inflammation of rabbit and guinea pig skin to *Staphylococcus aureus*. A reduction in the inflammatory process was noted when test animals were given levan intraperitoneally. Histopathological analysis showed that levan-treated rats were afforded significant protection from damage to the aorta despite a high cholesterol diet (Dahech et al. 2013).

3.7 Immunostimulant

The immunostimulant activity of levan has been demonstrated repeatedly. *Curcuma kwangsiensis* levan stimulated macrophage proliferation and enhanced phagocytosis at concentrations below 200 μg/ml. *Paenibacillus bovis* levan enhanced spleen lymphocytes proliferation and increased the concentration of tumor necrosis factor (TNF-α). Levan from *Bacillus licheniformis* stimulated IL6 expression even at very low levels. A practical application of this immunostimulatory effect was demonstrated on pigs. Supplementing the diet with 0.1% levan improved growth performance and augmented the immune response to an inflammatory response (Li and Kim 2013). When the diet of juvenile *Cyprinus carpio* (common carp) diet was supplemented with 0.5% (w/w) levan from *Bacillus megaterium*, hemoglobin and the total erythrocyte count of the fish were significantly increased, and 100% of them survived an *Aeromonas hydrophila* challenge (Rairakhwada et al. 2007). In another study, *Epinephelus coioides* H. (orange-spotted grouper) were fed varying amounts of levan (0–50 g/kg) obtained from *Bacillus licheniformis* FRI MY-55, and it was seen that in all groups fed with levan, the count of toxic *Vibrio* spp. and total viable aerobic bacteria in the intestines were significantly decreased. Moreover, the survival rate of orange-spotted groupers fed 25 g/kg levan was significantly higher compared to the control group (Huang et al. 2015). Xu et al. (2006) assessed the immunostimulating effects of levan purified from soybean mucilage (originating from *B. subtilis* (Natto) and found that the production of IL-12 p40 and TNF-α were strongly induced in macrophage cell lines in vitro. Their experiments also demonstrated that levan was recognized by Toll-like receptor 4 (TLR4) in mice and a

human cell line. When levan was orally administrated to ovalbumin-immunized mice, ovalbumin-specific IgE serum levels and Thr2 response were significantly reduced. It is evident that levan is a powerful immunostimulator, and once its production is made more cost-effective, it will doubtlessly find a solid place in health-related applications.

3.8 Description of the Advanced Functionalization and Modification Methods

Levan can be chemically derivatized to modify its molecular structure, resulting in altered physicochemical properties and biological activity. Most common derivatization methods used on levan are aldehyde-activation, sulfation, and phosphonation.

Sulfation is usually carried out by mixing a suspension of levan in pyridine with chlorosulfonic acid ($ClSO_3H$). Since hydroxymethyl groups on the fructofuranosyl rings are sterically more available, they are better targets for sulfation compared to hydroxyl groups. The sulfation process creates S=O, C-O-S, and C-O-SO_3 linkages on the levan chain. In recent years, sulfated levan has been the subject of various biomedical applications. Levan from *Bacillus subtilis* NRC1aza and its sulfated derivatives were all shown to be strong antioxidants. Moreover, sulfated levan in this study was shown to induce apoptosis in a liver cancer cell line, HepG2. This indicates the potential of sulfated levan as a promising antitumor component (Abdel-Fattah et al. 2012). Erginer et al. (2016) used levan from *Halomonas smyrnensis* AAD6 as a target for sulfation and evaluated its anticoagulant activity. The results showed that sulfated *Halomonas* levan was an exceptionally potent anticoagulant, demonstrating even higher anticoagulant activity via thrombin inhibition than heparin at certain concentrations. In vitro experiments with mouse fibroblast L929 cell line showed that sulfated *Halomonas* levan was biocompatible. When treated with concentrations of sulfated *Halomonas* levan as high as 1000 μg/ml, more than 70% of the cells remained viable. Gomes et al. (2018) developed adhesive, free-standing, multilayer films containing chitosan, alginate, and sulfated *Halomonas* levan. The presence of sulfated *Halomonas* levan provided higher shear strength and tensile strength to the films. When compared to films developed without sulfated levan, films with sulfated levan exhibited four times higher adhesion strength. Myoblast cells were grown on sulfated levan-containing films, which turned out to be both cytocompatible and myoconductive. In a study carried out by Avsar et al. (2018), sulfated-hydrolyzed *Halomonas* levan (ShHL) was used with polycaprolactone (PCL) and polyethyleneoxide (PEO) to develop electrospun fibrous scaffolds for tissue engineering applications. Increasing concentrations of ShHL in the matrices provided an elongation at break value tenfold greater than to matrices without ShHL. L929 fibroblast and HUVEC cells were successfully grown on the fibrous scaffolds, pointing to their potential in tissue engineering.

Phosphonation of levan is another derivatization method that has been assessed in a number of studies. The method for the phosphonation of levan is similar to that of sulfation. However, in this case, phosphorus trichloride (PCl_3) is used as the

phosphorus donor, creating P-H, P=O, P-O-H, and P-O-C linkages on the levan chain (Costa et al. 2013). Layer-by-layer films containing chitosan and phosphonated *Halomonas* levan (pHL) were developed. When compared to the control group, in which pHL was replaced with alginate, chitosan-pHL films showed about three times higher detachment force. As verified with scanning electron microscopy, surfaces of chitosan-pHL films were homogeneous and smooth, providing significantly strong adhesion to L929 fibroblast cells (Costa et al. 2013). The potential of pHL to remove bisphenol A from aqueous solutions was also investigated. Bisphenol A is a monomer of the plastic polycarbonate and is an endocrine disrupting compound widely encountered in sewage sludge, sediments, and surface water. In the study conducted by Haciosmanoğlu et al. (2019), pHL was shown to be a promising adsorbent for removal of bisphenol A from water. pHL was able to adsorb 104.8 ± 5.02 mg/g bisphenol A at 25 °C, and it could be reused for three cycles of adsorption-desorption.

Periodate oxidation is another common chemical modification method for levan. Potassium periodate (KIO_4) is the preferred oxygen donor. This method is also called "aldehyde activation," since aldehyde groups are formed on fructofuranosyl rings as a result of oxidation. Several studies are present in the literature where the physicochemical properties and biological activity of oxidized levan are identified. Vina et al. (2001) used levan from *Zymomonas mobilis* as an immobilizing matrix for the antileukemic enzyme from *Erwinia carotovora,* L-asparaginase. Levan was first oxidized via KIO_4, followed by reductive alkylation by $NaBH_4$. A gentle oxidation around 24% yielded the highest residual enzyme activity. The K_m value of the immobilized enzyme was higher than that of native enzyme. Conjugation with levan increased the pH tolerance, thermal stability, and storage stability of L-asparaginase. Sima et al. (2011) developed nanostructured thin films using oxidized levan from *Halomonas smyrnensis* AAD6. It was revealed that oxidation increased the hydrophilic behavior of levan, due to the presence of acidic aldehyde-hydrogen bonds. Specific surface areas of the films showed high potential for proliferation of SaOs2 osteoblast cells. Maciel et al. (2012) synthesized magnetic levan particles for trypsin immobilization. Magnetic levan particles were oxidized via $NaIO_4$. Trypsin immobilized on these particles was used 10 times, and only lost 16% of its initial specific activity. In another study, Sarilmiser and Öner (2014) investigated the effect of oxidation on anticancer activity of *Halomonas* levan, and oxidized levan showed higher anticancer activity compared to its unmodified form for all cell lines tested, namely, A549 (human lung adenocarcinoma), AGS (human gastric adenocarcinoma), HepG2/C3A (human liver hepatocellular carcinoma), and MCF-7 (human breast adenocarcinoma). The underlying mechanism of levan's anticancer activity was shown to be apoptosis, as demonstrated by the luminogenic-based caspase-3/7 activity assay.

All these results point out that chemical modification is a valuable tool to endow levan with novel physicochemical properties and biological activities. In the future, a better understanding of the structural aspect of levan such as molecular weight and branching pattern on the efficiency of chemical modifications may yield even more striking results.

4 Applications and Uses in Medical and Pharmaceutical Fields

The inherent safety of levan makes it attractive for use in medicine. An array of beneficial activities has fired up the imagination of researchers. From treating burns, ulcers, and leishmania to decreasing cholesterol and drug toxicity, lab studies offer tantalizing glimpses into potential utilization in medicine and pharmaceuticals.

4.1 Healing Damaged Tissue

Levan has been tested for efficacy in healing damaged tissue. Incorporated into thin films, levan enhances the process of healing stubborn wounds, burns, and bone fractures not responding to conventional treatments. What is there about levan that suggests it could be of value to wounded patients? Nearly a decade ago, Sturzoiu et al. (2011) selected levan for studies of the healing process based on the known benefits of polysaccharides in traditional medicine. They found that levan has a role in metalloproteinase activation, a key step in the healing of tissues that have been burned or mechanically damaged. Metalloproteinases are secreted as proenzymes so require activation before they effectively remove damaged cells, clearing the way for migration of new cells, cell proliferation, and development of new vasculature. They determined that active metalloproteinases were present at a high level when test animals were treated with levan suggesting that levan activated the metalloproteinases, the critical step for initiation of the healing process.

Since then, several groups have incorporated levan into films for use in medical applications. Matrix-assisted pulsed laser evaporation (MAPLE) was used to prepare films with a gradient of levan and oxidized levan. Looking at cell proliferation across the gradient, they noticed that certain areas had a greater capacity to modulate the intracellular signaling events in focal adhesion, the site where the cells preferentially interacted with the engineered matrix. Intracellular signaling appeared to initiate gene expression leading to cellular differentiation. Cellular proliferation was greatest on oxidized levan with a substantial amount of growth observed around the boundary area between the oxidized levan and the native levan (Axente et al. 2014; Sima et al. 2011).

In another approach, levan-phosphonate and chitosan were electrostatically self-assembled layer-by-layer to form films on glass slides. These films had remarkable lap shear strength of 2500 ± 300 kPa, 3 times the strength of similar films when alginate was substituted for the derivatized levan. Dermabond®, a cyanoacrylate-based glue approved for medical use, has a shear adhesive strength of 180 kPa on bovine pericardium. While differences in test conditions certainly would affect these values, the work does suggest the phosphorylated levan-chitosan films may be competitive with the cyanoacrylate adhesive. Additionally, it was noted that cells adhered in far greater numbers to the films including levan as compared to those where the levan was replaced with chitosan (110 cells per mm^2 for levan versus 20 cells per mm^2 for chitosan) (Costa et al. 2013). Sulfated levan, chitosan, and alginate were later formed layer-by-layer into free standing films (Gomes et al. 2018).

A third method was to form cast films of levan blended with chitosan and poly (ethylene oxide) (PEO). While chitosan, PEO and levan have film forming capabilities, all have shortcomings. Varying the ratios of the three components, blends with enhanced stability, flexibility, and transparency were prepared.

Experiments in healing were not limited to tissue damaged mechanically or by burns. It was found that levan could be of value in bone regeneration when poor adhesion or development of a fibrous capsule created problems for regrowth of the bone. A supercritical CO_2 assisted technique was used to add levan to either cellulose acetate or polyvinylidene fluoride-co-hexafluoropropylene. The resulting film or foam structures were deemed potentially suitable for tissue engineering (Tabernero et al. 2019).

A blend of levan, gelatin, and a biodegradable polyester has been used as the feedstock for three-dimensional bioprinting (Duymaz et al. 2019). Low molecular weight *Halomonas* levan was obtained via mild acid hydrolysis, where its molecular weight was reduced from 4.247×10^6 Da to 1.051×10^5, thus providing better water solubility. Density and viscosity values of 3D scaffolds were directly proportional to levan concentration in the mixture. The scaffolds were uniform in pore size and homogeneous in structure. To assess the biocompatibility of these 3D scaffolds, human osteoblast (HOb) cell line G-292 was used. Scaffolds containing levan provided better proliferation than the control group (scaffolds without levan). Scanning electron microscopy images revealed that cells were randomly distributed on and inside the scaffolds, creating more dense populations in some areas where levan concentration is probably higher. The results indicate that levan-based 3D bioprinted scaffolds are promising biomaterials for bone tissue engineering.

4.2 Cholesterol

The ability of levan to suppress blood lipids and body fat even when high-calorie foods were consumed was described in a patent 25 years ago. Since then, several projects have arrived at similar conclusions. An extensive laboratory study found that when compared with controls, rats on a diet that included 1% or 5% (w/w) levan had a significantly lower cholesterol accompanied by an increase in fecal excretion of sterols and lipids. The authors pondered possible mechanisms for the observed effects on serum cholesterol. There was no clear-cut answer but one theory was simply that levan prevented sterol absorption (Yamamoto et al. 1999). The beneficial effect of levan on lipid profiles of rats has been reported by several others including most recently by Bahroudi et al. (2020) who documented a decreased serum total cholesterol and LDL in rats treated with 5% levan. An added bonus was that the levan appeared to control body weight gain.

4.3 Diabetes

Nearly nine percent of the world population, ages 20–79, are living with diabetes. Using diabetic rats dosed with levan, Dahech et al. (2011) looked at the effect of the

polysaccharide on the glucose level and the effect on organs adversely affected by diabetes. Perhaps the most remarkable result was the impact on the glucose concentration. Control animals averaged 1.2 gm/L glucose and diabetic rats averaged 3.6 gm/L glucose. The plasma glucose in diabetic animals treated with levan averaged 1.7 gm/L, a 52% decrease. Importantly, levan had no effect on glucose levels in normal rats. Increases of superoxide dismutase, catalase, and glutathione peroxidase activities in both pancreas and liver were observed. Histological work confirmed protective activity of the levan treatment. At first it might seem counter-intuitive that a polysaccharide could be useful in the treatment of diabetes. Understanding the roles of oxidative stress, autoimmunity, and glucose absorption in diabetes, the researchers proposed possible mechanisms to account for the effect of levan: (1) inhibition of the auto-immune reaction leading to less pancreatic β-cell damage, (2) antioxidant activity of levan decreasing damage to the pancreas, and (3) prebiotic activity increasing the amount of short chain fatty acids which might inhibit glucose absorption (Dahech et al. 2011). Surprisingly, other than an in vitro study that found levan alleviated oxidative stress on pancreatic cells, little work has been done to follow up on this potentially game-changing approach to treatment.

4.4 Anticancer Activity

Many polysaccharides are known to have anticancer and cancer preventive properties and levan is no exception. Decades ago, a group led by Leibovici in Israel published extensively about tumor inhibition by levan. In the last few years, levan has reappeared in anticancer research. An attempt has been made to develop a better understanding of the mechanism by which levan inhibits the development of malignancies. One question is why some studies were much more successful than others. One thought was that the molecular weight of the levan determined its efficacy as an antitumor agent but tests on a series of four different molecular weight levans quashed that idea. As more data was gathered, the complexity of factors determining the efficacy of levan antitumor properties became apparent. Some levan derivatives were more effective than the parent compound and some cell lines were more susceptible to treatment than others. Abdel-Fattah et al. (2012) found that sulfated levan was selectively cytotoxic against Hep G2 cells. The effective dose was more than an order of magnitude higher than for paclitaxel (Taxol®) but the potential for the levan derivative to present fewer side effects cast the results in a more favorable light. Quarternized levan was found to be only slightly less effective against a human breast cancer cell line, MCF-7, than the reference standard cisplatin. Mice treated with levan sulfate along with a cancer promoting chemical were found to have tumor necrosis factor levels well below those not receiving the levan derivative and even below the controls. The possibility was raised that levan anticancer activity resulted from deregulation of energy pathways and cellular homeostasis leading to the accumulation of lactate. Since an increase in lactate is also related to oncogenesis, this intriguing theory brings up more questions. Another explanation recently explored is that the antiproliferative effects of levan are mediated by oxidative stress.

Breast cancer MCF-7 cells were treated with levan from *Halomonas smyrnensis* AAD6. Cancer cell proliferation was decreased by induction of apoptosis facilitated by oxidative stress.

4.5 Calcium Absorption

Difructose anhydride IV (DFA IV) promotes calcium absorption and bone growth, potentially inhibiting the development of osteoporosis. This cyclic disaccharide is obtained by levan conversion with bacterial levan fructotransferase (EC 4.2.2.16). It was postulated that the mechanism was an increased permeability due to an opening of the tight junctions. No et al. (2007) reported that when a low-calcium diet (0.1% calcium) was supplied with 5% (w/w) levan, rats exhibited a higher net calcium absorption compared to the control group (no levan supplementation), though femoral weights and calcium contents were not significantly different.

4.6 Toxicity Decrease

One of the earliest tests of levan in the role of protective agent was described in a 1959 publication. In a study of tissue resistance to toxic substances, sulfated levan was deemed to be highly protective against lethal doses of the drugs 48/80, polymyxin B and stilbamidine. More recently, as part of a biocompatibility assessment of levan, the polymer was added to jars of brine shrimp. The LD_{50} of the antiviral sesquiterpene hydroquinone (avarol) was increased dramatically from 0.18 to 10 ppm (Poli et al. 2009). Others have found levan decreases the toxicity of copper and an insecticide.

4.7 Antimicrobial

Most studies examining levan activity against various microorganisms have shown effective doses of levan are far higher than drugs already on the market. However, there are some mitigating factors that favor the polysaccharide. Levan is nontoxic, the production process is substantially more environmentally friendly, and the mechanism of action is usually not the same as that for the commercially available treatments.

Parasites and Viruses: For example, miltefosine, a drug with teratogenic potential and the only oral drug available to treat some forms of leishmania, the IC_{50} of levan is 700 times higher than that of miltefosine (Al-Halbosiy et al. 2018) but without the serious side effects. Similarly, antiviral drugs can cause adverse reactions while levan is anticipated to display fewer negative outcomes. Of the few known trials to date, one reported on the value of sulfated levan to treat *Herpes simplex* type 1 and influenza virus type A.

Fungi: Antifungal agents present a particular challenge because, like their hosts, fungi are eukaryotes; a drug that is toxic to a fungus is also toxic to the patient. Fungal diseases kill more than 1.5 million people annually and affect over a billion people. Salman et al. (2019) found the minimum inhibitory concentration (MIC) of levan for all isolates of *Candida albicans* tested ranged from 50 to 100 mg/ml. While this dose is far above that of commercial antifungal agents (0.5 micrograms per ml), it showed inhibitory effects on the growth and virulence of the yeast potentially without the harmful downside.

Bacteria: Kimberly-Clark Worldwide Inc. received a patent for use of levan, inulin, or graminan to inhibit the adherence of flora to skin, mucosa, or inanimate surfaces like wood and countertops. The fructose polymers either attached to the surface, thereby displacing microorganisms, or they coated the microorganisms preventing their adherence effectively precluding microbial diseases. Nanocomposites of levan-iron oxide, levan-titanium oxide, and levan-zinc oxide (Taran et al. 2019) effectively inhibited the growth of *Escherichia coli* and *Staphylococcus aureus* while levan-silver nanoparticles displayed antibacterial activity against the same two bacteria as well as *Klebsiella pneumonia* and *Salmonella typhimurium*.

4.8 Ulcer Treatment

It has been estimated that 10% of adults are affected by peptic ulcers at least once in their lifetime, most often traced to *Helicobacter pylori*. Levan was tested as a potential antibacterial agent. Surprisingly, levan was inefficacious against *H. pylori* but 200 mg/kg levan effectively healed ulcers in lab rats. Histological observations found marked necrosis of the upper mucosal layer and edema, inflammatory cell infiltration, and congestion of the mucosa in the stomach of control rats. Stomach sections of animals treated with levan showed marked restoration of the gastric mucosal layers. It was proposed that the protective coating of levan and possibly prebiotic activity led to the beneficial outcome (Ragab et al. 2019).

4.9 Pharmaceuticals

Drug delivery to the colon can be hampered by degradation as it passes along the digestive tract. One approach was to encapsulate the drug in a film of a methacrylate polymer blended with levan. The film remained stable until it reached the colon. At that point, colonic microbes metabolized the levan and the encapsulated drug was released into the colon. In a second tactic to deliver a drug to the distil end of the intestine, derivatized levan was used to cross link a temperature responsive polymer. The release rate of the encapsulated drug could be tuned by changing the formulation (Osman et al. 2017).

The value of levan in another formulation has been explored for potential delivery of a topical drug. Pantelić et al. (2020) used levan to form a matrix-like microstructure to convey an amphiphilic model drug to the skin. Levan contributed to the

formation of a matrix-like environment and promoted the stability of two types of colloidal systems.

An interesting approach to overcoming allergic reactions to drugs such as penicillin was covered by a 1979 Wellcome Foundation patent application describing the conjugation of allergenic drugs with levan to avoid an allergic reaction. A number of experiments involving laboratory animals suggested some success with the procedure.

One other trial identified a role uniquely filled by fructans. Some cancers overexpress the fructose transporter GLUTS making a fructose polymer a nearly specific homing device. Tabernero et al. (2017) developed a drug delivery system for the antitumor drug 5-fluorouracil bound to the surface of levan taking advantage of the fact that the fructose residues of levan might draw the drug-levan complex directly to the cancer site.

5 Conclusions

As its remarkable properties are still being discovered, levan is gaining more and more popularity in the medical and pharmaceutical arenas. As is true for most polysaccharides, levan is not only benign but in some cases actively beneficial. But it is the combination of extremely low intrinsic viscosity, range of specific viscosities and high adhesive strength that distinguish levan from other polysaccharides. The main bottleneck for levan-related applications is its relatively high production costs. The substrate for levan biosynthesis is sucrose; thus, there are few alternative feedstock sources other than molasses which adds impurities particularly problematic for health-related uses. New microbial strains and novel enzymatic production methods are being researched to obtain higher levan yields. As more cost-effective strategies are developed, it is certain that the full potential of levan will come to light. Another issue is the lack of knowledge about details of the levan structure, namely, the branches. The length of branches and the possible presence of subbranching of the biopolymer are simply not known, leaving a large gap in our understanding of how levan interacts with other biological systems.

References

Abdel-Fattah AM, Gamal-Eldeen AM, Helmy WA, Esawy MA. Antitumor and antioxidant activities of levan and its derivative from the isolate *Bacillus subtilis* NRC1aza. Carbohydr Polym. 2012;89:314–22. https://doi.org/10.1016/j.carbpol.2012.02.041.

Al-Halbosiy MMF, Thabit ZA, Al-Qaysi SAAS, Moussa TAA. Biological activity of levan produced from rhizospheric soil bacterium *Brachybacterium phenoliresistens* KX139300. Baghdad Sci J. 2018;15(3):238–43. https://doi.org/10.21123/bsj.2018.15.3.0238.

Avsar G, Agirbasli D, Agirbasli MA, Gunduz O, Öner ET. Levan based fibrous scaffolds electrospun via co-axial and single-needle techniques for tissue engineering applications. Carbohydr Polym. 2018;193:316–25.

Axente E, Sima F, Sima LE, Erginer M, Eroglu MS, Serban N, Ristoscu C, Petrescu SM, Toksoy Öner E, Mihailescu IN. Combinatorial MAPLE gradient thin film assemblies signaling to human osteoblasts. Biofabrication. 2014;6:1–9. https://doi.org/10.1088/1758-5082/6/3/035010.

Bahroudi S, Shabanpour B, Combie J, Shabani A, Salimi M. Levan exerts health benefit effect through alteration in bifidobacteria population. Iranian Biomed J. 2020;24(1):54–9. https://doi.org/10.29252/ibj.24.1.54.

Benigar E, Dogsa I, Stopar D, Jamnik A, Cigić IK, Tomšič M. Structure and dynamics of a polysaccharide matrix: aqueous solutions of bacterial levan. Langmuir. 2014;30:4172–82. https://doi.org/10.1021/la500830j.

Bersaneti GT, Pan NC, Baldo C, Celligoi MAPC. Co-production of fructooligosaccharides and levan by levansucrase from *Bacillus subtilis* natto with potential application in the food industry. Appl Biochem Biotechnol. 2018;184(3):838–51.

Chen X, Gao H, Ploehn HJ. Montmorillonite-levan nanocomposites with improved thermal and mechanical properties. Carbohydr Polym. 2014;101:565–73. https://doi.org/10.1016/j.carbpol.2013.09.073.

Chiang CJ, Wang JY, Chen PT, Chao YP. Enhanced levan production using chitin-binding domain fused levansucrase immobilized on chitin beads. Appl Microbiol Biotechnol. 2009;82(3):445.

Costa RR, Neto AI, Calgeris I, Correia CR, Pinho AC, Fonseca J, . . ., Mano JF. Adhesive nanostructured multilayer films using a bacterial exopolysaccharide for biomedical applications. J Mater Chem B. 2013;1(18):2367–74. https://doi.org/10.1039/c3tb20137f.

Dahech I, Belghith KS, Hamden K, Feki A, Belghith H, Mejdoub H. Antidiabetic activity of levan polysaccharide in alloxan-induced diabetic rats. Int J Biol Macromol. 2011;49:742–6. https://doi.org/10.1016/j.ijbiomac.2011.07.007.

Dahech I, Harrabi B, Hamden K, Feki A, Mejdoub H, Belghith H, Belghith KS. Antioxidant effect of nondigestible levan and its impact on cardiovascular disease and atherosclerosis. Int J Biol Macromol. 2013;58:281–6. https://doi.org/10.1016/j.ijbiomac.2013.04.058.

Djurić A, Gojgić-Cvijović G, Jakovljević, D, Kekez B, Kojić JS, Mattinen M-L, Harju IE, Vrvić MM, Beškoski VP. *Brachybacterium* sp. CH-KOV3 isolated from an oil-polluted environment – a new producer of levan. Int J Biol Macromol. 2017; 104:311–21. https://doi.org/10.1016/j.ijbiomac.2017.06.034.

Duymaz BT, Erdiler FB, Alan T, Aydogdu MO, Inan AT, Ekren N, . . ., Selvi SS. 3D bio-printing of levan/polycaprolactone/gelatin blends for bone tissue engineering: characterization of the cellular behavior. Eur Polym J. 2019;119: 426–37.

Erginer M, Akcay A, Coskunkan B, Morova T, Rende D, Bucak S, . . ., Öner ET. Sulfated levan from *Halomonas smyrnensis* as a bioactive, heparin-mimetic glycan for cardiac tissue engineering applications. Carbohydr Polym. 2016;149:289–96.

Esawy MA, Abdel-Fattah AM, Ali MM, Helmy WA, Salama BM, Taie HAA, Hashem AM, Awad GEA. Levansucrase optimization using solid state fermentation and levan biological activities studies. Carbohydr Polym. 2013;96:332–41. https://doi.org/10.1016/j.carbpol.2013.03.089.

Euzenat O, Guibert A, Comber D. Production of fructo-oligosaccharides by levansucrase from *Bacillus subtilis* C4. Process Biochem. 1997;32(3):237–43. https://doi.org/10.1016/S0032-9592(96)00058-1.

Gao S, Qi X, Hart DJ, Gao H, An Y. Expression and characterization of levansucrase from *Clostridium acetobutylicum*. J Agric Food Chem. 2017;65(4):867–71.

Gojgić-Cvijović GD, Jakovljević DM, Lončarević BD, Todorović NM, Pergal MV, Ćirić J, Loos K, Beškoski VP, Vrvić MM. Production of levan by *Bacillus licheniformis* NS032 in sugar beet molasses-based medium. Int J Biol Macromol. 2019;121:142–51. https://doi.org/10.1016/j.ijbiomac2018.10.019.

Gomes TD, Caridade SG, Sousa MP, Azevedo S, Kandur MY, Öner ET, Alves NM, Mano JF. Adhesive free-standing multilayer films containing sulfated levan for biomedical applications. Acta Biomater. 2018;69:183–95. https://doi.org/10.1016/j.actbio.2018.01.027.

Haciosmanoğlu GG, Doğruel T, Genç S, Öner ET, Can ZS. Adsorptive removal of bisphenol A from aqueous solutions using phosphonated levan. J Hazard Mater. 2019;374:43–9.

Hernández L, Arrieta J, Menéndez C, Vazquez R, Coego A, Suárez V, Selman G, Petit-Glatron MF, Chambert R. Isolation and enzymic properties of levansucrase secreted by *Acetobacter*

diazotrophicus SRT4, a bacterium associated with sugar cane. Biochem J. 1995;309:113–8. https://doi.org/10.1042/bj3090113.

Huang MY, Chang CI, Chang CC, Tseng LW, Pan CL. Effects of dietary levan on growth performance, nonspecific immunity, pathogen resistance and body composition of orange-spotted grouper (*E pinephelus coioides H*.). Aquac Res. 2015;46(11):2752–67.

Jakob F, Pfaff A, Novoa-Carballal R, Rübsam H, Becker T, Vogel RF. Structural analysis of fructans produced by acetic acid bacteria reveals a relation to hydrocolloid function. Carbohydr Polym. 2013;92:1234–42. https://doi.org/10.1016/j.carbpol.2012.10.054.

Joaquim EO, Hayashi AH, Torres L, Figueiredo-Ribeiro RC, Shiomi N, de Sousa FS, et al. Chemical structure and localization of levan, the predominant fructan type in underground systems of *Gomphrena marginata* (*Amaranthaceae*). Front Plant Sci. 2018;9:1745.

Kazak H, Barbosa AM, Baregzay B, Alves da Cunha MA, Toksoy Öner E, Dekker RFH, Khaper N. Biological activities of bacterial levan and three fungal β-glucans botryosphaeran and lasiodiplodan under high glucose condition in the pancreatic β-cell line INS-1E. 2014.

Kim SJ, Chung BH. Antioxidant activity of levan coated cerium oxide nanoparticles. Carbohydr Polym. 2016;150:400–7.

Kırtel O, Lescrinier E, Van den Ende W, Öner ET. Discovery of fructans in Archaea. Carbohydr Polym. 2019;220:149–56.

Küçükaşik F, Kazak H, Güney D, Finore I, Poli A, Yenigün O, Nicolaus B, Toksoy Öner E. Molasses as fermentation substrate for levan production by *Halomonas* sp. Appl Microbiol Biotechnol. 2011;89:1729–40. https://doi.org/10.1007/s00253-010-3055-8.

Lammens W, Le Roy K, Schroeven L, Van Laere A, Rabijns A, Van den Ende W. Structural insights into glycoside hydrolase family 32 and 68 enzymes: functional implications. J Exp Bot. 2009;60(3):727–40.

Li J, Kim IH. Effects of levan-type fructan supplementation on growth performance, digestibility, blood profile, fecal microbiota, and immune responses after lipopolysaccharide challenge in growing pigs. J Anim Sci. 2013;91:5336–43. https://doi.org/10.2527/jas2013-6665.

Liu Q, Yu S, Zhang T, Jiang B, Mu W. Efficient biosynthesis of levan from sucrose by a novel levansucrase from *Brenneria goodwinii*. Carbohydr Polym. 2017;157:1732–40.

Maciel JC, Andrad PL, Neri DFM, Carvalho Jr LB, Cardoso CA, Calazans GMT, . . ., Silva MPC. Preparation and characterization of magnetic levan particles as matrix for trypsin immobilization. J Magn Magn Mater. 2012;324(7):1312–6.

Ni D, Xu W, Bai Y, Zhang W, Zhang T, Mu W. Biosynthesis of levan from sucrose using a thermostable levansucrase from *Lactobacillus reuteri* LTH5448. Int J Biol Macromol. 2018;113:29–37.

No JR, Park SY, Kim MK, Jo HY, Lee IY, Ly SY. The effects of levan on blood lipids and the absorption of calcium in rats fed a low calcium diet. J Korean Soc Food Sci Nutr. 2007;36(1):51–7.

Öner ET, Hernández L, Combie J. Review of levan polysaccharide: from a century of past experiences to future prospects. Biotechnol Adv. 2016;34:827–44.

Osman A, Toksoy Öner E, Eroglu MS. Novel levan and pNIPA temperature sensitive hydrogels for 5-ASAcontrolled release. Carbohydr Polym. 2017;165:61–70. https://doi.org/10.1016/j.carbpol.2017.01.097.

Pantelić I, Lukić M, Gojgić-Cvijović G, Jakovljević D, Nikolić I, Lunter DJ, Daniels R, Savić S. *Bacillus licheniformis* levan as a functional biopolymer in topical drug dosage forms: from basic colloidal considerations to actual pharmaceutical application. Eur J Pharm Sci. 2020; 142: 105109. https://doi.org/10.1016/j.ejps.2019.105109.

Poli A, Kazak H, Gürleyendağ B, Tommonaro G, Pieretti G, Toksoy Öner E, Nicolaus B. High level synthesis of levan by a novel *Halomonas* species growing on defined media. Carbohydr Polym. 2009;78:651–7. https://doi.org/10.1016/j.carbpol.2009.05.031.

Ragab TIM, Shalaby ASG, Awdan SAE, El-Bassyouni GT, Salama BM, Helmy WA, Esawy MA. Role of levan extracted from bacterial honey isolates in curing peptic ulcer: *in vivo*. Int J Biol Macromol. 2019;142:564–73. https://doi.org/10.1016/j.ijbiomac.2019.09.131.

Rairakhwada D, Pal AK, Bhathena ZP, Sahu NP, Jha A, Mukherjee SC. Dietary microbial levan enhances cellular non-specific immunity and survival of common carp (*Cyprinus carpio*) juveniles. Fish Shellfish Immunol. 2007;22(5):477–86.

Salman JAS, Ajah HA, Khudair AY. Analysis and characterization of purified levan from *Leuconostoc mesenteroides* ssp. *cremoris* and its effects on *Candida albicans* virulence factors. Jordan J Biol Sci. 2019;12(2):243–9.

Sarilmiser HK, Öner ET. Investigation of anti-cancer activity of linear and aldehyde-activated levan from *Halomonas smyrnensis* AAD6T. Biochem Eng J. 2014;92:28–34.

Sarilmiser HK, Ates O, Ozdemir G, Arga KY, Toksoy Öner E. Effective stimulating factors for microbial levan production by *Halomonas smyrnensis* AAD6^{T}. J Biosci Bioeng. 2015;119(4): 455–63. https://doi.org/10.1016/j.jbiosc.2014.09.019.

Sima F, Mutlu EC, Eroglu MS, Sima LE, Serban N, Ristoscu C, . . ., Mihailescu IN. Levan nanostructured thin films by MAPLE assembling. Biomacromolecules. 2011;12(6):2251–6.

Srikanth R, Siddartha G, Reddy CHS, Harish BS, Ramaiah MJ, Uppuluri KB. Antioxidant and anti-inflammatory levan produced from *Acetobacter xylinum* NCIM2526 and its statistical optimization. Carbohydr Polym. 2015;123:8–16.

Sturzoiu C, Petrescu M, Galateanu B, Anton M, Nica C, Simionca G, Dinischiotu A, Stoian G. *Zymomonas mobilis* levan is involved in metalloproteinases activation in healing of wounded and burned tissues. Sci Pap: Anim Sci Biotechnol. 2011; 44:453–8.

Tabernero A, González-Garcinuño Á, Sánchez-Álvarez JM, Galán MA, Martín del Valle EM. Development of a nanoparticle system based on a fructose polymer: stability and drug release studies. Carbohydr Polym. 2017;160:26–33. https://doi.org/10.1016/j.carbpol.2016.12.025.

Tabernero A, Baldino L, González-Garcinuño Á, Cardea S, Martín del Valle EM, Reverchon E. Supercritical CO_2 assisted formation of composite membranes containing an amphiphilic fructose-based polymer. J CO2 Util. 2019;34:274–81. https://doi.org/10.1016/j.jcou.2019.06.014.

Taran M, Lotfi M, Safaei M. Optimal conditions for levan biopolymer production and its use in the synthesis of bactericidal levan-ZnO nanocomposite. Biotechnologia. 2019;100(4):397–405. https://doi.org/10.5114/bta.2019.90240.

Vina I, Karsakevich A, Bekers M. Stabilization of anti-leukemic enzyme L-asparaginase by immobilization on polysaccharide levan. J Mol Catal B Enzym. 2001;11(4–6):551–8.

Xu Q, Yajima T, Li W, Saito K, Ohshima Y, Yoshikai Y. Levan (β-2, 6-fructan), a major fraction of fermented soybean mucilage, displays immunostimulating properties via Toll-like receptor 4 signalling: induction of interleukin-12 production and suppression of T-helper type 2 response and immunoglobulin E production. Clin Exp Allergy. 2006;36(1):94–101.

Yamamoto S, Iizuka M, Tanaka T, Yamamoto T. The mode of synthesis of levan by *Bacillus subtilis* levansucrase. Agric Biol Chem. 1985;49(2):343–9. https://doi.org/10.1080/00021369.1985.10866727.

Yamamoto Y, Takahashi Y, Kawano M, Iizuka M, Matsumoto T, Saeki S, Yamaguchi H. *In vitro* digestibility and fermentability of levan and its hypocholesterolemic effects in rats. J Nutr Biochem. 1999; 10:13–8. https://doi.org/10.1016/S0955-2863(98)00077-1.

Zhang T, Li R, Qian H, Mu W, Miao M, Jiang B. Biosynthesis of levan by levansucrase from *Bacillus methylotrophicus* SK 21.002. Carbohydr Polym. 2014;101:975–81.

Gums

4

Weslley Felix de Oliveira, Priscilla Barbosa Sales Albuquerque, Priscila Marcelino dos Santos Silva, Luana Cassandra Breitenbach Barroso Coelho, and Maria Tereza dos Santos Correia

Contents

Abstract

Microbial gums are hydrophilic homo- or hetero-polysaccharides of high molecular weight, which can be produced and secreted by yeast and bacteria cells. Production of these biopolymers by microorganisms with stable chemical characteristics and having biomedical properties may be carried out on a large scale. Gums can cause the death of microorganisms, and chemical modifications can be useful to improve the antimicrobial action. Antioxidant effects are reported for different exopolysaccharides (EPSs) by their scavenging ability on different free

Luana Cassandra Breitenbach Barroso Coelho and Maria Tereza dos Santos Correia contributed equally with all other contributors.

W. F. de Oliveira · P. M. dos Santos Silva · L. C. B. B. Coelho (✉) · M. T. dos Santos Correia
Departamento de Bioquímica, Centro de Biociências, Universidade Federal de Pernambuco, Recife, PE, Brazil

P. B. S. Albuquerque
Departamento de Medicina, Universidade de Pernambuco, Garanhuns, PE, Brazil

J. M. Oliveira et al. (eds.), *Polysaccharides of Microbial Origin*,
https://doi.org/10.1007/978-3-030-42215-8_4

radicals. EPSs already displayed effective action against topical wounds acting as micro-/nanocarrier and immobilizing matrix. EPSs produced mainly by fungi and bacteria were also investigated in terms of its antitumor effects via in vitro cell line studies and in vivo protocols using animal models. At least four mechanisms could be associated to anticancer activity by EPSs, enabling some polysaccharides to act through multiple pathways. Innovative materials based on microbial gums have been developed to promote controlled drug delivery in pharmaceutical research because of their functional properties, biodegradability, and biosafety characteristics. Despite their potential, some difficulties in their production and purification processes may result in high cost and lower yields, reducing their commercial applications. Several studies based on different methods have been developed to improve the viability of microbial gums production and their applications. Thus, this chapter aims to report on important biological properties of microbial gums, ways in which such actions can be improved, and the main challenges in working with these polysaccharides.

Keywords

Exopolysaccharide · Antimicrobial · Antioxidant · Antitumor · Healing · Drug delivery

1 Introduction

Polysaccharides are natural polymers extracted from plants, algae, animals, fungi, and via microbiological fermentation, with excellent yield and promising biotechnological applications. Independent of their origin, polysaccharides are characterized as carbohydrate polymers with high molecular weight and composed of more than 20 common substituent units of sugars when one counts different monosaccharides plus their variable pyranose (six-atom) and furanose (five-atom) ring forms (Porter and Martens 2017). Gums are hydrophilic polysaccharides and when treated with hot or cold water form viscous colloidal solutions or dispersions (Valencia et al. 2019). Natural gums have potential uses in biotechnology in addition to the food, environment, and pharmaceutical industries since these biopolymers can be used as thickeners and emulsifiers to control or improve the rheological properties and stability of products (Valencia et al. 2019; Ahmad et al. 2019). These biopolymers, also called hydrocolloids, can be found as storage materials, exudates, cell wall components, and extracellular substances from plants, animals, seaweeds, fungi, and other microbial sources (Izydorczyk et al. 2005; Taheri and Jafari 2019). Microbial polysaccharides can be obtained with high yield at an industrial scale when compared to source algae and plant biopolymers. In addition, strains of genetically modified microorganisms can lead to the production of gums with single characteristic (Habibi and Khosravi-Darani 2017; Ng et al. 2020).

Regarding the group of polysaccharides obtained from microbial origin, they are classified as intracellular, structural, and extracellular polysaccharides (Liu et al. 2017a). Intracellular polysaccharides act as a glycidic source for microbial cells to carry out fermentation when nutrients are depleted (Busuioc et al. 2009). Structural polysaccharides include the lipopolysaccharides (LPS), which are found in the bacterial outer membrane and are associated with virulence factors produced by several species.

Extracellular polysaccharides can still be divided into two subgroups: capsular polysaccharides (CPS) that are tightly bound to the cell envelope, mostly associated with the pathogenicity of bacteria and virulence-promoting factors; and exopolysaccharides (EPSs), which are produced by prokaryotic and eukaryotic species and released into the ambient medium. Mostly, EPSs provide cell adhesion and protection against negative environmental conditions, in addition to serving as carbon and energy reserves (Tallon et al. 2003; Liu et al. 2017a; Yildiz and Karatas 2018). In industrial applications, EPSs are the microbial polysaccharides of greatest value due to the ability to obtain them through simpler extraction processes and with high yield (Paulo et al. 2012). Several microbial gums are EPSs that can be homopolysaccharides or heteropolysaccharides and are constituted of monosaccharides and noncarbohydrate substitutes such as phosphate and succinate (Yildiz and Karatas 2018). Under controlled growth, microorganisms can produce a wide variety of polysaccharides with interest in biomedical research, for example, xanthan secreted by *Xanthomonas campestris*, hyaluronic acid from *Streptococcus* sp., sphingans (welan, diutan, and gellan, among others) from *Sphingomonas* sp., pullulan from *Aureobasidium pullulans,* and alginate from *Pseudomonas* sp. (Kaur et al. 2014).

Gums can have different biomedical properties, for example, their probable antimicrobial action can be a viable alternative to treat drug-resistant microorganisms. In addition, free hydroxyl groups in the structure of these polysaccharides can interact with different molecules; this factor, associated with their bioavailability, biodegradability, and low cost, allows them to suffer chemical changes to improve their antimicrobial action (Braz et al. 2020). Moreover, biopolymers can also have antioxidant effects; this bioactivity is of great importance since the imbalance between pro- and antioxidant molecules can contribute to the emergence of different pathologies. This property can vary among microbial EPSs due to their composition, structure, extraction methods, and chemical modifications (Sivakanthan et al. 2020).

EPSs gained more attention as new sources for cancer treatment since conventional therapies, such as surgery, chemotherapy, and radiotherapy, can cause side effects; in addition, EPSs might be associated to multidrug resistance of the combined medicine therapy. In view of this, researches of the last 20 years have been focused on the use of microbial polysaccharides in order to circumvent the issues occurred in the clinical application of cancer treatments (Abdelnasser et al. 2017).

The development of new treatments and materials (especially the natural sourced ones) that can provide benefits for animal or human patients is a priority for biomedical and pharmaceutical industries beyond the ones focused on cancer disease. Malignant tumors often develop at sites of chronic injury, and tissue injury

has an important role in the pathogenesis of malignant disease, with chronic inflammation being the most important risk factor (Xiang et al. 2020). In this context, the use of EPSs as new wound-dressing systems is preferred due to their appropriate biocompatibility and biodegradability with living tissues, which promotes a faster and more secure healing process (Tabernero and Cardea 2020).

The use of microbial gums to achieve control of drug release is of great interest in pharmaceutical research due to their nontoxicity, biodegradability, and easy obtention from renewable sources. However, these polysaccharides can present functional characteristics, such as viscosity, stability, and swelling, that require physical and chemical modification to improve their properties for biomedical applications (Rana et al. 2015; Shanmugam and Abirami 2019). In this context, several new materials based on microbial gums are being proposed as drug delivery vehicles, such as polymers, hydrogels, and micro-/nanoparticles (Saidin et al. 2018).

Limitations in the production and purification of microbial gums are important factors that contribute to their reduced commercialization. Progresses in microbial gums research highlight approaches to improve the yield and cost of their obtaining, such as the isolation of new strains, the use of low-cost substrates, and optimization of process conditions (Barcelos et al. 2019). In addition, microbial gums have been modified via physical and chemical reactions to improve their functional properties and maximize their applications (Ahmad et al. 2015).

Therefore, this chapter of microbial origin gums focuses mainly on their possible biomedical applications such as antimicrobial, antioxidant, antitumor, and healing agents. In addition, gums' potential use in drug delivery systems and the challenges faced in investigating the bioactivities and use of these polysaccharides are also addressed.

2 Production of Microorganism Gums

Biotechnological production of microbial polysaccharides is more advantageous than from other sources, such as plants, animals, and seaweed. Since these polysaccharides can be obtained from microorganisms in a controlled manner, in large scale, with stable chemical characteristics, and regardless of location and season. There is still a sustainable feature by the possibility of using agricultural and industrial wastes as substrates in the production. However, a high production cost can be considered a limiting factor in this obtaining process (Delattre et al. 2011; Giavasis 2013; Barcelos et al. 2019).

A good recovery of EPS in the production requires the use of a suitable culture medium to allow high yields and not interfere in the detection and quantification methods of the EPS (Leroy and De Vuyst 2016). The most frequent media are skim milk, whey, and whey-based media (Ruas-Madiedo and De Los Reyes-Gavilán 2005), which can still be optimized, for example, by adding diverse carbon and nitrogen sources, ideal pH, and mineral salts, to increase the amount of EPS

produced (Leroy and De Vuyst 2016; Almansoory et al. 2020). For example, the extraction yield of the EPS from *Lactobacillus sakei* increased 2.16 times over the original yield in the fermentation process under optimized medium composed with 127.80 g/L of sucrose, initial pH of 6.87, and an inoculation volume of 3.15% (Wang et al. 2019). The yield of dextran from *Leuconostoc mesenteroides* was 31.24% higher than the obtention in the nonoptimized study in a medium containing sucrose (117.48 g/L), sodium acetate (4.10 g/L), and initial pH of 6.88 (Du et al. 2017).

The two types of fermentation frequently used in EPS extraction are solid-state fermentation (SSF) and submerged fermentation (SmF). In technical SSF, microorganisms grow under solid substrates, for example, paper pulp, white, agricultural wastes, bagasse, and chicken feather. While the SmF method employs liquid substrates for fermentation useful for the growth of microorganisms that require high moisture content (Osemwegie et al. 2020), the SSF is a method widely used in the production of EPS by lactic acid bacteria, and the biopolymer produced accumulates around the cells grown in the solid medium, whereas in SmF strategy, microorganisms use nutrients of the liquid medium to synthesize and secret the EPS in the culture broth (Chaisuwan et al. 2020). Thereafter, some conditions in the fermentation process can also affect the production and structural characteristics of gums, such as temperature, agitation speed, dissolved oxygen, and oxygen transfer capacity (Prajapati et al. 2013; Kaur et al. 2014).

The EPS recovery after fermentation is performed by different protocols based on precipitation and conditioning poster; the choice of the best method depends, among other factors, on the culture medium composition or food matrix and microbial strain (Leroy and De Vuyst 2016). EPS isolation process usually starts by heating the culture broth, for example, to >90 °C, to kill microbial cells, reduce the broth viscosity, facilitate mixing during precipitation, and inactivate the enzymes that can modify the polymer in the following steps (Prajapati et al. 2013; Liu et al. 2017a). After removal of the cells by filtration or centrifugation, precipitation of the polymer in cell-free filtrate or supernatant occurs often by adding alcohols (ethanol, isopropanol, and methanol) and acetone (Palaniraj and Jayaraman 2011; Giavasis 2013).

Extraction solution containing the precipitated polysaccharide still may have impurities, so additional steps include removal of proteins (deproteinization) and pigments (decoloration). Sewage and trichloroacetic acid methods cause protein denaturation, while hydrogen peroxide, activated carbon, dialysis, and macroporous resin techniques are useful for the removal of colored substances (Liu et al. 2017a, 2020). After this cleaning of impurities, the process of purification of the EPS fraction commonly encompasses membrane separation methods, such as microfiltration and ultrafiltration, which promote the separation of the polymer based on molecular weights through synthetic membranes with different pore sizes (Ruas-Madiedo and De Los Reyes-Gavilán 2005; Liu et al. 2020). Final purification stage involves the use of chromatographic columns, such as ion exchange and gel filtration chromatography, promoting separation of the polymers based on charge and molecular weight, respectively (Liu et al. 2017a).

3 Antimicrobial Properties

Antimicrobial agents present in antibiotics, food preservatives, and disinfectants can cause inhibition of growth or death of microorganisms. However, microbial resistance is a reality, and these compounds can no longer cause metabolic and/or physiological alterations in the pathogenic microorganisms (Abushaheen et al. 2020). Moreover, the US Centers for Disease Control and Prevention (CDC) have estimated that antibiotic-resistant bacteria are responsible for causing about more than two million illnesses and over 23,000 deaths per year (Cao et al. 2020a). Thus, natural products may have effects against pathogenic microorganism strains and even action on those resistant to synthetic antimicrobials. Various microorganisms can produce chemicals with antimicrobial activity, such as protein or peptide compounds called bacteriocins and reuterin produced from glycerol by some strains of *Lactobacillus reuteri* (Gyawali and Ibrahim 2014). In addition, polysaccharides from different natural sources can have antipathogenic action (Albuquerque et al. 2020), such as the gums. For example, an EPS characterized as branched heteropolysaccharide constituted of galactoglucan and levan, produced by *Lactococcus lactis*, exerted a wide inhibitory activity against different species of bacteria and the fungus *C. albicans* (Nehal et al. 2019).

Levan is a neutral homopolysaccharide constituted of d-fructo-furanosyl residues joined by β-2,6 linkages in core with lateral branches by β-2,1 linkages, and for its biosynthesis, the enzyme levansucrase is necessary, which is also called sucrose 6-fructosyltransferase (Srikanth et al. 2015a). Antimicrobial activity of levans produced by different microorganisms has been reported. Three levan compounds showed action against various foodborne pathogenic bacteria; these β-2,6-fructan compounds were the following: high-molecular-weight levan with molecular weight (MW) of 3×10^6 Da, and low-molecular-weight levan (MW $= 5 \times 10^4$ Da) synthesized by the levansucrase from *Zymomonas mobilis* and difructose dianhydride IV (DFA IV) produced by a levan fructotransferase from *Arthrobacter ureafaciens* (Byun et al. 2014). An exopolysaccharide produced by *Bacillus tequilensis*-GM was identified as levan, and this polysaccharide was able to inhibit biofilm formation and disrupt preformed biofilms from *E. coli*, *S. au*reus, and *Enterococcus faecalis* (Abid et al. 2019). Antiviral action has been reported for levan produced by different *Bacillus* sp. strains, which showed antiviral effect against respiratory virus (HPAI and H5N1) and enteric virus (adenovirus type 40) (Esawy et al. 2011).

Dextran is an exopolysaccharide α-D-glucan, mainly composed of α-1,6-glycosidic linkage and in lower proportion of α-1,2; α-1,3; and α-1,4 branched linkages, obtained by different lactic acid-producing bacteria (Sajna et al. 2015) that can act against microorganisms. A dextran extracted from *Leuconostoc pseudomesenteroides*, for example, had an inhibitory activity against *Escherichia coli* and *Staphylococcus aureus* (Ye et al. 2019). However, some dextrans may not have any antimicrobial action as soon as they are isolated and may need to undergo chemical changes in their structure in order to exhibit this action (McCarthy et al. 2019). For example, amphiphilic dextran esters were obtained by the reaction between this polysaccharide and different substituted 1,2,3-triazoles-4-carboxylates. Antimicrobial analyzes by disc-diffusion assay revealed that the presence of chemical groups $-CH_3$ and $-OCH_3$ as

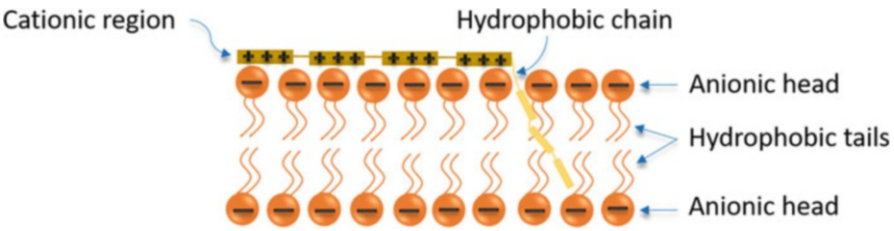


Fig. 1 Schematic representation of the mechanism of action from a cationic amphiphilic polysaccharide on the microorganism membrane surface

substituent groups on para position of aromatic ring had action against some microorganisms, such as *S. aureus* and *C. albicans* (Stanciu et al. 2020). Moreover, an approach that has been employed to make gums with antimicrobial activity is to make them cationic and amphiphilic. As shown in Fig. 1, death induced by these polysaccharides occurs since the positively charged layer can interact by electrostatic adsorption with the negative surface of the cell membrane followed by the weakening of the membrane by the interaction of a hydrophobic chain in the nonpolar tails of the membrane (Pan et al. 2019). Cationic amphiphilic polymers were obtained from dextran backbone containing hydrophobic alkyl chain and quaternary ammonium groups. These polymers had antimicrobial action against different species of bacteria and fungi (Tuchilus et al. 2017). A similar approach was carried out with the neutral polysaccharide curdlan, a linear β-1,3-glucan produced by *Agrobacterium biovar*, which upon receiving quaternary ammonium groups generated an amphiphilic polyelectrolyte with antibacterial action (against *E. coli* and *P. aeruginosa*) and antifungal (in assays with *C. albicans*) (Popescu et al. 2019).

Xanthan gum is an anionic polysaccharide secreted by *Xanthomonas campestris* composed of a β-1,4-D-glucose backbone and side chains of D-mannose and D-glucuronic acid (Elella et al. 2020). The action against pathogenic microorganisms of xanthan has been attributed when this polysaccharide was grafted with poly (*N*-vinyl imidazole). The imidazole ring has cationic nature and confers antibacterial properties since it is able to interact electrostatically and hydrophobically with the cytoplasmic membrane; the penetration of this ring in the bacterial cell can cause its binding and destabilization of the bacterial DNA (Elella et al. 2017). Although this carbohydrate has a negative charge, xanthan-oligosaccharide exerted antibacterial action against *S. aureus* probably brought by its low molecular weight and hydroxyl groups in its structure; this oligosaccharide was also able to affect the cell membrane permeability and inhibit the biofilm formation of this microorganism (Wang et al. 2020).

4 Antioxidant Action

Free radicals, such as reactive oxygen species (ROS) and reactive nitrogen species (RNS), are compounds that have one or more unpaired electrons and can participate in several pathological processes (Oliveira et al. 2018). However, natural

compounds, such as polysaccharides from bacteria and fungi, can combat these oxidizing agents (Wang et al. 2013).

A levan produced by *Acetobacter xylinum* had its antioxidant activity revealed by the ability to reduce the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Srikanth et al. 2015b); this happens when the unpaired electron of nitrogen in DPPH receives a hydrogen atom from antioxidant compound (Kedare and Singh 2011). Furthermore, the antioxidant mechanism of gums can also be characterized through scavenging assays of other free radicals besides DPPH. Another levan obtained from *Bacillus megaterium* was able to scavenge the DPPH, superoxide anion, hydroxyl, and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radicals in a dose-dependent manner (Pei et al. 2020).

Ability of gums, such as dextran, to reduce free radicals can be affected by their monosaccharide composition, concentration, content of carboxy and hydroxy groups, and hydrogen or electron donation capacity. The ion chelation assay can also be used to evaluate the antioxidant action of these polysaccharides since the presence of -O- and -OH- groups in dextran may be able to chelate Fe^{2+} (Du et al. 2018). It is important to evaluate the antioxidant activity in different assays since the singular experimental chemical conditions may indicate that the gum may have action in microenvironments of different chemical composition inside the cell. For example, beside testing the ability to reduce free radicals, the capacity to donate electrons from dextran was also determined by total antioxidant capacity assay, which assesses the property of reduction of the Mo^{+6} to Mo^{+5} ions and the reducing power test that estimates the potential to reduce potassium ferricyanide (Soeiro et al. 2016).

Xanthan gum may also be able to fight oxidizing agents due to its pyruvate, acetyl, and glucuronic acid groups; the addition of the furfural radical in this polysaccharide increased the hydroxyl radical scavenging (Kang et al. 2019). A paradoxical factor can be attributed to the molecular weight of bioactive polysaccharides, since these macromolecules with high molecular weight can find barriers to penetrate the cell and exert their pharmacological action; nevertheless, their very low molecular weight can suppress its bioactivity (Li et al. 2016). Therefore, the antioxidant property of gums should also be evaluated when there is a reduction in their structure. Thus, a low-molecular-weight xanthan obtained by the biodegradation of this gum by the fungus *Chaetomium globosum* was able to exert scavenging effects on different free radicals due to the increase of carboxyl and hydroxyl groups that occurred by decrease in molecular weight (Hu et al. 2019). In other approaches, xanthan-derived oligosaccharides obtained by hydrolysis of xanthan in alkaline conditions also exhibited antioxidant activity (Xiong et al. 2013; Wu et al. 2013).

Gellan gum is an anionic polysaccharide composed of repeating units of glucose, glucuronic acid, and rhamnose purified from *Sphingomonas elodea*. There are reports of low antioxidant activity for native gellan, which possibly stems from strong intermolecular and intramolecular hydrogen bond in their structure. However, carboxylated derivatives of gellan with different uronic acid content did improve the activity of scavenging radicals (Redouan et al. 2011). Another way to improve the antioxidant performance of gellan gum is by forming

composite films based on lignin, a biopolymer consisting of aromatic groups, beside hydroxyl and methoxy functional groups that can donate hydrogens to free radicals (Rukmanikrishnan et al. 2020).

Nonconventional EPS has been purified from bacteria and fungi, and its antioxidant capacity has been evaluated through different assays, mainly by the reduction of free radicals, as shown in Table 1. It is also noted in the Table that even due to the diversity in the monomeric composition of these polysaccharides, concentrations below 10 mg/mL can have the maximum efficiency of combating oxidizing agents. EPS with different saccharide compositions can be obtained from the same source, as can be seen from *Weissella cibaria*; however, they can have antioxidant effects in similar concentrations. Furthermore, it is observed that the genera *Leuconostoc* and *Lactobacillus* can be important suppliers of antioxidant EPS.

5 Anticancer Activity

An abnormal mass of tissues originated by abnormal growth or division of cells is called a neoplasm or tumor. The tumor can be classified as benign or malignant. When the tumor does not extensively invade the healthy surrounding tissues or spread to other parts of the body, it is classified as benign, whereas a malignant tumor is an uncontrolled growth of cells, and it can become progressively invasive. In this case, the term cancer refers specially to this malignant tumor (Hanahan and Weinberg 2011). Cancer could be considered the most aggressive disease worldwide despite the advancing technology and intensive researches about therapy and cure; cancer still ranks among the first cause of death in the world (Yildiz and Karatas 2018).

Conventional cancer therapies, such as surgery, chemotherapy, and radiotherapy, present important limitations, such as poor prognosis and negative side effects (Bao et al. 2013). In view of this, researches of the last 20 years focused on the use of natural products in order to circumvent the toxic effects of anticancer agents. The question of identifying natural polymers with potential anticancer activity has contributed to carbohydrates from various sources being investigated. Some mechanisms for the antitumor effects of polysaccharides have been identified via in vitro cell line studies and in vivo protocols using animal models: NO pathway, cell cycle arrest (related to G0, G1, G2, and S cell cycle phases), depolarization of the mitochondrial membrane, and immunomodulation. Some polysaccharides act through multiple pathways, while others do not have the mechanism of action well defined (Albuquerque et al. 2020).

In order to understand the role of polysaccharides as antitumor agents, it is important to explain the hallmarks of cancer; they comprise six biological capabilities acquired during the multistep development of human tumors, which occur under an organizing principle for rationalizing the complexities of neoplastic disease. The hallmarks include sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, and activating invasion and metastasis (Hanahan and Weinberg 2011).

Table 1 Exopolysaccharides with antioxidant activity

EPS source	Monosaccharide composition	Mechanism of antioxidant action	Concentration with higher activity (mg/mL)	References
Weissella cibaria	Glucose and rhamnose	Capacity to reduce Fe^{3+}/ferricyanide complex and by scavenging effect on superoxide anion, DPPH, and hydroxyl radicals	4	Adesulu-Dahunsi et al. (2018)
	Mannose, glucose, galactose, arabinose, xylose, and rhamnose	Reducing power and scavenging ability on hydroxyl, DPPH, and superoxide anion radicals	5	Zhu et al. (2018a)
Weissella confusa	Mannose	Capacity to reduce Fe^{3+}/ferricyanide complex and by scavenging effect on superoxide anion, DPPH, and hydroxyl radicals	1.25	Lakra et al. (2020)
Leuconostoc pseudomesenteroides	Glucose	Scavenging ability on hydroxyl, DPPH, and ABTS radicals	5	Farinazzo et al. (2020)
Leuconostoc mesenteroides	Fructose	Scavenging ability on hydroxyl radical	6	Taylan et al. (2019)
Lactobacillus plantarum	Mannose, glucose, and galactose	Ferric reducing power, scavenging capacity on DPPH and ABTS radicals	8	Bomfim et al. (2020)
Lactobacillus acidophilus	Glucose, galactose, and maltose	Scavenging ability on DPPH radical	0.45	Abedfar et al. (2020)
Lactobacillus helveticus	Galactose, glucose, and mannose	Metal ion-chelating activity and scavenging ability on DPPH, hydroxyl, and superoxide anion radicals	4	Xiao et al. (2020)
Microbacterium aurantiacum	Glucuronic acid, glucose,	Reducing power and scavenging ability on	0.75–3.5	Sran et al. (2019)

(continued)

Table 1 (continued)

EPS source	Monosaccharide composition	Mechanism of antioxidant action	Concentration with higher activity (mg/mL)	References
	mannose, and fucose	hydroxyl, DPPH, and superoxide anion radicals		
Bacillus sp.	Galactose, glucose, and mannose	Ferric reducing power and scavenging ability on hydroxyl, DPPH, and superoxide anion radicals	7	Hu et al. (2019)
Aspergillus sp.	Mannose and galactose	Inhibiting the lipid peroxidation, scavenging ability on DPPH and superoxide anion radicals	10	Chen et al. (2011)
Oidiodendron truncatum	Glucose, mannose, and galactose	Inhibiting the lipid peroxidation, scavenging ability on DPPH and superoxide anion radicals	4	Guo et al. (2013)
Lasiodiplodia sp.	Glucose and mannose	Reducing power, inhibiting the lipid peroxidation, and scavenging ability on DPPH and superoxide anion radicals	0.2	Kumar et al. (2018)
Fusarium equiseti	Mannose, glucose, fucose, rhamnose, xylose, and arabinose	Reducing power and scavenging ability on hydroxyl radical	3	Prathyusha et al. (2018)
Chaetomium sp.	Glucose, mannose, arabinose, and galactose	Scavenging ability on hydroxyl and DPPH radicals	10	Zhang et al. (2017)

DPPH: 2,2-diphenyl-1-picrylhydrazyl. ABTS: 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)

The progression of the aberrant proliferative cell cycle and the resistance of the cell death are hallmarks closely related to the activation of proto-oncogenes and/or inactivation of tumor suppressor genes. Regulators of cell cycle phase transitions are important targets for cancer treatment to reduce clonogenic capacity of tumor cells (Mastbergen et al. 2000). The two tumor suppressor genes encode the proteins RB

(retinoblastoma associated) and TP53; they operate as central control points within two key complementary cellular regulatory circuits that govern the decisions of cells to proliferate or, alternatively, activate programs of apoptosis and senescence (Hanahan and Weinberg 2011). In relation to programmed cell death, the mitochondrial apoptotic pathway is one of the major routes of apoptosis, and mitochondria play a crucial role in processes of apoptosis signal. In this pathway, the key event is associated with the loss of the mitochondrial membrane potential, which allows the release of proapoptotic factors, such as cytochrome c, Smac/DIABLO, Omi/Htra2, endonuclease G, and apoptosis-inducing factor (Yu et al. 2016).

Still above the response of the apoptotic stimuli, regulatory proteins of the Bcl-2 family, which contains pro- and antiapoptotic members, control the trigger that transmits signals between regulators and effectors. The archetype, Bcl-2, and their relatives Bcl-xL, Bcl-w, Mcl-1, and A1 are apoptosis inhibitors, acting most of the time by suppressing two proapoptotic triggering proteins: Bax and Bak. These are proteins of the mitochondrial outer membrane and, when out of the Bcl-2 inhibition action, can disrupt the integrity of the outer mitochondrial membrane, causing the release of proapoptotic signaling proteins. The most important proapoptotic protein is the cytochrome c, whose release is associated to the activation of a cascade of caspases that act via their proteolytic activities to induce the multiple cellular changes associated with the apoptotic program. In addition, Bax and Bak share domains of proteins, termed BH3 motifs, with the antiapoptotic Bcl-2-like proteins, thus mediating various physical interactions (Hanahan and Weinberg 2011).

Up to this point, it was possible to define the hallmarks of cancer as acquired functional capabilities that allow cancer cells to survive, proliferate, and disseminate; these are the functions essentially acquired by different tumor types via distinct mechanisms and at various times during the course of multistep tumorigenesis. Modern knowledge about enabling characteristics and emerging hallmarks depicts other distinct attributes of cancer cells, being two of them particularly compelling. The first involves major reprogramming of cellular energy metabolism in order to support continuous cell growth and proliferation, thus replacing the metabolic program that operates in most normal tissues and fuels the physiological operations of the associated cells. The second one deals with the evasion by cancer cells from attack and elimination by immune cells, a capability that highlights the dichotomous roles of an immune system that both antagonizes and enhances tumor development and progression (Hanahan and Weinberg 2011).

It is possible to predict that the most important bioactivities displayed by natural polysaccharides acting as immunoceuticals in clinical cancer therapies should be associated with their immunostimulant and antitumor effects. Most polysaccharides use specific receptors, for example, toll-like receptors 2 and 4, on the macrophage cell surface, and stimulate the cells via the nuclear factor kappa B (NF-κB) and mitogen-activated protein kinase (MAPK) signaling pathways. NF-κB is a protein complex of inducible transcription factors responsible to regulate the expression of many genes encoding enzymes, chemokines, cytokines, and adhesion molecules involved in innate and adaptive immunity, antiapoptosis, inflammation, and proliferation. The MAPK family is a serine-threonine kinase that plays important roles in

the regulation of cellular activities such as gene expression, cell proliferation, differentiation, development, and apoptosis (Chaisuwan et al. 2020). A comprehensive explanation of the main antitumor mechanisms displayed when microbial bioactive exopolysaccharides stimulate macrophages is shown in Fig. 2. Thereafter, the anticancer activity is demonstrated in various cancer cell lines through different mechanisms.

Sarcoma is a nonepithelial tumor currently treated by chemotherapy, which is often characterized by serious side effects. Antineoplastic agents from natural sources can be suggested as the new generation of products from sustainable materials that can combine both ecological and health aspects. Therefore, exopolysaccharides from secure natural sources, for instance, *Bacillus*, might be a good alternative to synthetic antineoplastic drugs. CPS from *B. velezensis* SN-1, isolated in Da-jiang, China, was made and extensively characterized, in addition to being evaluated by antioxidant and antitumor activities against hepatocellular carcinoma (HepG-2). The antineoplastic analysis showed that the exopolysaccharide displayed significant antitumor activity toward HepG-2 tumor cells, which suggests the CPS generated by *B. velezensis* SN-1 as a natural antitumor drug (Cao et al. 2020b).

Still in view of the genus *Bacillus*, the endophytic bacterium, MD-b1, isolated from the medicinal plant *Ophiopogon japonicas* and identified as the *B. amyloliquefaciens*

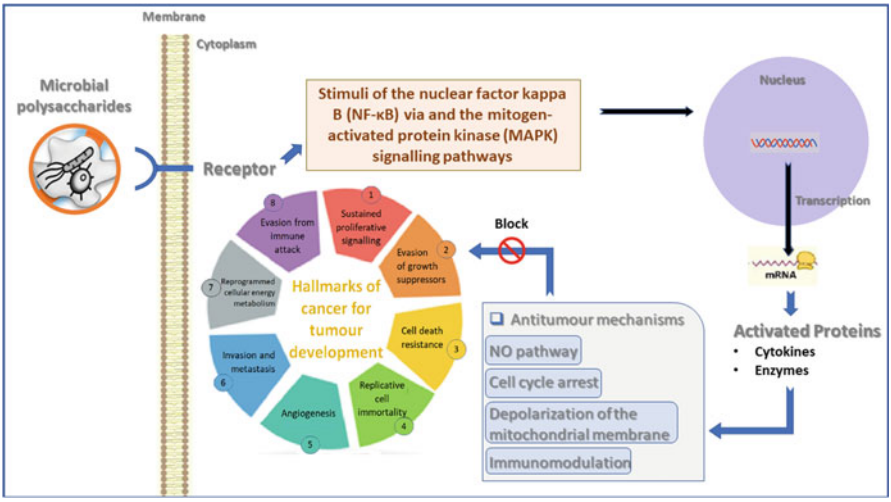


Fig. 2 A comprehensive explanation of the main antitumor mechanisms displayed by microbial exopolysaccharides. Polysaccharides bind to specific receptors on the macrophage cell surface and then stimulate important vias: the nuclear factor kappa B (NF-KB) and the mitogen-activated protein kinase (MAPK) signaling pathway. They are able to block the tumorigenesis through signals that translocate to the nucleus and activate transcription factors that regulate the gene expression of important proteins. Activated proteins, such as cytokines and enzymes, could initiate different mechanisms of action, including the NO pathway, modification of the cell cycle, depolarization of the mitochondrial membrane, and modulation of the immune system. Some polysaccharides act through more than one of the abovementioned mechanisms

sp., produced EPSs with significant therapeutic activities against gastric carcinoma cell lines (MC-4 and SGC-7901). It is important to mention that this was the first study investigating the endophytic microorganism isolated from *O. japonicas* and also the first discovery of antitumor activity for EPSs derived from the genus *Bacillus* (Chen et al. 2013). Other Bacillus strains, in this case the *B. circulans* isolated from the slimy layer of coconut, produced appreciable amounts of an exopolysaccharide composed of glucose, mannose, and galactose that showed bioactivities including antioxidant, anti-inflammatory, and antitumor activities. The strong antioxidant activity exhibited by this EPS forecasts its ability to serve as an antitumor compound against cultures of VERO cells and HepG-2 and Hep-2 cancer cell lines. The results clearly indicate that the EPS from *B. circulans* can serve as a potential candidate for the development of medical formulations (Vidhyalakshmi et al. 2016).

Another study used seven mud samples (named as BS) collected from a gas station in Giza governorates, Egypt; the samples were located at the discharge of water from station that loaded with large quantities of gasoline spilled on the ground. The molecular identification using 16S rRNA gene fragments of the isolate BS4 belonged to the genus *Bacillus* with 99% identity as *Bacillus mycoides* strain ATCC 6426. Then, different concentrations of EPSs produced by *B. mycoides* strain BS4 were evaluated against normal cell baby hamster kidney (BHK) and tumor cells (HepG-2 and colorectal adenocarcinoma cells, Caco-2). The results provided a promising microbial BS4-derived EPS with significant therapeutic antitumor activities against hepatic and colon cancer; however, authors highlighted the importance of further works to study the relationship between the structure and the EPS function (Farag et al. 2020).

Among the wide range of bacterial species able to produce EPSs, lactic acid bacteria (LAB) are a major group of species whose compositions, structure, and properties have been extensively studied. They usually have low cytotoxicity and side effects and may serve as an efficient alternative to the synthetic antitumor agents (Rahbar Saadat et al. 2019). For example, EPSs extracted from *Lactobacillus casei* 01 exerted antiproliferation effect on human colon cancer cell, HT-29 (Liu et al. 2011). The EPS isolated from *L. helveticus* MB2-1 was characterized and demonstrated to be composed of glucose, mannose, galactose, rhamnose, and arabinose. Preliminary in vitro tests revealed that EPS significantly inhibited the proliferation of HepG-2, BGC-823, and especially HT-29 cancer cells (Li et al. 2015). Also, the EPS of *L. plantarum* 70810 showed a significant antitumor activity on HepG-2, BGC-823, and HT-29 cancer cells (Wang et al. 2014). The EPSs from *L. acidophilus* have shown the antitumor properties against colon cancer cell lines, HCT15 and $CaCo_2$ (Deepak et al. 2016).

Colon cancer affects many organs and tissues and could be considered the most common type of cancer. Studies have shown that EPSs produced by probiotic strains can be effective against this disease. Probiotics are living microorganisms, preponderantly lactobacilli and bifidobacterial, or living microbial food ingredients that provide useful effects to consumers when applied in sufficient amounts. Some labs produce EPS, whose health benefits are related to their biological activities; in this case, EPSs might contribute to human health as prebiotics, i.e., nondigestible food

ingredients/components/supplements that confer health benefits to the host upon specific stimulatory modulation of selected populations (Ruas-Madiedo et al. 2002; Yildiz and Karatas 2018).

Leukemia, another important type of cancer and commonly known as cancer of the blood, is a malignant disease of the blood cell-forming tissue. There are important findings suggesting the use of EPSs as potential adjuvant chemotherapeutic and chemopreventive agents, also as effective drugs against human leukemia. For example, the anticancer activity of the EPS from Antarctic bacterium *Pseudoalteromonas* sp. S-5 was tested in human leukemia K562 cells. The EPS significantly inhibited the proliferation of K562 cells by morphological characteristics of apoptosis, such as condensation of chromatin and the formation of apoptotic bodies. EPS induced collapse of mitochondrial membrane potential and activation of caspase-9, suggesting that the intrinsic apoptotic signaling pathway was involved in apoptosis induced by the K562-treated cells. In addition, the ratio of Bax/Bcl-2 increased, and the calcium signal was associated with the cytotoxicity of the EPS against K562 cells (Chen et al. 2015).

An EPS was isolated from the fermentation broth of *Trichoderma pseudokoningii,* and its anticancer activities on human leukemia K562 cells were studied. The results demonstrated that EPS significantly inhibited K562 cell proliferation in a time- and concentration-dependent manner, and this was mainly caused by apoptosis. Meanwhile, the dissipation of mitochondrial membrane potential, increased production of ROS, enhancement of the concentration of intracellular calcium, upregulation of Bax and p53 mRNA, and downregulation of Bcl-2 mRNA were detected, characteristics primarily involved in the mitochondrial pathways (Huang et al. 2012). Other study found that the halophilic bacterium *Halomonas stenophila*, strain B100, produced a heteropolysaccharide that, when oversulfated, exerted antitumor activity on T cell lines deriving from acute lymphoblastic leukemia. B100S was the first bacterial EPS that has been demonstrated to exert a potent and selective proapoptotic effect on T leukemia cells (Ruiz-Ruiz et al. 2011).

In what concerns the group of eukaryotic microorganisms that have been reported to produce polysaccharides, fungi are demonstrated to produce EPSs with interesting bioactivities. For instance, an exopolysaccharide (EPS1-1) composed of glucose, mannose, galactose, and fructose was purified from the fermentation broth of the filamentous fungus *Rhizopus nigricans* and suggested as an antitumor drug against human colorectal carcinoma. EPS1-1 demonstrated significant inhibition of the proliferation of human colorectal carcinoma HCT-116 cells in vitro, in addition to induced S phase cell cycle arrest and increased sub-G0/G1 population, which could be considered a hallmark of apoptosis. It was also demonstrated that the mitochondrial pathway was involved in the EPS1-induced apoptosis since the polymer caused dissipation of mitochondrial membrane potential, accumulation of reactive oxygen species, up-regulation of Bax and p53 mRNA expression, and down-regulation of Bcl-2 mRNA expression (Yu et al. 2016).

The anticancer activity of the polysaccharide extracted from the *Auricularia polytricha* fungus (APPs) was evaluated toward A549 human lung cancer cells, and its underlying mechanisms were investigated. APPs significantly inhibited the

proliferation and DNA synthesis of A549 cells in a concentration-dependent manner. Also, the polysaccharide induced apoptosis in A549 cells by arresting cell cycle progression at the G0/G1 phase and significantly increased the expression of cyclin-dependent kinase (CDK) inhibitors p53 and p21, whereas the expression of cyclin A, cyclin D, and CDK2 was decreased by treatment with the polymer. The apoptotic induction in APPs-treated A549 cells could also be associated with the release of cytochrome c from mitochondria to cytosol, which in turn resulted in the activation of caspases-9 and -3, and the cleavage of poly (ADP-ribose) polymerase. Furthermore, the inhibitory effect of APPs on the growth in BALB/c-nu nude mice bearing A549 cells was also proven (Yu et al. 2014).

Some bioactivities, including the antitumor activity, of the EPS extracted from an endophytic fungus of *Crocus sativus* L. (saffron), a scarce plant used in Chinese traditional herbal medicine, was examined for the first time. EPS exhibited remarkable cytotoxicity against K562, A549, HL-60, and human cervical cancer (HeLa) cells in a dose-dependent manner (Wen et al. 2018). The antitumor activity of the EPS extracted from other endophytic fungi, in this case the JY25, *Chaetomium* sp., isolated from the leaves of the Chinese medicinal plant *Gynostemma pentaphylla*, was evaluated against A549 cells and demonstrated good inhibitory effects (Zhang et al. 2017).

A neutral water-soluble polysaccharide (HPA), mainly composed of mannose, was isolated from the marine fungus *Hansfordia sinuosae,* and its antitumor effect in vitro was tested. HPA showed a remarkable inhibitory effect on human cervical carcinoma HeLa cells and human breast carcinoma MCF-7 cells. Furthermore, for HeLa cells, HPA was able to increase intracellular ROS levels, induce cell apoptosis, decrease mitochondrial membrane potential, and elevate the expression of caspase-3; all these findings are significant for suggesting HPA as a potential antitumor agent (Li et al. 2018). Another neutral polysaccharide (LGPS-1) had its anticancer efficacy tested; it was isolated from *Lentinus giganteus*, an edible mushroom with high protein content. The antitumor activity of LGPS-1 was assessed using HepG-2 cells and showed an inhibition proliferation rate of the carcinoma cells due to induced apoptosis through intrinsic mitochondrial apoptosis and PI3K/Akt signaling pathways (Tian et al. 2016).

The strain *Neopestalotiopsis* sp. SKE15 was isolated and used for the production of an EPS tested against three cell lines: HeLa, human stomach cancer AGS gastric carcinoma, and human breast cancer MDA-MB-231. The EPS treatment (at the dose of 100 μg/mL) inhibited both the HeLa cells and breast cancer cells proliferation in the rate of around 61% and 56%, respectively, indicating the performance of the EPS as promising therapeutic agent since it was safe and active at low concentrations (Fooladi et al. 2019).

The antiproliferative and apoptosis-inducing activities of an acidic polysaccharides fraction from *Pholiota dinghuensis* Bi mycelium (PDP-3) against human breast cancer MCF-7 cells were investigated. Interesting results displayed a significant prevention of cell growth toward MCF-7 cells by the p38/MAPK signal transduction pathway, which was the potential mechanism of PDP-3 in regulating both cell proliferation and apoptosis. The protein expressions of MCF-7 cells were

upregulated p21 and downregulated cyclin D1, CDK4 and PCNA, upregulated Bax and downregulated Bcl-2, and caspase-9 and caspase-3. In MCF-7 cells treated with PDP-3, downregulated TRAF2, and upregulated ASK1, phosphorylated of p38 and p53 were also found (Gan et al. 2015).

A crude polysaccharide extract obtained after nuclear fusion in *Ganoderma lucidum* and *Polyporus umbellatus* mycelia (Khz-cp) was able to inhibit the growth of cancer cells by inducing apoptosis preferentially in transformed cells. The main mechanism was reported to be the increase of intracellular calcium concentration and the activation of P38 to generate ROS via NADPH oxidase. The apoptosis was caspase dependent and occurred via a mitochondrial pathway (Kim et al. 2014).

Two polysaccharides (termed PLPS-1 and PLPS-2) were isolated from mycelia of cultured *Phellinus linteus*, structurally characterized and submitted to in vitro antitumor assays. The monosaccharide composition, the molar ratio, and the carbohydrate components of their side branches were different, thus suggesting that the distinct antitumor activity between the two PLPS evidently occurred due to their structural alterations. In vitro antitumor assays for PLPS-1 displayed strong antiproliferative effect against S-180 sarcoma cells through apoptosis, whereas PLPS-2 had no such effect. Thus, PLPS-1 was considered a potential anticancer agent (Mei et al. 2015).

The action of microbial polysaccharides on malignant cells was mainly demonstrated via immunomodulation and apoptosis. The anticancer activity was reported in various cancer cell lines, such as hepatocellular carcinoma, colorectal adenocarcinoma, human colon cancer cells, human leukemia cells, human lung cancer cells, human cervical cancer cells, and human breast carcinoma, the growth of the cells being directly suppressed by the polysaccharides. In addition, different anticancer efficacies might be involved with the molecular weight, composition, structural conformation, and charge characteristics of the polysaccharides.

6 Healing Capacity

The wound-healing process is a tissue survival mechanism initiated after an injury and roughly involves four integrated stages, including hemostasis, inflammation, proliferation, and remodeling; it is the guarantee that damaged or destroyed tissues are removed and the breach in tissue integrity is restored. The wound repair is performed spontaneously or by a connective tissue repair, both of them being independent of how the injury has been sustained, or whether the injury is minor, major, or confined to soft tissue trauma or is a surgical wound (Beldon 2010). Figure 3 presents the report for the normal acute physiological wound-healing process.

In the course of a normal wound-healing process, immune-inflammatory cells appear transiently and then disappear. The innate immune system is involved in cleaning dead cells and the cellular debris; the adaptive immunity, which is accomplished by inflammatory cells such as macrophages, neutrophils, and myeloid progenitors, is engaged in the wound healing itself. The abovementioned immune cells

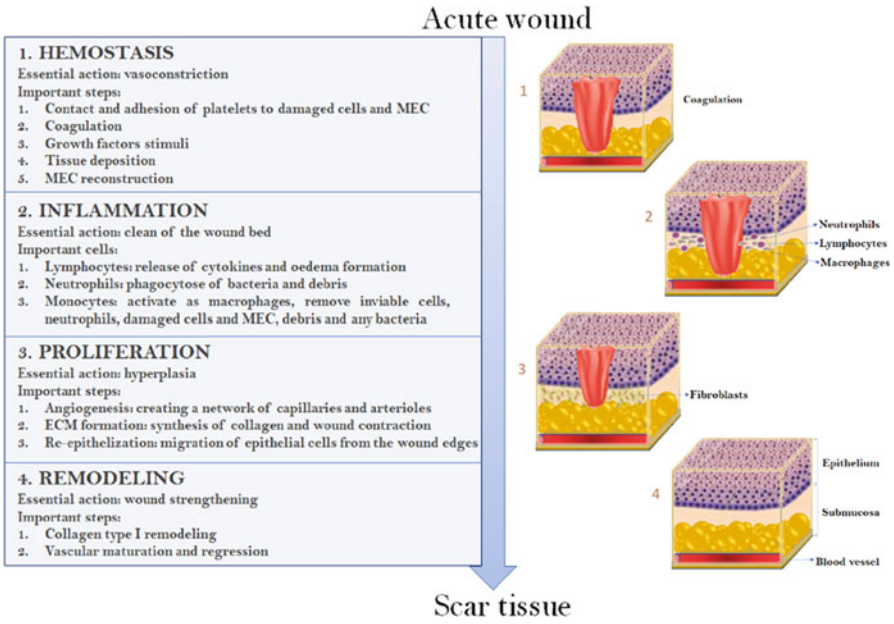


Fig. 3 Normal wound-healing process and particular characteristics of its four orchestrated phases

are one of the major sources of the angiogenic, epithelial, and stromal growth factors and matrix-remodeling enzymes that are needed for wound healing; similarly, these factors are also needed to support neoplastic progression. In addition, lymphocytes B and T may facilitate the recruitment, activation, and persistence of both wound healing and tumor-promoting immune cells (Hanahan and Weinberg 2011).

Given the last information, one could ask what is the main relationship between the wound-healing process and the development of cancer? This motivating question was already proposed by Schäfer and Werner (2008). In their review article, they highlighted some similarities between tumor generation and wound healing, including the abovementioned immune factors. They also pointed crucial differences, such as the altered metabolism, impaired differentiation capacity, and invasive growth of malignant tumors.

In view of the sophisticated repair process, the reactions are synergic and ordered, contributing to a wound repair without interruption, for example, how it occurs in acute wounds. Differently, chronic or inert wounds are those interrupted by an orderly process for some reason, infected with microorganisms, or unable to heal because of a potential disease (Xiang et al. 2020). Defects in wound repair are considered a severe health problem and frequently affect aged patients, diabetics, immunosuppressed, and individuals submitted to chemotherapy or radiotherapy. They often develop painful, nonhealing ulcers. Neoplasia is a particularly dangerous complication of nonhealing ulcers, and the development of cancer in fibrotic tissue is a usual event. This fact was reported by Rudolf Virchow in 1863, who postulated

that chronic irritation and previous injuries are a precondition for tumorigenesis (Schäfer and Werner 2008).

Some important factors allow wounds to become one of the leading causes of death worldwide, for example, the high cost for wound care and the strong correlation between chronic wounds and increasing age. In view of this, there is a growing pressure for the development of advanced wound care that reaches the soaring demands; then, wound dressings are developed with the aim to protect the wound bed and promote skin regeneration to accelerate wound healing (Xiang et al. 2020).

When choosing a wound dressing, many factors must be considered, including the stage of the current wound, the frequency of dressing replacement, the cost of the treatment, and associated drugs (such as antibiotics and painkillers). Characteristics of nontoxicity, biocompatibility, viscosity, and adequate mechanical properties must be considered when choosing an ideal wound dressing; it is essential that it could absorb the exuded tissue fluid, allow the exchange gas in time, prevent bacterial infection, and maintain a humid environment around the wound interface to allow the cells to adhere and proliferate properly, thereby promoting the wound healing (Xiang et al. 2020).

The majority of the currently available wound dressings are either passive or interactive materials such as hydrogels, hydrocolloids, and films that provide a controlled environment for the site of healing. However, there are some of them whose ability to initiate the cellular responses is deficient, and hence they require the necessity of combining with other molecules that can stimulate and trigger target cell responses crucial to the wound-healing process (Mayet et al. 2014). Biopolymers able to interact with innate cells and promote wound repair have an advantage over the existing synthetic dressings, being an advantageous alternative for the current wound care sector. Polysaccharides are the major class of biomolecules with ideal physical, chemical, mechanical, and biological properties for wound dressings. Besides the mentioned properties of polysaccharides, they can be molded in different scaffolds that are crucial in developing functional biomaterials (Sahana and Rekha 2020). In particular, polysaccharides from microbial sources have emerged as economical and sustainable biomaterials for this field due to their fast and high-yielding production, and the excellent biocompatibility with human tissues (Ng et al. 2020).

It is important to mention that the wound repair might be analyzed besides the antioxidant activity of the wound dressing. It is well known that, when the tissue is injured, short-term process of inflammation occurs because of the release of inflammatory mediators and ROS by the macrophages; thus, some steps of the wound healing process are impaired or delayed, retarding the repair. In view of this, the inhibition of ROS production is an important point to guarantee the proliferative phase of repair by fibroblasts (Trabelsi et al. 2017).

The majority of works reporting microbial polysaccharides as wound-healing agents were developed using hydrogels scaffolds. For a better understanding, it is important to explain that hydrogels are artificial biomaterial scaffolds able to offer a much-favored 3D microenvironment for regenerative medicine. Exopolysaccharide-based hydrogels have been proposed as efficient dressings given their excellent water-reserving ability, and biocompatibility, i.e., because they do not interact

biologically with human tissues, a critical limitation hampering their translation into paradigmatic scaffolds for tissue engineering (Ng et al. 2020).

For example, a hydrogel composed of dextran-hyaluronic acid (Dex-HA) enriched with sanguinarine (SA) incorporated in the gelatin microsphere (GMs) was successfully evaluated in a rat full-thickness burn infection model. The wound-healing effects and antibacterial properties were analyzed; overall, the hydrogel exhibited the best healing effect when analyzed by in vivo wound healing; additionally, the inflammation reaction was lower than the other groups, and the epithelization, collagen deposition, and uniform distribution were faster when compared with the others. The results suggested that the SA/GMs/Dex-HA hydrogel provides a potential way for infected burn treatment with high-quality and efficient scar inhibition (Zhu et al. 2018b).

A highly stretchable hydrogel composed of hyaluronan and sodium alginate was suggested as a matrix that could favor the anchorage of keratinocytes and thus enhance its cell carrier role for tissue regeneration (Murphy et al. 2012). A customized dextran-based was used for clinical translation and its regenerative response mechanisms in wound healing were determined. The hydrogel alone, with no additional growth factors, cytokines, or cells, was developed to treat third-degree burn wounds on mice. Results demonstrated that the treatment promoted remarkable neovascularization and skin regeneration, which was suggested as a lead to treatments for dermal wounds (Sun et al. 2011). More recently, another hydrogel based on dextran demonstrated an accelerated healing mechanism, this time in a third-degree porcine burn model. The results suggested that the hydrogel may substantially improve healing quality and reduce skin grafting incidents, thus serving as a base for clinical studies to improve the care of severe burn injury patients (Shen et al. 2015).

A thermo-reversible hydrogel composed of xanthan gum-konjac glucomannan (at different concentrations and ratios) was produced and characterized. The blends were considered hydrophilic and suitable for their future application as wound dressings. The ability to provide a moist local wound environment that absorbs excess exudate and promotes a proper wound healing was evidenced. Besides, the hydrogels also possessed adequate biological properties for supporting cell adhesion, migration, and proliferation, thereby promoting the cell secretion of extracellular matrix components to accelerate the granulation process (Alves et al. 2020).

The wound-healing effects of the EPS produced by *Lactobacillus* sp.Ca_6 (EPS-Ca_6) were reported using an excision wound model in rats, which demonstrated that the gel of EPS-Ca_6 accelerated significantly the wound-healing activity when compared to the nontreated group, and a total closure was achieved after 14 days of wound induction. Furthermore, histological examination of biopsies showed fully re-epithelialized wound with a complete epidermal regeneration. The antibacterial and antioxidant activities of this EPS were also evaluated through different assays, the results being compatible with a potential antioxidant activity (Trabelsi et al. 2017).

The in vivo wound-healing performance, the in vitro antioxidant activity, and the rheological characterization of the EPS produced by *Pseudomonas stutzeri* AS22 (EPS22) were investigated. EPS22 showed good chelating ability and a

pseudoplastic behavior, high elasticity, good mechanical strength, and stability with high water-absorption ability at 0.5% concentration, thus being considered a gel. The application of the PES22 gel was tested on dermal full-thickness excision wounds in a rat model every 2 days and enhanced significantly wound-healing activity and total closure (Maalej et al. 2014).

An EPS produced and purified from *Streptococcus zooepidemicus* MTCC 3523 was identified as hyaluronic acid (HA) and showed significant wound-healing activity in a rat model. It is important to mention that HA is the major component of the extracellular matrix and directly contributes to wound healing through its role in repair and increasing strength of tissues. According to the authors, the obtained results of wound healing by treatment of HA were encouraging and in perfect agreement with the abovementioned HA information (Patil et al. 2011).

Several bacteria from marine niche are adapted to synthesize EPSs with unique chemical compositions. When compared to polysaccharides sourced from plants and animals, they present advantages such as the ease of downstream processing, higher yield, purity, and lower cost (Moscovici 2015). An EPS isolated from a marine bacteria *Alteromonas* sp. PRIM-28 was used for EPS production. The polysaccharide, namely EPS-A28, was extensively characterized and tested for its bioactivities using in vitro models. EPS-A28, reported as an anionic heteropolysaccharide, showed biocompatibility and induced proliferation and migration of dermal fibroblasts and keratinocytes. In addition, EPS-A28 was able to increase the S-phase of cell cycle and to induce nitric oxide and arginase synthesis in macrophages. The results suggested that EPS-A28 could be potentially used as a multifunctional bioactive polymer in wound care (Sahana and Rekha 2019).

Another marine bacterium, this time identified as *Pantoea* sp. YU16-S3 (EPS-S3), was evaluated as wound dressing by studying the key-molecular mechanisms in vitro and in vivo. The results demonstrated that EPS-S3 activated macrophages, facilitated cell migration in fibroblasts, and accelerated the phases of the cell cycle. In vivo experiments in rats showed the re-epithelialization of injured tissue with increased expression of growth factors in EPS-treatment. EPS-63 biosynthesized by the marine bacterium was a potential biomolecule for cutaneous wound-healing applications (Sahana and Rekha 2020).

Considering that correct moisture levels are required for efficient recovery times in wound-healing processes, bacterial cellulose presents a high-water holding ability, which allows for the wound site to have the ideal moisture conditions. Due to the network of its nanofibers, scaffolds of bacterial cellulose (BC) might prevent infection by creating a physical barrier, thus preventing bacteria colonization into the wound (Ahmed et al. 2020).

BC possesses an exceptional water vapor permeability which can be hugely beneficial in wound dressings due to the multitude of hydroxyl groups (Fu et al. 2013). Other publications highlighted BC suitable as a skin substitute material or a temporary wound treatment, for example, the ones who reported BC similar to skin (Lee and Park 2017) and able to maintain an optimal moisture content for the proliferation and regular function of epidermal cells and fibroblasts in a three-dimensional culture model (Xu et al. 2016).

Microbial polysaccharides with multifunctional activities targeted to the distinct phases of wound healing, i.e., hemostasis, inflammation, proliferation, and remodeling, have great promise as a single treatment to the complex physiological process of healing. EPS-based hydrogels have been considered the most ideal scaffold for the production of wound dressings since they display a three-dimensional structure that mimics the native extracellular matrix of the injured tissue; additionally, their high-water content is essential in conferring a moist environment at the wound site, thus aiding the wound repair.

7 Drug Delivery

Conventional methods to deliver drugs as oral and injection often present limitations in a particular therapy related to high dosages and repeated administration that can result in lower patient compliance, severe side effects, and toxicity. Other drug delivery alternatives including transdermal, transmucosal, ocular, and pulmonary pathways have been developed using biological polymeric films, hydrogels, micro-/nanoparticles, beads, and tablets (Fig. 4) to address controlled drug release to cells and tissues over time and in space. These delivery routes can be more efficient,

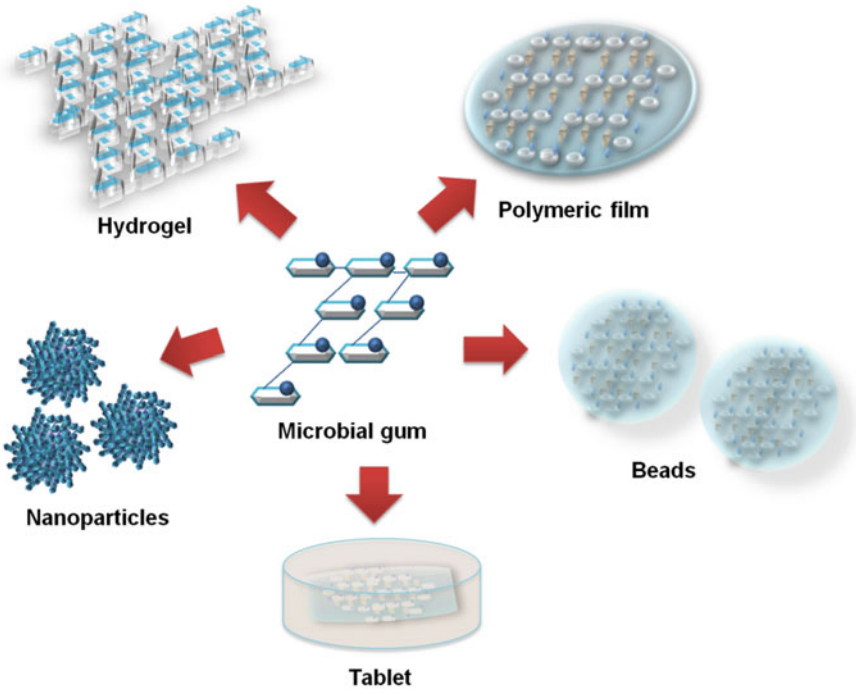


Fig. 4 Examples of microbial gums biobased formulations for controlled drug delivery

promoting an increase in the patient compliance, and reduction in the required dosage and side effects (Shirsath and Goswami 2019).

Materials based on microbial gums have some functional properties, biocompatibility and biosafety required for the delivery of various drugs, which can be metabolized by the intestinal microflora and enzymes. However, gums in their native form have showed some disadvantages that include pH-dependent swelling/solubility, alteration in viscosity degree on storage, and uncontrolled rates of hydration that can interfere in their use as drug delivery (Rana et al. 2011). Thus, gums have been modified to minimize these limitations and improve their drug release properties, through grafting with polymers, cross-linking, and derivatization of functional groups, among others (Rana et al. 2015; Bhatia 2016).

Xanthan gum is an important exopolysaccharide produced by *Xanthomonas campestris* bacteria with unique rheological properties for pharmaceutical formulations. The use of xanthan gum in various drug delivery systems has been reported highlighting this potential to control drug release due to its gelling property and ability to maintain the drug within the gel (Verma et al. 2017). A study investigated the application of a xanthan-based hydrogel to drug delivery for colon treatment. Xanthan gum was hydrolyzed and grafted with acrilamide using microwave irradiation. The grafting process has been used to alter its swelling and drug release properties (Rana et al. 2011). Morphological and thermal analysis showed that this modified-xanthan gum became somewhat smoother and more heat tolerant than nonmodified xanthan. The grafted xanthan was bound to a triamcinolone drug and showed a reduction in its swelling in media similar to the gastro-enteric and colonic systems, as well as a controlled drug release and noncytotoxicity, suggesting an efficient and safe drug delivery system for human use (Anjum et al. 2015).

Xanthan gum hydrogels were also developed using xanthan gum/polyvinylpyrrolidone polymer cross-linked with acrylic acid for controlled delivery of 5-fluorouracil at colon-specific site. Cross-linking is an alternative to reduce the swelling degree of the gums, since the hydrophilic swell of gums can result in the leaking out of the entrapped drug prior to arrival at its specific site (Rana et al. 2011). Drug release studies revealed the controlled release pattern of 5-fluorouracil at colon-specific site for prolonged time period, and nontoxicity (Anwar et al. 2020). Another study has presented a cross-linked injectable hydrogel based on aldehyde-modified xanthan and carboxymethyl-modified chitosan for drug delivery. This hydrogel demonstrated self-healing, biocompatibility, biodegradability, and stable drug release within 10 h after injection in liquids. Controlled drug release was also observed, especially in tissues with abundant excretion and exudation. When loading a vascular endothelial growth factor in rats, the hydrogel allowed an interaction between this biomaterial and the animal, accelerating the angiogenesis and reconstruction of the abdominal wall tissue (Huang et al. 2018). Chitosan/xanthan gum-based hydrogels were prepared as drug carrier system cross-linked for controlled acyclovir delivery. Acyclovir is an antiviral drug used against herpes simplex virus infections. The characterization analyses revealed that acyclovir was efficiently encapsulated into hydrogels, and the drug release behavior was pH dependent (Malik et al. 2020).

Cationic polymeric nanoparticles based on gums as dextrans have showed great potential for drug delivery (Nguyen and O'Rear 2017). Biocompatible cross-linked chitosan-dextran sulfate nanoparticles have been synthesized for ocular controlled release of ciprofloxacin antibiotic drug, in order to increase the bioavailability compared to conventional eye drops. The therapeutic value of nanoparticles was evaluated in simulated tear fluid at pH 7.4. Ocular irritancy was evaluated and revealed that chitosan-dextran sulfate nanoparticles did not induce any vascular response, being nonirritant to the ocular surface. They also showed monotonous controlled-release for duration of 21 h. Antimicrobial assays done against ophthalmic microbes showed good results of minimum inhibitory concentration and minimum bactericidal concentration, confirming the drug efficacy (Chavan et al. 2017). Thus, chitosan-dextran sulfate nanoparticles can be considered a ciprofloxacin vehicle nonirritant that promotes prolonged time of drug availability, offering efficient therapeutic value.

The use of dextran-sulfate as drug delivery platform for drug-coated balloons was also investigated. Drug-coated balloons are a promising alternative for peripheral artery disease treatment, but a significant drug loss from the balloon surface is observed during balloon tracking, reducing the therapeutic dose required at the diseased site (Kaule et al. 2015). To minimize this limitation, dextran sulfate films were prepared to carry an antiproliferative drug paclitaxel, and the drug release was investigated. Studies revealed that 10–20% of the drug loaded was lost during the time period of balloon tracking (1 min), allowing that a clinically relevant dose of drug be released during the typical time period of balloon inflation and treatment (from 1 min to 4 min). These data suggest the potential of dextran-sulfate films for controlled drug delivery from balloons (Lamichhane et al. 2016).

Magnetic nanoparticles were synthesized with cationic dextran conjugated with the oligoamine spermine to improve hydrophilic capecitabine antineoplastic delivery to negatively charged cancerous cells. Dextran-spermine nanoparticles showed an attractive sustained release behavior up to 56% after 24 h at neutral pH, and a burst release up to 98% after 3 h in acidic pH. The in vitro cytotoxicity of the drug-loaded nanoparticles was evaluated in glioblastoma U87MG cells and indicated promising theranostic effect when compared to free capecitabine, showing the need of more studies for glioblastoma-therapeutic application (Ghadiri et al. 2017). Other approach using dextran consisted in cross-linked poly(ethylene glycol)-*graft*-dextran nanoparticles for reduction and pH dual response drug delivery into cancer cells. These nanoparticles were loaded with the antineoplastic drug doxorubicin and were efficiently internalized into cancer cells, when the drug was released in response to acidic pH or reduction environment. In addition, they showed good biocompatibility and significant inhibition of cancer cells proliferation, suggesting their potential for safe cancer therapeutics (Lian et al. 2017).

Curdlan-based nanoparticles were also developed for biocompatible and safe therapeutic nucleic acid delivery. iRGD peptide-conjugated 6-amino-6-deoxy curdlan nanoparticles loading short interfering RNA (siRNA) were designed for delivery to integrin-expressing cancer cells. Curdlan was chemically modified in order to bind to siRNA and specifically enter integrin-expressing HepG2 cancer

cells. The siRNA was effectively carried and released in the cytoplasm. Moreover, it induced significant inhibition of the polo-like kinase-1 (Plk1) gene, related to mitotic events and frequently over-expressed in human cancers (Ganbold et al. 2019). It suggests that iRGD peptide-functionalized curdlan nanoparticles for siRNA delivery are a promising therapeutic strategy for cancer.

Other study investigated the efficiency of multiparticulate regioselective curdlan gum-based drug delivery system of moxifloxacin hydrochloride, an antibiotic absorved in the anterior part of the gastrointestinal tract, and therefore with a narrow therapeutic absorption window in this tract. Moxifloxacin beads were prepared via cross-linking of calcium, sodium alginate, and curdlan gum, and their release behavior of floating in gastric fluid and controlled release were investigated in vitro. More than 90% drug release was observed in about 10 h, and the beads remained buoyant more than 12 h. The kinetics modulation was controlled by the extent of cross-linked formation and bead sizes. These data suggest the potential of curdlan-based moxifloxacin drug delivery system for regioselective release and to improve bioavailability and therapy (Gurav and Sayyad 2019a). Curdlan gum was also used for design gastroretentive tablets of lamotrigine as regioselective drug delivery system. Lamotrigine is an antiepileptic agent exclusively absorbed from the upper region of gastrointestinal tract, and its unregulated plasma concentrations induce toxic effects, which can be prevented by a controlled gastroretentive drug delivery system. The lamotrigine tablets based on curdlan showed significant floating ability to release the drug regioselectively in stomach and proximal part of small intestine, and a floating time of at least 12 h (Gurav and Sayyad 2019b).

Pullulan is another microbial gum that has been also reported as an efficient matrix for fast oral dissolving drug delivery applications due to its bioadhesive, mechanical strength and good properties of film formability (Prajapati et al. 2018). A pullulan film-loading enalapril maleate, an antihypertensive agent, was developed and exhibited good physicochemical properties, no interaction between drug and polymer, and high dissolution rate, favoring this use for mucoadhesive buccal drug delivery (Gherman et al. 2016). An electrically responsive poly(acrylamide)-*graft*-pullulan copolymer was synthesized and evaluated under electric stimulation for transdermal drug delivery of rivastigmine tartarate through skin. When electric stimulation was applied, the drug diffusion rate was significantly enhanced in comparison with no electric stimulation condition. Analyses of skin histopathology revealed a reversible alteration in skin structure when it was submitted to electric stimulation, being an efficient biomaterial for transdermal drug delivery (Patil et al. 2020).

Levan has been applied as a capping agent for gold and silver salt solutions in order to obtain polymer-capped nanoparticles (Sathiyanarayanan et al. 2017). Levan-capped silver nanoparticles showed bactericide effect against *Escherichia coli* and *Bacillus subtilis*. These nanoparticles were also introduced in an alginate gel in order to create a silver release system with bactericidal activity. The system reduced the bacterial survival up to 20% in 5–10 h depending on the bacteria genera, being useful for drug delivery applications, such as wound dressing (González-Garcinuño et al. 2019).

β-glucan has also been used for the delivery of drug molecules (Zhang et al. 2018). Particles based on β-glucan were prepared to contain a payload of rifabutin, an antituberculosis drug, for macrophage-targeted delivery. β-glucan particles were extracted from yeast cells by acidic and alkaline extraction and were either spray dried or lyophilized, followed by rifabutin loading and alginate sealing. Spray-dried β-glucan particles showed great results of thermal stability, drug entrapment, and release. The formulation was also phagocytosed by macrophages within 5 min of exposure, being a promise for targeted drug delivery to macrophage in the tuberculosis therapeutics (Upadhyay et al. 2017).

Gellan gum is another microbially derived polysaccharide that has been applied in drug delivery formulations (Cardoso et al. 2017). A mucoadhesive in situ gel based on gellan gum and hydroxylpropyl methyl cellulose was developed for nasal delivery of the antiasthmatic drug salbutamol sulfate. In situ gel formulations are attractive for nasal applications due to their low viscosity into the nasal cavity, prolonged contact time, and continuous drug releasing (Gu et al. 2020). In this study, the drug release was found to be approximately 98% in 11 h. Viscosity and pH range were compatibles for nasal administration, and no histopathological damage was observed during ex vivo permeation studies. In this context, the in situ gel of gellan gum may be an efficient vehicle for nasal drug delivery (Salunke and Patil 2016). Other preparation of in situ gel of gellan gun was formulated for ocular delivery of brinzolamide, an antiglaucoma drug, in order to improve its therapeutic efficacy. In vitro drug release showed controlled and prolonged effect and less irritation degree than brinzolamide solutions after administration (Sun and Zhou 2018).

A low-acyl gellan macrobeads-carrying meloxicam was evaluated for oral delivery in the prophylaxis of colorectal cancer. Nonalteration on meloxicam structure was observed during production of the beads. Swelling and release studies showed a pH-dependent behavior of the meloxicam release from gellan gum-based beads in vitro, allowing the application for specific delivery in the distal parts of the gastrointestinal tract (Osmałek et al. 2017). The structural properties of microbial gums make them promising materials for new drug delivery formulations for prevention and treatment of different diseases with more specificity and efficiency.

8 Challenges in Microbial Gums

There is a great diversity of molecular structures for microbial gums; however, a few have been commercialized due to limitations related to production, extraction, and purification, including substrate availability, high costs, and cultivation challenges. With growing interest in microbial gums, research focused on approaches to solve the issues and challenges in their obtaining and applications. Many studies are taking place to isolate new strains, to use low-cost substrates, optimizing the process conditions involved in the extraction and purifications (Barcelos et al. 2019).

The improvement of curdlan production from *Agrobacterium* sp. ATCC 31749 was evaluated with the addition of juice of discarded bottom part of green asparagus

spear on culture medium. An increase of *Agrobacterium* sp. ATCC 31749 cell mass and enhancement of the curdlan production yield were observed, as well as a reduction in the cost of production and environmental waste resulting from the large-scaled discarded bottom parts of green asparagus spear (Anane et al. 2017). The high cost of the gellan gum production from *Sphingomonas paucimobilis* may limit its commercialization. A study showed the use of corn steep liquor as a cost-effective nutrient source in culture medium to improve gellan gum yields, interesting for industrial production (Huang et al. 2020).

A promise for increasing purity and high quality, these polysaccharides are metabolic engineering, which can manipulate the genes involved in encoding of the catalytic enzymes of the reactions in the pathway, or by altering a regulatory pathway (Shanmugam and Abirami 2019). This approach has been attempted for the production of some microbial gums, such as xanthan gum. Large amounts of ethanol are required to extract xanthan gum from *Xanthomonas campestris* fermentation broth, as well as to remove xanthomonadin impurities. In order to reduce the amount of ethanol and the production cost, a xanthomonadin-deficient strain of *X. campestris* was constructed by inserting the *Vitreoscilla* globin gene into the region of the *pigA* gene, resulting in the reduction of xanthomonadin synthesis. The insertion also induced an increase in the metabolism of *X. campestris*, allowing that the product ion of xanthan gum reaches wild-type levels. Thus, the engineered bacteria promote a reduction in the use of ethanol and the downstream-processing cost in xanthan gum production (Dai et al. 2019). This same strategy has also showed an enhancement of welan gum production in *Sphingomonas* sp., being of great importance for industrial applications (Liu et al. 2017b).

Moreover, microbial gums have been modified via physical and chemical reactions to improve their functional properties for biomedical applications, such as antimicrobial agents, antitumor drugs, and polymeric matrices to produce hydrogels, coatings, and nanoparticles useful for controlled drug delivery, tissue engineering, and wound dressings (Ahmad et al. 2015; Shanmugam and Abirami 2019). For example, gellan gum oxidized with hydrogen peroxide showed an antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli,* and *Aspergillus niger*, growing with the oxidation level, having a potent application as antimicrobial coating material in the food industry (Lu et al. 2019). Other study developed a formulation of xanthan gum and polyvinylpyrrolidone polymer chemically cross-linked with acrylic acid monomer to fabricate pH-sensitive hydrogels for nontoxic and controlled drug delivery (Anwar et al. 2020). Thus, the scale of production, purity, and quality of microbial gums may be improved, contributing for industrial demands and commercialization.

9 Conclusions

Gums of microorganisms are polysaccharides capable of forming viscous dispersions or gels in water, and many of these biopolymers have already been well characterized, structurally and biologically. EPSs can be produced on a large scale

by microorganisms applying SSF and SmF methods, which use solid and liquid substrates, respectively. Optimization of this production can still be achieved through changes in parameters in the fermentation processes. Microbial gums, such as dextran and xanthan, had their antimicrobial action improved when they became cationic and amphiphilic, capable of interacting with the negative surface and hydrophobic tails of microbial cell membranes to cause its death. Antioxidant potential of EPSs is another biological property that these biopolymers can have and is generally determined by assays involving the scavenging of free radicals. It is very meaningful to review different sources of microbial polysaccharides which possess splendid activities in health, such as the activity of inducing apoptosis in oncotherapy and adjuvant therapy, and the ability to hasten the wound-healing process. Microbial gums-based delivery formulations are promising candidates for more specific, efficient, and controlled release of drug compounds, allowing the direct treatment of the cause of the disease. Despite challenges, progresses in the use of microbial gums have been registered through understanding the complexity and biomedical applications of these polysaccharides to shape and improve their various biological, physical, and chemical properties.

Acknowledgments Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) is recognized for fellowships (LCBBC and MTSC) and grants. This work was also supported by the Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco (FACEPE) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

References

Abdelnasser SM, Yahya SMM, Mohamed WF, et al. Antitumor exopolysaccharides derived from novel marine Bacillus: isolation, characterization aspect and biological activity. Asian Pac J Cancer Prev. 2017;18:1847–54.

Abedfar A, Abbaszadeh S, Hosseininezhad M, Taghdir M. Physicochemical and biological characterization of the EPS produced by *L. acidophilus* isolated from rice bran sourdough. LWT. 2020;127:109373.

Abid Y, Azabou S, Casillo A, et al. Isolation and structural characterization of levan produced by probiotic *Bacillus tequilensis*-GM from Tunisian fermented goat milk. Int J Biol Macromol. 2019;133:786–94.

Abushaheen MA, Muzaheed FAJ, et al. Antimicrobial resistance, mechanisms and its clinical significance. Dis Mon. 2020;66:100971.

Adesulu-Dahunsi AT, Sanni AI, Jeyaram K. Production, characterization and *In vitro* antioxidant activities of exopolysaccharide from *Weissella cibaria* GA44. LWT. 2018;87:432–42.

Ahmad NH, Mustafa S, Man YBC. Microbial polysaccharides and their modification approaches: a review. Int J Food Prop. 2015;18:332–47.

Ahmad S, Ahmad M, Manzoor K, et al. A review on latest innovations in natural gums based hydrogels: preparations & applications. Int J Biol Macromol. 2019;136:870–90.

Ahmed J, Gultekinoglu M, Edirisinghe M. Bacterial cellulose micro-nano fibres for wound healing applications. Biotechnol Adv. 2020;41:107549.

Albuquerque PBS, Oliveira WF, Silva PMS, et al. Epiphanies of well-known and newly discovered macromolecular carbohydrates – a review. Int J Biol Macromol. 2020;156:51–66.

Almansoory AF, Al-Baldawi IA, Hazaimeh M. Optimization of the EPS production of a bacterial floc consortium using different parameters. Biocatal Agric Biotechnol. 2020;23:101466.

Alves A, Miguel SP, Araujo ARTS, Jes D. Xanthan gum – Konjac glucomannan blend hydrogel. Polymers (Basel). 2020;12(1):99.

Anane RF, Sun H, Zhao L, et al. Improved curdlan production with discarded bottom parts of Asparagus spear. Microb Cell Factories. 2017;16:59–67.

Anjum F, Bukhari SA, Siddique M, et al. Microwave irradiated copolymerization of xanthan gum with acrylamide for colonic drug delivery. BioRes. 2015;10(1):1434–51.

Anwar M, Pervaiz F, Shoukat H, et al. Formulation and evaluation of interpenetrating network of xanthan gum and polyvinylpyrrolidone as a hydrophilic matrix for controlled drug delivery system. Polym Bull. 2020;78:1–22.

Bao X, Yuan H, Wang C, et al. Antitumor and immunomodulatory activities of a polysaccharide from *Artemisia argyi*. Carbohydr Polym. 2013;98:1236–43.

Barcelos MCS, Vespermann KAC, Pelissari FM, Molina G. Current status of biotechnological production and applications of microbial exopolysaccharides. Crit Rev Food Sci Nutr. 2019;60: 1475–95.

Beldon P. Basic science of wound healing. Surgery. 2010;28:409–12.

Bhatia S. Microbial polysaccharides as advance nanomaterials. In: Bhatia S, editor. Systems for drug delivery. Switzerland: Springer International Publishing; 2016. p. 29–54.

Bomfim VB, Pereira Lopes Neto JH, Leite KS, et al. Partial characterization and antioxidant activity of exopolysaccharides produced by *Lactobacillus plantarum* CNPC003. Lwt. 2020;127: 109349.

Braz EMA, Silva SCCC, Brito CARS, et al. Modified chicha gum by acetylation for antimicrobial and antiparasitic applications: characterization and biological properties. Int J Biol Macromol. 2020;160:1177–88.

Busuioc M, Mackiewicz K, Buttaro BA, Piggot PJ. Role of intracellular polysaccharide in persistence of *Streptococcus mutans*. J Bacteriol. 2009;191:7315–22.

Byun BY, Lee SJ, Mah JH. Antipathogenic activity and preservative effect of levan (β-2,6-fructan), a multifunctional polysaccharide. Int J Food Sci Technol. 2014;49:238–45.

Cao Y, Naseri M, He Y, et al. Non-antibiotic antimicrobial agents to combat biofilm-forming bacteria. J Glob Antimicrob Re. 2020a;21:445–51.

Cao C, Li Y, Wang C, et al. Purification, characterization and antitumor activity of an exopolysaccharide produced by *Bacillus velezensis* SN-1. Int J Biol Macromol. 2020b;156: 354–61.

Cardoso VMO, Cury BSF, Evangelista RC, et al. Development and characterization of cross-linked gellan gum and retrograded starch blend hydrogels for drug delivery applications. J Mech Behav Biomed Mater. 2017;65:317–33.

Chaisuwan W, Jantanasakulwong K, Wangtueai S, et al. Microbial exopolysaccharides for immune enhancement: fermentation, modifications and bioactivities. Food Biosci. 2020;35:100564.

Chavan C, Bala P, Pal K, et al. Cross-linked chitosan-dextran sulphate vehicle system for controlled release of ciprofloxacin drug: an ophthalmic application. OpenNano. 2017;2:28–36.

Chen Y, Mao W, Tao H, et al. Structural characterization and antioxidant properties of an exopolysaccharide produced by the mangrove endophytic fungus *Aspergillus* sp. Y16. Bioresour Technol. 2011;102:8179–84.

Chen YT, Yuan Q, Shan LT, et al. Antitumor activity of bacterial exopolysaccharides from the endophyte *Bacillus amyloliquefaciens* sp.isolated from *Ophiopogon japonicus*. Oncol Lett. 2013;5:1787–92.

Chen G, Qian W, Li J, et al. Exopolysaccharide of Antarctic bacterium *Pseudoaltermonas* sp. S-5 induces apoptosis in K562 cells. Carbohydr Polym. 2015;121:107–14.

Dai X, Gao G, Wu M, et al. Construction and application of a *Xanthomonas campestris* CGMCC15155 strain that produces white xanthan gum. Microbiology. 2019;8:e631.

Deepak V, Ramachandran S, Balahmar RM, et al. In vitro evaluation of anticancer properties of exopolysaccharides from *Lactobacillus acidophilus* in colon cancer cell lines. In Vitro Cell Dev Biol Anim. 2016;52:163–73.

Delattre C, Fenoradosoa TA, Michaud P. Galactans: an overview of their most important sourcing and applications as natural polysaccharides. Braz Arch Biol Technol. 2011;54:1075–92.

Du R, Xing H, Yang Y, et al. Optimization, purification and structural characterization of a dextran produced by *L. mesenteroides* isolated from Chinese sauerkraut. Carbohydr Polym. 2017;174: 409–16.

Du R, Qiao X, Zhao F, et al. Purification, characterization and antioxidant activity of dextran produced by *Leuconostoc pseudomesenteroides* from homemade wine. Carbohydr Polym. 2018;198:529–36.

Elella MHA, Mohamed RR, ElHafeez EA, Sabaa MW. Synthesis of novel biodegradable antibacterial grafted xanthan gum. Carbohydr Polym. 2017;173:305–11.

Elella MHA, Sabaa MW, Hanna DH, et al. Antimicrobial pH-sensitive protein carrier based on modified xanthan gum. J Drug Deliv Sci Tec. 2020;57:101673.

Esawy MA, Ahmed EF, Helmy WA, et al. Production of levansucrase from novel honey *Bacillus subtilis* isolates capable of producing antiviral levans. Carbohydr Polym. 2011;86:823–30.

Farag MMS, Moghannem SAM, Shehabeldine AM, Azab MS. Antitumor effect of exopolysaccharide produced by *Bacillus mycoides*. Microb Pathog. 2020;140:103947.

Farinazzo FS, Valente LJ, Almeida MB, et al. Characterization and antioxidant activity of an exopolysaccharide produced by *Leuconostoc pseudomesenteroides* JF17 from juçara fruits (*Euterpe edulis* Martius). Process Biochem. 2020;91:141–8.

Fooladi T, Soudi MR, Alimadadi N, et al. Bioactive exopolysaccharide from *Neopestalotiopsis* sp. strain SKE15: production, characterization and optimization. Int J Biol Macromol. 2019;129:127–39.

Fu L, Zhang J, Yang G. Present status and applications of bacterial cellulose-based materials for skin tissue repair. Carbohydr Polym. 2013;92:1432–42.

Gan D, Zeng X, Hai R, Ye H. Potential mechanism of mycelium polysaccharide from *Pholiota dinghuensis* Bi in regulating the proliferation and apoptosis of human breast cancer MCF-7 cells through p38/MAPK pathway. J Funct Foods. 2015;12:375–88.

Ganbold T, Han S, Hasi A, et al. Receptor-mediated delivery of therapeutic RNA by peptide functionalized curdlan nanoparticles. Int J Biol Macromol. 2019;126:633–40.

Ghadiri M, Vasheghani-Farahani E, Atyabi F et al. *In-vitro* assessment of magnetic dextran-spermine nanoparticles for capecitabine delivery to cancerous cells. Iran J Pharm Res. 2017;16(4):1320–1334.

Gherman S, Zavastin D, Ochiuz L, et al. Enalapril maleate loaded pullulan film for mucoadhesive buccal drug delivery applications. Cellul Chem Technol. 2016;50(5–6):593–600.

Giavasis I. Production of microbial polysaccharides for use in food. In: McNeil B, Archer D, Giavasis I, Harvey L, editors. Microbial production of food ingredients, enzymes and nutraceuticals. 1st ed. Sawston: Woodhead Publishing; 2013. p. 413–68.

González-Garcinuño A, Masa R, Hernández M, et al. Levan-capped silver nanoparticles for bactericidal formulations: release and activity modeling. Int J Mol Sci. 2019;20:1502–21.

Gu F, Fan H, Cong Z, et al. Preparation, characterization, and *in vivo* pharmacokinetics of thermosensitive in situ nasal gel of donepezil hydrochloride. Acta Pharma. 2020;70:411–22.

Guo S, Mao W, Li Y, et al. Preparation, structural characterization and antioxidant activity of an extracellular polysaccharide produced by the fungus *Oidiodendron truncatum* GW. Process Biochem. 2013;48:539–44.

Gurav Y, Sayyad F. Design of multi-particulate regioselective drug delivery system of moxifloxacin using curdlan gum. Curr Pharm Res. 2019a;9(3):3101–10.

Gurav YA, Sayyad FJ. Design of regioselective drug delivery system of lamotrigine using curdlan gum. TPI. 2019b;8(4):88–93.

Gyawali R, Ibrahim SA. Natural products as antimicrobial agents. Food Control. 2014;46:412–29.

Habibi H, Khosravi-Darani K. Effective variables on production and structure of xanthan gum and its food applications: a review. Biocatal Agric Biotechnol. 2017;10:130–40.

Hanahan D, Weinberg RA. Review hallmarks of cancer: the next generation. Cell. 2011;144:646–74.

Hu X, Pang X, Wang PG, Chen M. Isolation and characterization of an antioxidant exopolysaccharide produced by *Bacillus sp*. S-1 from Sichuan Pickles. Carbohydr Polym. 2019;204:9–16.

Huang T, Lin J, Cao J, et al. An exopolysaccharide from *Trichoderma pseudokoningii* and its apoptotic activity on human leukemia K562 cells. Carbohydr Polym. 2012;89:701–8.
Huang J, Deng Y, Ren J, et al. Novel in situ forming hydrogel based on xanthan and chitosan re-gelifying in liquids for local drug delivery. Carbohydr Polym. 2018;186:54–63.
Huang J, Zhu S, Li C, et al. Cost-effective optimization of gellan gum production by *Sphingomonas paucimobilis* using corn steep liquor. Prep Biochem Biotechnol. 2020;50(2):191–7.
Izydorczyk M, Cui SW, Wang Q. Polysaccharide gums: structures, functional properties, and applications. In: Cui SW, editor. Food carbohydrates: chemistry, physical properties, and applications. 1st ed. Boca Raton: CRC Press; 2005. p. 43.
Kang Y, Li P, Zeng X, et al. Biosynthesis, structure and antioxidant activities of xanthan gum from *Xanthomonas campestris* with additional furfural. Carbohydr Polym. 2019;216:369–75.
Kaule S, Minrath I, Stein F, et al. Correlating coating characteristics with the performance of drug-coated balloons – a comparative *in vitro* investigation of own established hydrogel- and ionic liquid-based coating matrices. PLoS One. 2015;10(3):e0116080.
Kaur V, Bera MB, Panesar PS, et al. Welan gum: microbial production, characterization, and applications. Int J Biol Macromol. 2014;65:454–61.
Kedare SB, Singh RP. Genesis and development of DPPH method of antioxidant assay. J Food Sci Technol. 2011;48:412–22.
Kim TH, Kim JS, Kim ZH, et al. Khz-cp (crude polysaccharide extract obtained from the fusion of *Ganoderma lucidum* and *Polyporus umbellatus* mycelia) induces apoptosis by increasing intracellular calcium levels and activating P38 and NADPH oxidase-dependent generation of reactive oxygen species in SNU-1 cells. BMC Complement Altern Med. 2014;14:1–13.
Kumar CG, Mongolla P, Pombala S. Lasiosan, a new exopolysaccharide from *Lasiodiplodia* sp. strain B2 (MTCC 6000): structural characterization and biological evaluation. Process Biochem. 2018;72:162–9.
Lakra AK, Domdi L, Tilwani YM, Arul V. Physicochemical and functional characterization of mannan exopolysaccharide from *Weissella confusa* MD1 with bioactivities. Int J Biol Macromol. 2020;143:797–805.
Lamichhane S, Anderson J, Remund T, et al. Dextran sulfate as a drug delivery platform for drug-coated balloons: preparation, characterization, *in vitro* drug elution, and smooth muscle cell response. J Biomed Mater Res Part B. 2016;104B:1416–30.
Lee SE, Park YS. The role of bacterial cellulose in artificial blood vessels. Mol Cell Toxicol. 2017;13:257–61.
Leroy F, De Vuyst L. Advances in production and simplified methods for recovery and quantification of exopolysaccharides for applications in food and health. J Dairy Sci. 2016;99:3229–38.
Li W, Xia X, Tang W, et al. Structural characterization and anticancer activity of cell-bound exopolysaccharide from *Lactobacillus helveticus* MB2-1. J Agric Food Chem. 2015;63:3454–63.
Li S, Xiong Q, Lai X, et al. Molecular modification of polysaccharides and resulting bioactivities. Compr Rev Food Sci Food Saf. 2016;15:237–50.
Li H, Cao K, Cong P, et al. Structure characterization and antitumor activity of the extracellular polysaccharide from the marine fungus *Hansfordia sinuosae*. Carbohydr Polym. 2018;190:87–94.
Lian H, Du Y, Chen X, et al. Core cross-linked poly(ethylene glycol)-graft-dextran nanoparticles for reduction and pH dual responsive intracellular drug delivery. J Colloid Interface Sci. 2017;496:201–10.
Liu CT, Chu FJ, Chou CC, Yu RC. Antiproliferative and anticytotoxic effects of cell fractions and exopolysaccharides from *Lactobacillus casei* 01. Mutat Res Genet Toxicol Environ Mutagen. 2011;721:157–62.
Liu J, Wang X, Pu H, et al. Recent advances in endophytic exopolysaccharides: production, structural characterization, physiological role and biological activity. Carbohydr Polym. 2017a;157:1113–24.
Liu X, Zhu P, Jiang R, et al. Enhancement of welan gum production in *Sphingomonas* sp. HT-1 via heterologous expression of *Vitreoscilla* hemoglobin gene. Carbohydr Polym. 2017b;156:135–42.

Liu Z h, Niu F j, Xie Y x, et al. A review: natural polysaccharides from medicinal plants and microorganisms and their anti-herpetic mechanism. Biomed Pharmacother. 2020;129:110469.
Lu Y, Zhao X, Fang S. Characterization, antimicrobial properties and coatings application of gellan gum oxidized with hydrogen peroxide. Foods. 2019;8:31–43.
Maalej H, Moalla D, Boisset C, et al. Rhelogical, dermal wound healing and in vitro antioxidant properties of exopolysaccharide hydrogel from *Pseudomonas stutzeri* AS22. Colloids Surf B: Biointerfaces. 2014;123:814–24.
Malik NS, Ahmad M, Minhas MU, et al. Chitosan/ xanthan gum based hydrogels as potential carrier for an antiviral drug: fabrication, characterization, and safety. Front Chem. 2020;8: 50–66.
Mastbergen SC, Duivenvoorden I, Versteegh RT, Geldof AA. Cell cycle arrest and clonogenic tumor cell kill by divergent chemotherapeutic drugs. Anticancer Res. 2000;20:1833–8.
Mayet N, Choonara YE, Kumar P, et al. A comprehensive review of advanced biopolymeric wound. J Pharm Sci. 2014;103(8):2211–30.
McCarthy RR, Ullah MW, Booth P, et al. The use of bacterial polysaccharides in bioprinting. Biotechnol Adv. 2019;37:107448.
Mei Y, Zhu H, Hu Q, et al. A novel polysaccharide from mycelia of cultured *Phellinus linteus* displays antitumor activity through apoptosis. Carbohydr Polym. 2015;124:90–7.
Moscovici M. Present and future medical applications of microbial exopolysaccharides. Front Microbiol. 2015;6:1012.
Murphy SV, Skardal A, Atala A. Evaluation of hydrogels for bio-printing applications. J Biomed Mater Res – Part A. 2012;101(1):272–84.
Nehal F, Sahnoun M, Smaoui S, et al. Characterization, high production and antimicrobial activity of exopolysaccharides from *Lactococcus lactis* F-mou. Microb Pathog. 2019;132:10–9.
Ng JY, Obuobi S, Chua ML, et al. Biomimicry of microbial polysaccharide hydrogels for tissue engineering and regenerative medicine – a review. Carbohydr Polym. 2020;241:116345.
Nguyen HX, O'Rear EA. Modified dextran, heparin-based triggered release microspheres for cardiovascular delivery of therapeutic drugs using protamine as a stimulus. J Microencapsul. 2017;34(3):299–307.
Oliveira WF, Silva PMS, Coelho LCBB, et al. Plant antioxidants and mechanisms of action. Lett Drug Des Discov. 2018;15:1103–15.
Osemwegie OO, Adetunji CO, Ayeni EA, et al. Exopolysaccharides from bacteria and fungi: current status and perspectives in Africa. Heliyon. 2020;6:e04205.
Osmałek T, Milanowski B, Froelich A, et al. Design and characteristics of gellan gum beads for modified release of meloxicam. Drug Dev Ind Pharm. 2017;43(8):1314–29.
Palaniraj A, Jayaraman V. Production, recovery and applications of xanthan gum by *Xanthomonas campestris*. J Food Eng. 2011;106:1–12.
Pan Y, Xia Q, Xiao H. Cationic polymers with tailored structures for rendering polysaccharide-based materials antimicrobial: an overview. Polymers. 2019;11:1283.
Patil KP, Patil DK, Chaudhari BL, Chincholkar SB. Production of hyaluronic acid from *Streptococcus zooepidemicus* MTCC 3523 and its wound healing activity. J Biosci Bioeng. 2011;111:286–8.
Patil SB, Inamdar SZ, Das KK, et al. Tailor-made electrically-responsive poly(acrylamide)-*graft*-pullulan copolymer based transdermal drug delivery systems: synthesis, characterization, *in-vitro* and *ex-vivo* evaluation. J Drug Deliv Sci Tec. 2020;56:101525.
Paulo EM, Boffo EF, Branco A, et al. Production, extraction and characterization of exo-polysaccharides produced by the native *Leuconostoc pseudomesenteroides* R2 strain. An Acad Bras Ciênc. 2012;84:495–507.
Pei F, Ma Y, Chen X, Liu H. Purification and structural characterization and antioxidant activity of levan from *Bacillus megaterium* PFY-147. Int J Biol Macromol. 2020;161:1181–8.
Popescu I, Pelin IM, Ailiesei GL, et al. Amphiphilic polysaccharide based on curdlan: synthesis and behaviour in aqueous solution. Carbohydr Polym. 2019;224:115157.
Porter NT, Martens EC. The critical roles of polysaccharides in gut microbial ecology and physiology. Annu Rev Microbiol. 2017;71:349–69.

Prajapati VD, Jani GK, Zala BS, Khutliwala TA. An insight into the emerging exopolysaccharide gellan gum as a novel polymer. Carbohydr Polym. 2013;93:670–8.
Prajapati VD, Chaudhari AM, Gandhi AK, et al. Pullulan based oral thin film formulation of zolmitriptan: development and optimization using factorial design. Int J Biol Macromol. 2018;107:2075–85.
Prathyusha AMVN, Mohana Sheela G, Bramhachari PV. Chemical characterization and antioxidant properties of exopolysaccharides from mangrove filamentous fungi *Fusarium equiseti* ANP2. Biotechnol Rep. 2018;19:e00277.
Rahbar Saadat Y, Yari Khosroushahi A, Pourghassem Gargari B. A comprehensive review of anticancer, immunomodulatory and health beneficial effects of the lactic acid bacteria exopolysaccharides. Carbohydr Polym. 2019;217:79–89.
Rana V, Rai P, Tiwary AK, et al. Modified gums: approaches and applications in drug delivery. Carbohydr Polym. 2011;83:1031–47.
Rana V, Kamboj S, Sharma R, et al. Modification of gums synthesis techniques and pharmaceutical benefits. In: Thakur VK, Thakur MK, editors. Handbook of polymers for pharmaceutical technologies. 3rd ed. Beverly: Scrivener Publishing LLC; 2015. p. 299–364.
Redouan E, Emmanuel P, Michelle P, et al. Evaluation of antioxidant capacity of ulvan-like polymer obtained by regioselective oxidation of gellan exopolysaccharide. Food Chem. 2011;127:976–83.
Ruas-Madiedo P, De Los Reyes-Gavilán CG. Invited review: methods for the screening, isolation, and characterization of exopolysaccharides produced by lactic acid bacteria. J Dairy Sci. 2005;88:843–56.
Ruas-Madiedo P, Hugenholtz J, Zoon P. An overview of the functionality of exopolysaccharides produced by lactic acid bacteria. J Dairy Sci. 2002;12:163–71.
Ruiz-Ruiz C, Srivastava GK, Carranza D, et al. An exopolysaccharide produced by the novel halophilic bacterium *Halomonas stenophila* strain B100 selectively induces apoptosis in human T leukaemia cells. Appl Microbiol Biotechnol. 2011;89:345–55.
Rukmanikrishnan B, Ramalingam S, Rajasekharan SK, et al. Binary and ternary sustainable composites of gellan gum, hydroxyethyl cellulose and lignin for food packaging applications: biocompatibility, antioxidant activity, UV and water barrier properties. Int J Biol Macromol. 2020;153:55–62.
Sahana TG, Rekha PD. A bioactive exopolysaccharide from marine bacteria *Alteromonas* sp. PRIM-28 and its role in cell proliferation and wound healing in vitro. Int J Biol Macromol. 2019;131:10–8.
Sahana TG, Rekha PD. A novel exopolysaccharide from marine bacterium *Pantoea* sp. YU16-S3 accelerates cutaneous wound healing through Wnt/β-catenin pathway. Carbohydr Polym. 2020;238:116191.
Saidin NM, Anuar NK, Affandi MMRMM. Roles of polysaccharides in transdermal drug delivery system and future prospects. J Appl Pharm Sci. 2018;8(3):141–57.
Sajna KV, Gottumukkala LD, Sukumaran RK, Pandey A. White biotechnology in cosmetics. In: Pandey A, Höfer R, Taherzadeh M, Nampoothiri KM, Larroche C, editors. Industrial biorefineries & white biotechnology. 1st ed. Amsterdam: Elsevier; 2015. p. 607–52.
Salunke SR, Patil SB. Ion activated in situ gel of gellan gum containing salbutamol sulphate for nasal administration. Int J Biol Macromol. 2016;87:41–7.
Sathiyanarayanan G, Dineshkumar K, Yang Y-H. Microbial exopolysaccharide-mediated synthesis and stabilization of metal nanoparticles. Crit Rev Microbiol. 2017;43(6):731–52.
Schäfer M, Werner S. Cancer as an overhealing wound: an old hypothesis revisited. Nat Rev Mol Cell Biol. 2008;9:628–38.
Shanmugam M, Abirami RG. Microbial polysaccharides – chemistry and applications. JBAPN. 2019;9(1):73–8.
Shen Y, Song H, Papa A, et al. Acellular hydrogel for regenerative burn wound healing: translation from a porcine model. J Invest Dermatol. 2015;135(10):2519–29.
Shirsath NR, Goswami AK. Nanocarriers based novel drug delivery as effective drug delivery: a review. Curr Nanometer. 2019;4:71–83.

Sivakanthan S, Rajendran S, Gamage A, et al. Antioxidant and antimicrobial applications of biopolymers: a review. Food Res Int. 2020;136:109327.
Soeiro VC, Melo KRT, Alves MGCF, et al. Dextran: influence of molecular weight in antioxidant properties and immunomodulatory potential. Int J Mol Sci. 2016;17:1–15.
Sran KS, Bisht B, Mayilraj S, Roy Choudhury A. Structural characterization and antioxidant potential of a novel anionic exopolysaccharide produced by marine *Microbacterium aurantiacum* FSW-25. Int J Biol Macromol. 2019;131:343–52.
Srikanth R, Reddy CHSSS, Siddartha G, et al. Review on production, characterization and applications of microbial levan. Carbohydr Polym. 2015a;120:102–14.
Srikanth R, Siddartha G, Sundhar Reddy CHSS, et al. Antioxidant and anti-inflammatory levan produced from *Acetobacter xylinum* NCIM2526 and its statistical optimization. Carbohydr Polym. 2015b;123:8–16.
Stanciu MC, Belei D, Bicu E, et al. Novel amphiphilic dextran esters with antimicrobial activity. Int J Biol Macromol. 2020;150:746–55.
Sun J, Zhou Z. A novel ocular delivery of brinzolamide based on gellan gum: in vitro and in vivo evaluation. Drug Des Dev Ther. 2018;12:383–9.
Sun G, Zhang X, Shen Y, et al. Dextran hydrogel scaffolds enhance angiogenic responses and promote complete skin regeneration during burn wound healing. PNAS. 2011;108(52):20976–81.
Tabernero A, Cardea S. Supercritical carbon dioxide techniques for processing microbial exopolysaccharides used in biomedical applications. Mater Sci Eng C. 2020;112:110940.
Taheri A, Jafari SM. Gum-based nanocarriers for the protection and delivery of food bioactive compounds. Adv Colloid Interf Sci. 2019;269:277–95.
Tallon R, Bressollier P, Urdaci MC. Isolation and characterization of two exopolysaccharides produced by *Lactobacillus plantarum* EP56. Res Microbiol. 2003;154:705–12.
Taylan O, Yilmaz MT, Dertli E. Partial characterization of a Levan type exopolysaccharide (EPS) produced by *Leuconostoc mesenteroides* showing immunostimulatory and antioxidant activities. Int J Biol Macromol. 2019;136:436–44.
Tian Y, Zhao Y, Zeng H, et al. Structural characterization of a novel neutral polysaccharide from *Lentinus giganteus* and its antitumor activity through inducing apoptosis. Carbohydr Polym. 2016;154:231–40.
Trabelsi I, Ktari N, Ben Slima S, et al. Evaluation of dermal wound healing activity and *in vitro* antibacterial and antioxidant activities of a new exopolysaccharide produced by *Lactobacillus* sp.Ca6. Int J Biol Macromol. 2017;103:194–201.
Tuchilus CG, Nichifor M, Mocanu G, Stanciu MC. Antimicrobial activity of chemically modified dextran derivatives. Carbohydr Polym. 2017;161:181–6.
Upadhyay TK, Fatima N, Sharma D, et al. Preparation and characterization of beta-glucan particles containing a payload of nanoembedded rifabutin for enhanced targeted delivery to macrophages. EXCLI J. 2017;16:210–28.
Valencia GA, Zare EN, Makvandi P, et al. Self-assembled Carbohydr Polym for food applications: a review. Compr Rev Food Sci Food Saf. 2019;18(6):2009–24.
Verma SV, Kalyani S, Badwaik HR. Xanthan gum a versatile biopolymer: current status and future prospectus in hydro gel drug delivery. Curr Chem Biol. 2017;11(1):10–20.
Vidhyalakshmi R, Valli Nachiyar C, Narendra Kumar G, Sunkar S. *Bacillus circulans* exopolysaccharide: production, characterization and bioactivities. Int J Biol Macromol. 2016;87:405–414.
Wang H, Liu Y, Qi Z, et al. An overview on natural polysaccharides with antioxidant properties. Curr Med Chem. 2013;20:2899–913.
Wang K, Li W, Rui X, et al. Characterization of a novel exopolysaccharide with antitumor activity from *Lactobacillus plantarum* 70810. Int J Biol Macromol. 2014;63:133–9.
Wang B, Song Q, Zhao F, et al. Production optimization, partial characterization and properties of an exopolysaccharide from *Lactobacillus sakei* L3. Int J Biol Macromol. 2019;141:21–8.
Wang Z, Yang Q, Wang X, et al. Antibacterial activity of xanthan-oligosaccharide against *Staphylococcus aureus* via targeting biofilm and cell membrane. Int J Biol Macromol. 2020;153:539–44.

Wen L, Xu Y, Wei Q, et al. Modeling and optimum extraction of multiple bioactive exopolysaccharide from an endophytic fungus of *Crocus sativus* L. Pharmacogn Mag. 2018;14:36–43.

Wu SJ, Wu JH, Xia LZ, et al. Preparation of xanthan-derived oligosaccharides and their hydroxyl radical scavenging activity. Carbohydr Polym. 2013;92:1612–4.

Xiang J, Shen L, Hong Y. Status and future scope of hydrogels in wound healing: synthesis, materials and evaluation. Eur Polym J. 2020;130:109609.

Xiao L, Han S, Zhou J, et al. Preparation, characterization and antioxidant activities of derivatives of exopolysaccharide from *Lactobacillus helveticus* MB2-1. Int J Biol Macromol. 2020;145:1008–17.

Xiong X, Li M, Xie J, et al. Antioxidant activity of xanthan oligosaccharides prepared by different degradation methods. Carbohydr Polym. 2013;92:1166–71.

Xu R, Xia H, He W, et al. Controlled water vapor transmission rate promotes wound-healing via wound reepithelialization and contraction enhancement. Sci Rep. 2016;6:24596.

Ye G, Li G, Wang C, et al. Extraction and characterization of dextran from *Leuconostoc pseudomesenteroides* YB-2 isolated from mango juice. Carbohydr Polym. 2019;207:218–23.

Yildiz H, Karatas N. Microbial exopolysaccharides: resources and bioactive properties. Process Biochem. 2018;72:41–6.

Yu J, Sun R, Zhao Z, Wang Y. *Auricularia polytricha* polysaccharides induce cell cycle arrest and apoptosis in human lung cancer A549 cells. Int J Biol Macromol. 2014;68:67–71.

Yu W, Chen G, Zhang P, Chen K. Purification, partial characterization and antitumor effect of an exopolysaccharide from *Rhizopus nigricans*. Int J Biol Macromol. 2016;82:299–307.

Zhang H, Wang X, Li R, et al. Preparation and bioactivity of exopolysaccharide from an endophytic fungus *Chaetomium* sp. of the medicinal plant gynostemma pentaphylla. Pharmacogn Mag. 2017;13:477–82.

Zhang M, Kim JA, Huang AYC. Optimizing tumor microenvironment for cancer immunotherapy: β-glucan-based nanoparticles. Front Immunol. 2018;9:341–55.

Zhu Y, Wang C, Jia S, et al. Purification, characterization and antioxidant activity of the exopolysaccharide from *Weissella cibaria* SJ14 isolated from Sichuan paocai. Int J Biol Macromol. 2018a;115:820–8.

Zhu Q, Jiang M, Liu Q, et al. Enhanced healing activity of burn wound infection by dextran-HA hydrogel enriched with sanguinarine. Biomater Sci. 2018b;6:2472–86.

The Promise and Challenge of Microbial Alginate Production: A Product with Novel Applications

5

Wael Sabra

Contents

Abstract

Alginate is a polysaccharide that has various established applications in food, textile, and pharmaceutical industries for its viscosifying and gelling properties. In the last two decades, and owing to their biocompatibility, and nontoxicity as well as versatility in view of modifications, several novel applications have been emerged that can be the base of novel future markets. Currently, seaweeds are the largest source for alginate production, and despite the variations in quality and quantity due to changes of climate and sources, most of the commercially produced alginates are still based on brown seaweeds. Nevertheless, alginate can also be biotechnologically produced by species of two families of heterotrophic bacteria, namely *Pseudomonas* and *Azotobacter*. Efforts

W. Sabra (✉)
Faculty of Life Sciences, Rhine-Waal University of Applied Sciences, Kleve, Germany
e-mail: wael.sabra@hochschule-rhein-waal.de

J. M. Oliveira et al. (eds.), *Polysaccharides of Microbial Origin*,
https://doi.org/10.1007/978-3-030-42215-8_5

have been made in the past to produce alginate polymers from these bacteria; however, its production never left the lab scale. Herein, the biological function of microbial alginate and the current process aspects with regard to its industrial production as well as the bottlenecks that still exist are highlighted. Based on their unique biochemical and biophysical characteristics and the ability to upgrade microbial alginate polymers through in vitro modifications, the achievements for alginate polymers of the last 10 years in food, pharmaceutical, and biomedical applications are discussed.

Keywords

Azotobacter vinelandii · *Pseudomonas* · Tissue engineering · Scale-up challenges · Microaerobic culture · Alginate applications

1 Introduction

Alginate is a linear polysaccharide composed of (1–4)-β-D mannuronic acid (M) and its C-5 epimer α-l-guluronic acid (G). The distribution of the acids residues along the chain is nonrandom and involves relatively long sequences of each uronic acid arranged in a block-wise pattern (Fig. 1). The extent and composition of these sequences together with the relative molecular mass of the alginate chain determine the gel-forming ability and the physicochemical properties of the polymer. In the presence of divalent cations, such as calcium, alginate gels can be formed due to ionic cross-linking of G-blocks with some contribution from the MG-blocks by certain divalent cations which form bridges between L-guluronic acid residues on adjacent chains (Fig. 1). Alginate occurs as the main cell wall constituent of marine algae and comprises up to 50% of the dry weight. The different species of the brown seaweeds such as *Laminaria hyperborea, Macrocystis pyrifera,* and *Ascophyllum nodosum are the* main current source of all commercial alginate materials. Seaweeds are collected from coastal waters or cultivated seaweeds farms, dried, and crushed, and the polysaccharide is extracted and purified by several ion exchange operations. Although the quantities of brown seaweed exceed the global current demand, there is actually a lack of this raw material because they exist in remote areas where transport costs are excessive. In addition, alginates from seaweeds are subjected to product variations because of seasonal variations and growth conditions. It can even vary within the plant itself, with the frond (leaves), stipe (stem), and holdfast (anchor) showing progressively more poly-G. Indeed, algal alginates with predefined properties cannot be easily obtained from a particular algal species, thus limiting their use in the pharmaceutical and chemical industries. In food and beverage, paper, textile and printing, and various biomaterials industries, algal alginates are used as viscosifier, thickener, stabilizer, and gelling agent. In fact, there is a huge market for alginate with an estimated annual worldwide production of around 45,000 metric tons (Stanisci et al. 2020). Some of the manufacturers of sodium alginate include FMC Corporation; Cargill, Inc.; Qingdao Lanneret; and KIMICA and DANISCO.

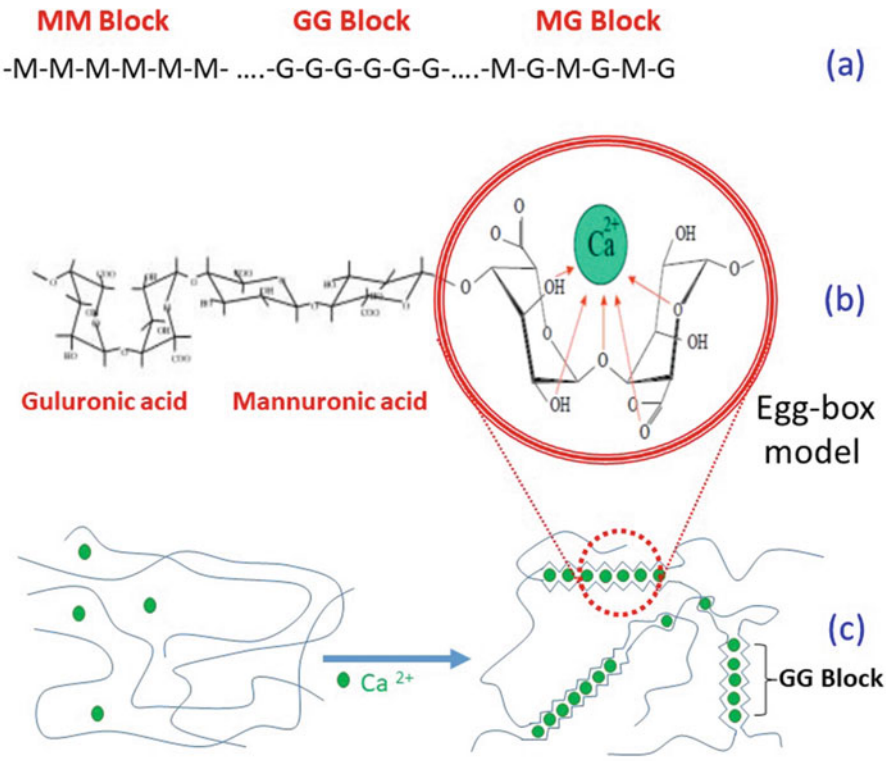


Fig. 1 Block structure of alginate (**a**) and its crosslinking with calcium cations in an eggbox model (**b**, **c**)

The price of alginate varies according to the source and quality, between 2 US$ and 40 US$/kg, and may reach more than 40,000 US$/kg for purified alginate for pharmaceutical applications (Pacheco-Leyva et al. 2016).

Alginate can also be biologically produced by bacteria such as *Azotobacter vinelandii*, *A. chroococcum,* and several species of *Pseudomonas* (Aarstad et al. 2019; Hay et al. 2013; Lotfy et al. 2018; Rehm and Valla 1997; Sabra et al. 2002). Unlike seaweed alginate, bacterial alginate production can be easily tailored to obtain molecules with different characteristics and unique compositions and, therefore, has the advantage that it may potentially be sold at higher prices, and this may open new markets for this polymer (Ertesvag 2015; Ertesvag et al. 2017; Gawin et al. 2020; Maleki et al. 2017). In fact, bacterial alginates are of potential commercial interest due to the fast development of pharmaceutical applications of this polymer as well as the discovery of its unique immunological properties (Aarstad et al. 2019). Certainly, the advances in biotechnological techniques have aroused the interest of industrial investors in developing a microbially optimized production process through controlling the growth conditions in a bioreactor while tailoring the production of biologically active high-value compounds.

2 Biosynthesis of Alginates and Their Biological Functions in Producing Bacteria

A. vinelandii is a diazotrophically strict aerobic bacterium that synthesizes two polymers during vegetative growth, alginate, and the intracellular polyesters – polyhydroxybutyrate (PHB) (Fig. 2) (Castillo et al. 2013; Sabra et al. 1999, 2001; Sabra and Zeng 2009). All of the steps involved in the conversion of central sugar metabolites into the alginate precursor in *A. vinelandii* have been previously identified and characterized (Flores et al. 2015; Pacheco-Leyva et al. 2016). Pyruvate is

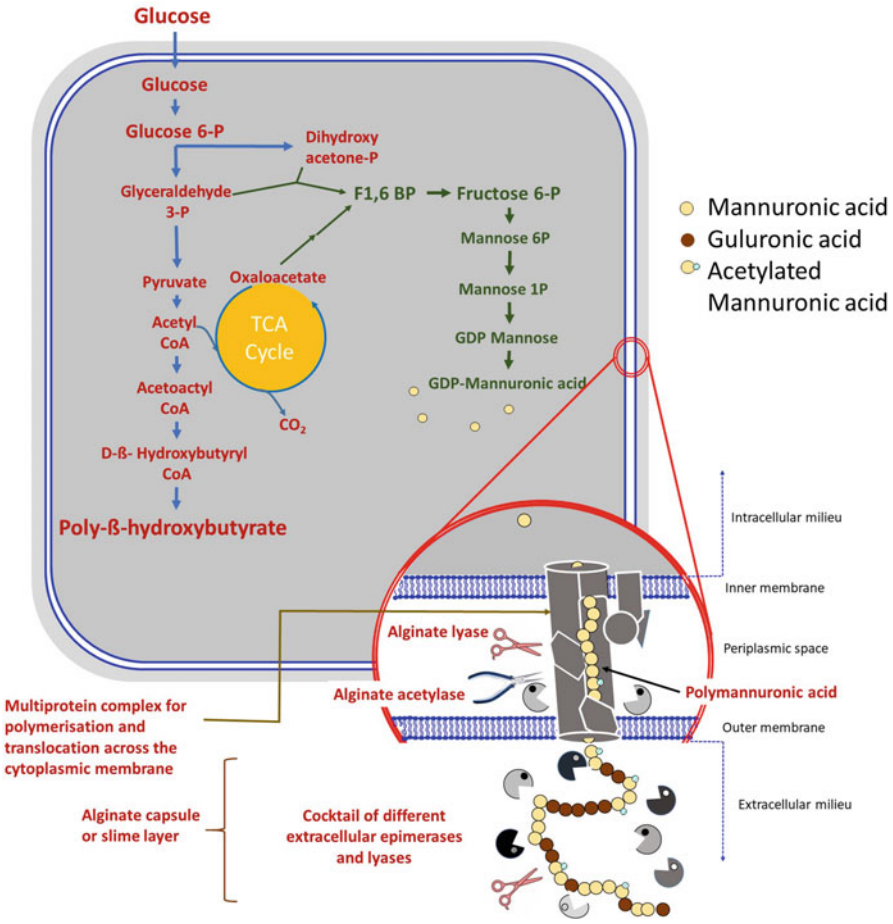


Fig. 2 Simplified metabolic pathway for the biosynthesis of alginate and poly-ß-hydroxybutyrate in Azotobacter vinelandii. The multiprotein complex involved in alginate polymerization and transport across the cell membrane is also shown. (Refer to Rehm and Moradali (2018), Urtuvia et al. (2017) for details about the protein-protein interactions and the complexity of alginate polymerization and transport)

first formed from the available C6 sugar through the Entner-Douderoff pathway, which is then channeled to the tricarboxylic acid (TCA) cycle, while oxaloacetate from the TCA cycle is converted to fructose-6-phosphate via gluconeogenesis. The formation of guanosine di-phosphate (GDP)-mannuronic acid, the active precursor for alginate synthesis, from fructose 6 phosphate requires the action of several cytosolic enzymatic steps (Fig. 2). Phosphomannose isomerase (AlgA) catalyzes the conversion of fructose-6-phosphate to mannose-6-phosphate, which is directly converted to its isomer form, mannose-1-phosphate, by phosphomanno-mutase (AlgC). The activated mannose-1-phosphate is converted to GDP-mannose with the hydrolysis of GTP by the GDP-mannose pyrophosphorylase (GMP) activity of AlgA. The GMP activity of this enzyme favors the reverse reaction, but AlgD (GDP-mannose-dehydrogenase) constantly converts GDP-mannose to GDP-mannuronic acid, and the reaction is shifted toward GDP-mannuronic acid and alginate production. This AlgD-catalyzed reaction is essentially irreversible and provides the direct precursor for polymerization, GDP-mannuronic acid. The high intracellular levels of GDP-mannose indicate that this AlgD-catalyzed step is a limiting step and was shown to represent the metabolic bottleneck in alginate-overproducing strains of *P. aeruginosa* (Rehm 2009). Polymerization is done by membrane-bound glycosyltransferases which catalyze the transfer of an activated sugar moiety onto a receptor molecule while forming a glycosidic bond. The polymannuronate polysaccharide resulting from polymerization and then translocation to the *A. vinelandii* periplasm is composed of M-residues, which can then be further modified during its passage across the periplasm.

2.1 Postpolymerization Modification of Alginate

A unique feature for alginate biosynthesis is its synthesis in the periplasm as a homopolymeric strand that contains solely mannuronic acid residues. During its passage across the periplasm, several alginate-modifying enzymes work in a coordinated action to create alginate polymers of different properties at different environmental conditions. This postpolymerization modification of the alginate strands represents the critical steps needed to create several polymers with unique characteristics like gel-strength, chain stiffness, water binding capacity, viscosity, immunogenicity, and biocompatibility (Ertesvag 2015). In *A. vinelandii, o*nly one periplasmic epimerase, which incorporates single G residues into the alginate during secretion of the polymer, was found (AlgG). Moreover, a family of seven calcium-dependent-mannuronan C5 epimerases (AlgE1–AlgE7) modifies the poly-mannuronate and produces specific epimerization patterns while introducing guluronic acid residue either as monomers or in blocks in the alginate strands. Recently, Aarstadt et al. (2019) showed the importance of the AlgE1 to form extralong G-blocks, when acting on poly-M, and concluded that *A. vinelandii* alginates can form gels with mechanical properties similar to the desired seaweed alginates and of comparable compositions, which strengthens the possibilities of commercial alginate production from microbial sources.

The action of the different epimerases on the polymannuronate strand depends on the acetylation pattern by O-acetylases. Acetylation can occur at the hydroxyl groups of either the C2 or C3 position of the mannuronic acid residue. Only nonacetylated M-residues can be epimerized to G-residues by epimerases, while O-acetylated M-residues are protected from epimerization. Finally, the length of the alginate strand is determined by the activity of different alginate lyases. A multiprotein complex that is anchored to the membrane is responsible for (1) the polymerization (forming poly M); (2) protecting the nascent alginate against lyase activity, epimerization, and acetylation; and finally (3) completing the translocation of the modified alginate strands across the outer membrane and secretion to the external milieu (Flores et al. 2015; Hay et al. 2014; Rehm and Moradali 2018).

Both alginate lyases and C-5-epimerases can be used in vitro to tailor alginates for specific purposes. Accordingly, the biochemical properties of these enzymes have been extensively characterized to understand the production mechanism (Aarstad et al. 2019; Castillo et al. 2013; Ertesvag 2015; Gawin et al. 2020; Stanisci et al. 2020), especially due to their potential biotechnological applications (Aarstad et al. 2013). Interestingly, most of these polymer-modifying enzymes in *A. vinelandii* were reported to be affected by the oxygen concentrations in culturing media (Castillo et al. 2013, 2020; Pacheco-Leyva et al. 2016). The molecular mechanism involved in alginate production linked to the availability of oxygen in *A. vinelandii* was recently discussed by Pacheco-Leyva et al. (2016).

2.2 Biological Role of Alginate Production

The role of exopolysaccharides (EPS) in nature has not been clearly established and is probably diverse and complex. It has been suggested that EPS may protect against desiccation, phagocytosis and phage attack, participate in uptake of metal ions, function as adhesive agents and stabilize membrane structure against the harsh external environment, or act as ATP sinks, or be involved in interactions between plants and bacteria. Alginates in phaeophyta are believed to act as a structure-forming component providing mechanical strength and flexibility. This explains the difference in alginate compositions in different algae or in different regions of the same alga. As an alternative source of algal alginate and for the many advantages of bacterial alginates, several studies have been performed for the production of alginate by different strains of *A. vinelandii* and *Pseudomonas* spp. (Aarstad et al. 2019). *A. vinelandii* is distinguished from the rest of the family Azoto bacteriaceae by the presence of a characteristic life cycle which includes the formation of a spherically dormant cyst at carbon-limited conditions (Galindo et al. 2007). For the successful formation of the cyst, both PHB and alginate are essential. Alginate production is, therefore, a mandatory requirement for cyst formation in *A. vinelandii* and helps to protect the cells from desiccation, mechanical stress, and represents a diffusion barrier against heavy metal contamination.

The alginate capsule was also shown to control the intracellular redox status of *Azotobacter* cells grown under N_2-fixing conditions (Sabra et al. 2000).

Diazotrophic-grown *Azotobacter* cells are able to grow under fully aerated conditions, despite the fact that the nitrogenase enzyme complex, which catalyzes the reduction of N_2 to ammonia, is highly sensitive to oxygen. Therefore, the growth of N_2-fixing *A. vinelandii* was shown to favor microaerobic condition (Sabra and Zeng 2009). Nevertheless, at high-oxygen concentrations, the bacteria have developed many mechanisms to protect their oxygen-sensitive nitrogenase. *A. vinelandii* was shown to create their microaerobic conditions by controlling the alginate capsules formation, and by varying their permeability properties through the actions of the different epimerases (Figs. 2 and 3). In a phosphate-limited continuous culture, it was shown that the specific rate of oxygen consumption (qO_2) of the cells increased

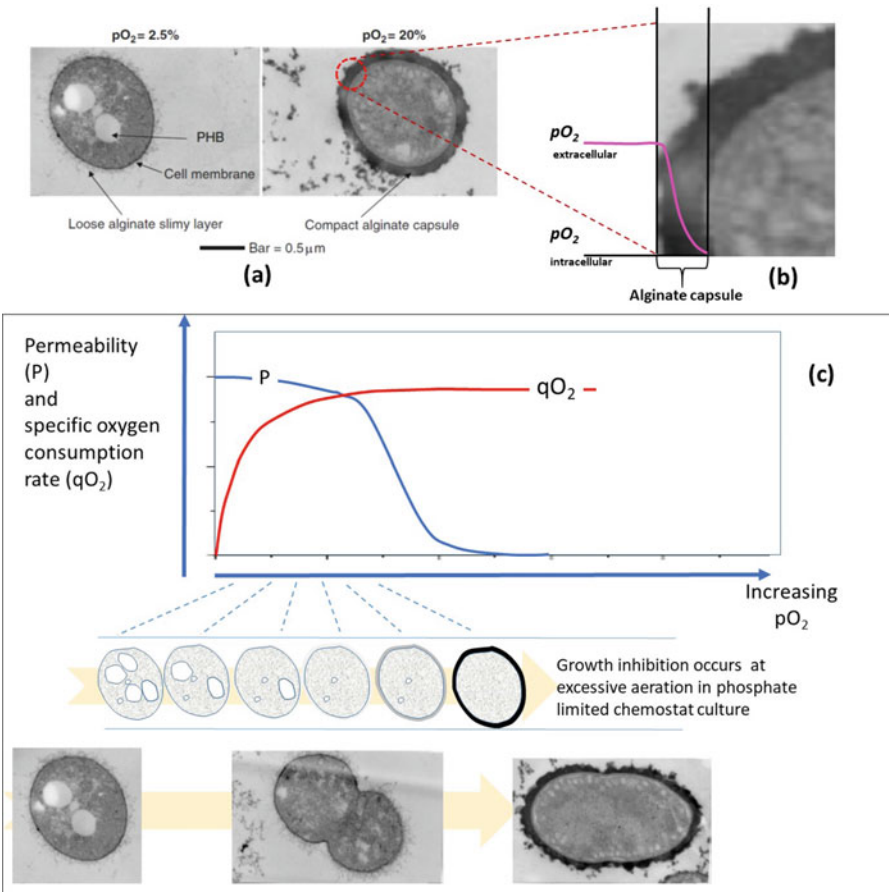


Fig. 3 Thin-section electron micrograph of diazotrophically growing A. vinelandii cells in phosphate-limited chemostat cultures at low- (2.5% of air saturation) and high- (20%) dissolved oxygen concentrations (pO_2, "**a**"), and the diffusion barrier exerted by a compact alginate capsule against oxygen at higher pO_2 to protect nitrogenase ("**b**"). At a constant intracellular pO_2 value suitable for nitrogenase, the difference in cell wall permeability as a function of extracellular pO_2 values is shown in "**c**"

as the dissolved oxygen tension (pO_2) was elevated from 1% to 5% air saturation (Sabra et al. 2000). The qO_2 remained, however, essentially constant when the pO_2 was controlled above 5%. These results cannot be interpreted in terms of the "respiratory protection" concept. This is in agreement with several studies that indicate that respiratory protection is not the prevailing mechanism for nitrogenase protection above a certain oxygen concentration (Oelze 2000). Indeed, at constant qO_2 values, while increasing the pO_2 values, the bacteria could keep the intracellular oxygen concentrations ($CO_{2,\ int}$), at a microaerobic or merely anaerobic range suitable for nitrogenase, if the permeability "P" of the cell wall to oxygen decreases sharply, as can be derived from (1).

$$qO_2 = qO_{2,\max} = P\left(pO_{2,ext} - CO_{2,int}\right) \approx \text{constant} \quad (1)$$

It was also observed that both the molecular weight and the guluronic acid content of the alginate formed in phosphate limited chemostat cultures monotonically increased with pO_2. The transmission electron microscopy of negatively stained *A. vinelandii* showed that cells at lower and higher pO_2 formed capsules with significant differences in the thickness and compactness of the polysaccharide (Fig. 3). Such an alginate capsule was proposed to function as diffusion barrier against oxygen for the O_2-sensitive nitrogenase enzyme complex (Sabra et al. 2000) (Fig. 3).

The fact that alginate production in *A. vinelandii* is dependent on oxygen concentration has attracted many scientists to understand the link between oxygen availability and alginate production at a molecular level (Aarstad et al. 2019; Castillo et al. 2013; Gawin et al. 2020; Pacheco-Leyva et al. 2016; Urtuvia et al. 2017). Interestingly, earlier studies also showed an oxygen-dependent alginate biosynthesis with *P. aeruginosa* (Bayer et al. 1990; Leitao and Sa-Correia 1997).

Concerning the biological role of alginate for the pathogenic bacteria *P. aeruginosa*, the causative agent of cystic fibrosis, it serves to protect the bacteria from adversity in its surrounding and also enhances adhesion to solid surfaces. Alginate is considered as a virulence factor, and as a result, biofilm develops which is advantageous to the survival of the bacterium in the lung (Hay et al. 2014). *P. aeruginosa* produces also alginate lyase which cleaves the polymer into short oligosaccharides resulting in increased detachment of the bacteria away from the surface, allowing them to spread and colonize new sites (Ertesvag 2015).

3 Industrial Production of Microbial Alginate: Challenges and Opportunities

3.1 Technical Challenges in Alginate Production from *A. vinelandii*

In aerobic industrial processes, limitation of oxygen during growth is a major technical problem. Increasing the oxygen transfer rate through increasing the agitation speed and/or the aeration rate is normally done. On the one hand, the stirrer

provides enough force to disperse the gas bubbles and increase their residence time in the medium. On the other hand, it provides homogeneity and mixing. Alginate production like many polysaccharides causes a drastic increase in viscosity. Moreover, alginate solution shows a non-Newtonian fluid behavior, in which the viscosity is shear rate dependent (Pandit et al. 2019). Whereas in lab-scale bioreactors turbulent flow condition is feasible by increased agitation, the high viscosities of polysaccharide fermentation broths make fully turbulent flow conditions impossible, or extremely difficult to achieve at large sale. During the growth of *A. vinelandii* in a bioreactor at microaerobic conditions, the rheological behavior of the broth changes from an "almost" Newtonian to a non-Newtonian behavior depending on the concentration of the polymer formed. In Newtonian fluids, it is manageable using reasonable techniques to relate operating variables (power number of the impeller, external power from the agitator, liquid density, impeller agitation speed, and impeller diameter) to power consumption and, hence, to the degree of agitation which proportionally affects the oxygen transfer rate. Hence an accurate control of the dissolved oxygen tension at an optimal value for polymer production can be achieved at the beginning of the fermentation. However, during alginate production, the broth behavior becomes non-Newtonian, and the apparent viscosities, which are not uniform within the fermentation vessel even at the same polymer concentrations, become extremely challenging for the process control. In such a solution, liquids-mixing intensity drops abruptly with distance from an impeller blade and, therefore, the apparent viscosities decrease at regions near the stirrer, while stagnant zones with higher apparent viscosities become near the vessel wall (Fig. 4). This rheological behavior of the alginate broth affects the oxygen transfer rates (k_La), the mixing characteristics of the fermentation, the power requirements, and scaling up. As a consequence, oxygen and nutrient limitations will mostly dominate near the bioreactor wall, while

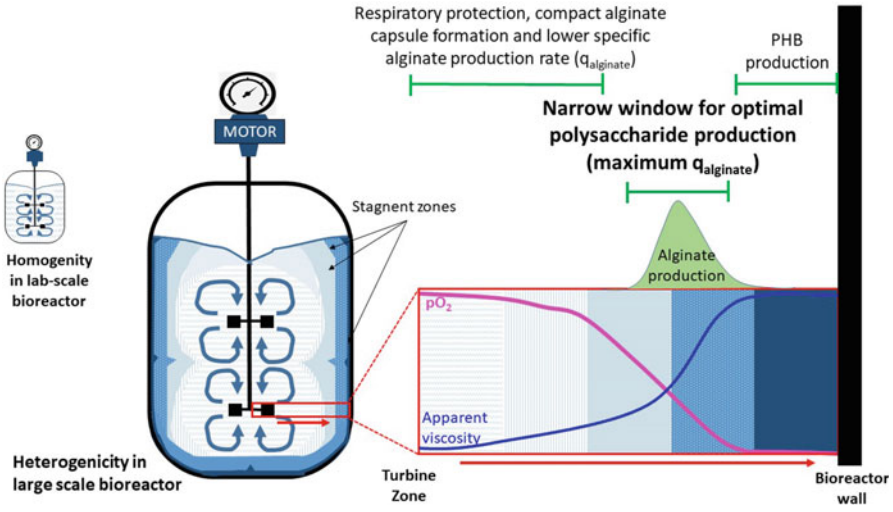


Fig. 4 Heterogeneities in viscous fermentation and challenges for the formation of alginate in large-scale bioreactors by *Azotobacter vinelandii*

oxygen and nutrient enrichments are to be found near the impeller zone. The insufficient mixing and mass transfer limitations with more gradient zones will escalate in industrial scale bioreactors (Fig. 4). Because of such heterogeneities, the reproducibilities in different scale bioreactors are challenging. Diaz- Barrera et al. (Diaz-Barrera et al. 2014) compared alginate production by *A. vinelandii* in two bioreactors with different scales (3 L and 14 L). While the two reactors had similar maximum oxygen transfer rates ($OTR_{max} = 19$ mmol L^{-1} h^{-1}) and produced the same alginate concentrations, the molecular weight of alginate obtained in the 3 L bioreactor was 1250 kDa compared to 590 kDa in the 14 L stirred fermenter (Diaz-Barrera et al. 2014).

Several studies have demonstrated that the G/M ratio, the molecular weight, and acetylation degree of alginates produced by *A. vinelandii* can be modified by the dissolved oxygen concentrations. In fact, a narrow range of dissolved oxygen tension, within the microaerobic range, is needed for the optimal alginate production with a defined quality (Fig. 4) (Castillo et al. 2020; Garcia et al. 2020; Pacheco-Leyva et al. 2016; Sabra et al. 1999, 2000, 2001; Sabra and Zeng 2009). Since the control of the dissolved oxygen tension (pO_2) at such a microaerobic range in a viscous pseudoplastic alginate broth is challenging, alternative process parameters (OTR, RQ) to monitor polymer production were investigated. Nevertheless, they have had limited success and were difficult to apply (Garcia et al. 2020; Sabra et al. 1999). Outside this narrow range for alginate production in *A. vinelandii, the cell* either exhibits a high-respiratory activity and hence wasting the C- source in respiration, or diverts the C-source to PHB synthesis at oxygen-limited conditions. In fact, this explains the complications associated with the biotechnological alginate production using *A. vinelandii*, and the additional risk accompanied by the process transfer to larger scales. It should be emphasized here that for other viable industrial polysaccharides processes like xanthan production by *Xanthomonas compestris* or gellan by *Sphingomonas* sp., such narrow ranges of pO_2 for the polymer production are lacking (Lobas et al. 1994; Peters et al. 1989).

Indeed, the deep understanding of bioreactor characteristics at different scales facilitates the successful development of any industrial processes. For the establishment of successful alginate fermentation processes using a productive *A. vinelandii* strain, a combination of strain improvements and process improvements should work in an integrated approach. While several studies on strain improvements and tailor-made alginate production by different mutants were intensively carried out in lab-scale bioreactors, process improvements in different bioreactors and scaling up studies are largely missing in the literatures. In fact, more fundamental and engineering studies are desired that deal with lowering the oxygen sensitivity of alginate production (Fig. 4), novel bioreactors, and bioreaction techniques for large-scale and long-term operation.

3.2 Nonpathogenic Strains of *Pseudomonas* as Promising Alginate Producers

The knowledge about biosynthesis of alginates was first gained in P. aeruginosa, which has been widely studied as a model organism for alginate production and biofilm formation in the lung, especially in patients suffering from the genetic

disease called cystic fibrosis (Rehm and Moradali 2018; Sabra et al. 2014). Although most studies have focused on clinical assays using *P. aeruginosa*, nonpathogenic species of the genus *Pseudomonas* were also studied for alginate production, and strains of *P. mendocina*, *P. syringae,* and *P. fluorescens* were shown to produce alginate under several growth conditions (Chang et al. 2007; Fan et al. 2019, 2020; Guo et al. 2012; Müller and Alegre 2006). *P. fluorescens*, a nonpathogenic plant-commensal strain was intensively investigated for alginate production by Ertestvag and her colleagues. While wild-type strains do not naturally produce alginate under laboratory conditions, the authors reported stable alginate production using mutant strains of *P. fluorescens* and reached up to 17 g/L alginate at a productivity of 0.35 gL^{-1} h^{-1} and a yield of 40% based on carbon (Gimmestad et al. 2014; Maleki et al. 2016). Unlike *A. vinelandii*, alginate production in *Pseudomonas* was constant within a wide range of pO_2 values. Such a difference is not surprising, especially because of the lack of respiratory protection in *Pseudomonas*. Nevertheless, alginates synthesized from pseudomonads lack the polyguluronic block structures normally found in *A. vinelandii* and in algae. It is well known that solutions of alginate polymers with the same molecular weights result in more viscous broth as the G/M ratio increases. While the low G/M ratio alginate polymers are undesirable in many industrial applications, their production process in bioreactors is easier to control with less high-viscosity-related upscaling problems.

Similar to A. *vinelandii*, the factors controlling alginate production and structure in *P. fluorescens* are well known, and different strains with different polymer characteristics can be obtained (Maleki et al. 2016, 2017). Gimmestad et al. (2003) produced a high-molecular weight polymannuronate polymer by using an epimerase negative strain of *P. fluorescens*. Such a polymer can be tailored for the production of particular alginate types for specialized applications by the action of different epimerases (Fig. 5). In fact, the in vitro use of different enzymes makes it possible to produce perfectly tailored alginate sequences not found in nature and thus improving polysaccharide performances for several applications. Recently, Gawin et al. (Gawin et al. 2020) used epimerase enzyme from *A. chroococcum* to introduce long G- blocks in the alginate strands. In general, alginates with a G-content of over 60% are highly desired for some medical and pharmaceutical applications. At the moment, such alginates with high G/M ratio can be extracted from the stem of *Laminaria hyperborean* which contains up to 70% G-content. However, the limited supply of such high G/M content alginates is currently limiting such applications. Interestingly, the alginate produced by the in vitro epimerization using the AlgE1 enzyme form *A. chroococcum* was reported to produce higher G-block polymers with a high degree of epimerization (up to 87%) (Gawin et al. 2020). This in vitro enzymatic approach to composition tailoring will definitely open the door for upgrading of various alginate samples into more valuable polymers.

Indeed, engineering more robust industrial strains to produce alginate at industrial scales is the future goal, especially due to the growing interest in synthetic biology, which has great potential for enhancing wide range of industrial applications. Beside the quality of alginate in terms of molecular weight and the G/M ratio, the stable production of the polymer in large-scale bioreactors should be taken as a critical factor for evaluating the alginate-producing bacterium. The use of a microbial host,

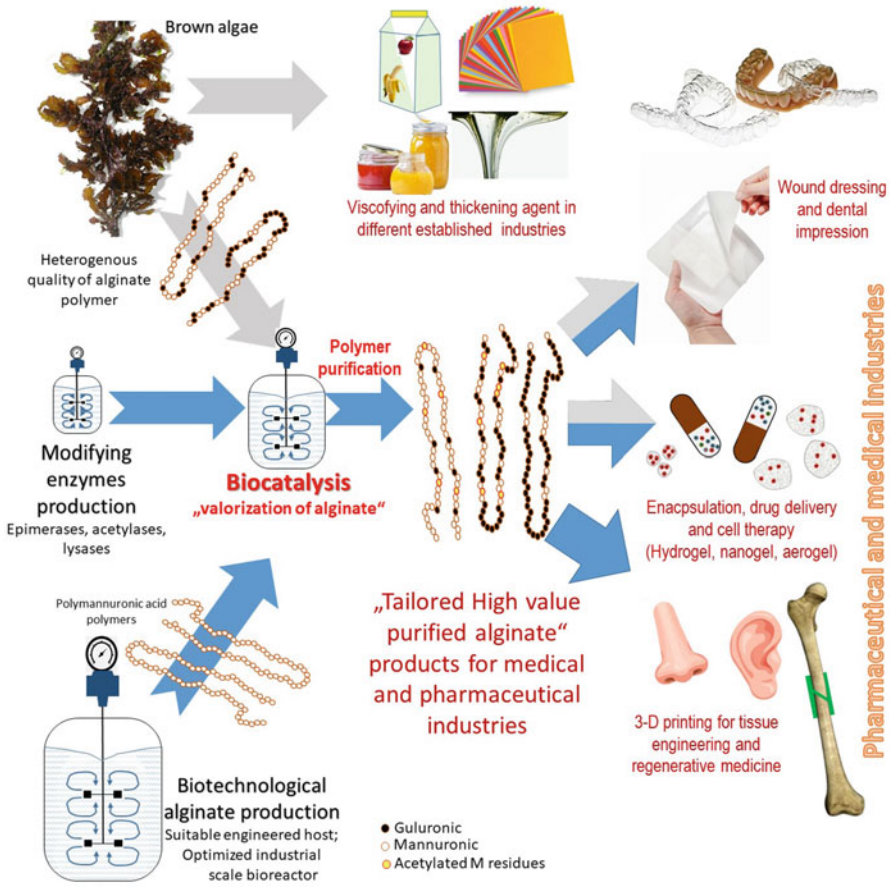


Fig. 5 Alginate production form brown seaweed are currently used in some established industries (shown in gray arrows). However, the seasonal variations, the sea current, the water temperature, and processing method are all factors that affect important structural features of the algal alginate produced, and hence heterogeneous qualities of alginate polymer are produced from brown algae. The advantage of using controlled growth conditions in bioreactors for bacterial alginate production, followed by the biocatalytic steps and valorizing alginate by the action of different modifying enzymes, will result in the formation of different high-value-tailored alginate products. This will open a new market for novel pharmaceutical and biomedical industries (shown in blue arrows)

whether the soil bacteria *A. vinelandii* or a nonpathogenic *Pseudomonas* strain, for alginate and/or high-value polymers production is clearly entering a new phase right now. The winner of such a race will definitely emerge in the next few years and will depend on developing a production process at the lowest possible cost. Designing new-generation bioprocesses that depend on engineering process-compatible microorganisms is the new roadmap. To achieve these goals, innovative fermentation process design that can reduce downstream-processing costs significantly, designing integrated systems with more fundamental knowledge about the control

mechanisms, and process dynamics in large bioreactor are needed. Only by developing cost-efficient processes through integration of fermentation in large-scale and downstream processing, the microbial production of alginates can fulfill their potentials as biotechnologically produced high-value chemicals. On this journey, chemical engineers, metabolic/genetic engineers, system biologists, and microbial physiologists will have to work all together in an integrated cooperative approach.

4 Applications of Alginate in Food, Pharmaceutical, and Biomedical Sectors

From the standpoint of the commercial use of alginate, three different criteria are important:

- Alginate's ability to form viscous solutions in aqueous media, which is in turn directly affected by the molecular weight, the number of M or G residues, and the solution strength. It is well known that the viscosity increases as the stiffness of the constituent chain blocks increases in the order MG<MM<GG, based on intramolecular steric hindrance.
- Gelation ability which can be induced by the presence of divalent ions, which cross-link the polymer chains through the "eggbox" model (Fig. 1), or by lowering the pH value below the pKa values of alginate monomers. Gel formation is thus dependent on the G/M ratio. Alginate rich in guluronate residues forms strong but brittle gels, while that rich in mannuronate residues is weaker but more flexible.
- For commercial applications of alginate, its polyelectrolyte nature is an important feature. The presence of carboxylic acid moieties in both monomer residues makes alginate a polyanion at neutral pH. The electrostatic repulsion present between the charged groups on the polymer chain increases with chain extension and affects the overall dimension in solution, which is then influenced by the presence of added supporting salts. A linear relationship was found between the intrinsic viscosity of alginate, as a polyanion, and the ionic strength (Smidsrod and Haug 1971).

Nowadays, more than 200 different grades of sodium alginate from different alginate suppliers (Tonnesen and Karlsen 2002) and with a wide range of molecular weight (33–400 kDa) and M/G ratios (ranging from 0.28 extracted from the stipe *of Lessonia trabeculata* to 3.17 from the seaweed *Durvillaea potatorum*) can be obtained commercially. This huge diversity in alginate polymers will definitely affect the critical physiochemical characters which has to be taken into consideration for the various commercial applications.

4.1 Alginate Applications in Food Sectors

Alginates are used in a number of large-scale industrial processes, mainly in the food, dye, and paper industries. Foods sold in the EU have had full ingredient

labeling since the mid-1980s. These include standard codes (E numbers) that accurately describe additives used in the production of food. As food additives, alginates are approved for use in EU and have been demonstrated as safe. As food ingredient, alginic acid is E400, while the sodium, potassium, ammonium, and calcium salts are E401–E404, respectively. Esters of alginic acid are also used as food additives and coded under E405. Though alginates have no nutritional value for human, they are used in food industries mainly as gelling and viscosifying agents. The availability of a wide range of polymers, with different viscosities, offers stability to foodstuffs under both high and low temperatures. This is particularly useful in the restructuring of foodstuffs that may become damaged or oxidized under high temperatures, such as meat products, fruits, and vegetables (Hay et al. 2013). Indeed, restructured foods can be produced in any shape or size, which allows for the production of foodstuffs that are of a more uniform or attractive esthetic nature (Longergan et al. 2019). The polymers are used in thickening agents in jams and juices, either on their own or as a blend with other thickening agents like pectin (Qin et al. 2018). While the viscosities of sodium alginate solutions are higher than those made from other thickening agents, they also display a remarkable shear-thinning effect, which is important in that the shearing applied during processing can lower the viscosity of the food mixture to assist in smoother flow (Qin et al. 2018). The hydrophilic nature of alginate aids retention of moisture and improves food texture, resulting in an improvement to the organoleptic qualities of the food products, thus improving consumer acceptance. Alginate polymers are also used as a carrier for protective coating of prepacked, cut, or prepared fruits and vegetables. These coatings act as effective carriers of antimicrobials for treating food surfaces which represent the typical point of entry of pathogens and likely the location of maximum microbial contamination. Ester of alginate, namely, propylene glycol alginate (PGA), is largely used as an emulsifying agent to maintain foam stability in brewing industry, in mousse and other desserts. As a water-soluble polysaccharide, alginate exhibits many of the physiological characteristics commonly associated with dietary fibers, such as laxation and stool bulking, while being indigestible in the human stomach and small intestine. The unique biochemical characteristics also offer alginate a range of novel physiological functions, which can be the basis for many new product opportunities in the future functional food industry.

4.2 Pharmaceutical and Biomedical Applications of Alginate

Alginate is a biodegradable hydrophilic polymer, nontoxic, nonimmunogenic, and biocompatible with body fluids, and therefore, for the past 40 years it has been one of the most widely used polymers in biomedical, bioengineering, and pharmaceutical industries without any considerable side effects. They have long been used for generating materials in dental impression. One of the early uses of alginate in biomedical sectors was its use in wound dressings (Barnett and Varley 1987). Alginate polymer maintains a moist microenvironment and allows evaporation of the exudates through the dressing while creating a barrier against different external

harmful microorganisms (Abasalizadeh et al. 2020). Alginate dressings can absorb 15–20 times their own weight in exudates and are manufactured in a range of products including flat sheets, ropes, and ribbons. Different polymers can be used in wound dressing; however, the hydrophilicity and the higher gel-forming characteristics of alginate gave it some more advantages over other natural materials. In fact, several alginate-based wound dressings are commercially produced by different companies (Rehm and Moradali 2018; Shakeel 2019; Szekalska et al. 2016). Furthermore, one of the backbones of the polymer, namely the ß-D-mannuronic acid, is considered as a nonsteroidal anti-inflammatory drug which has been tested in phase 1/2 clinical trial in ankylosing spondylitis patients (registered No. IRCT2013062213739N1) and in rheumatoid arthritis patients (registered No. IRCT2014011213739N2) without displaying neither side effects nor cardiovascular problems (Rehm and Moradali 2018). Alginates are also used to treat patients with gastroesophageal reflux disease. In fact, they were superior to placebo and antacid treatment (Leiman et al. 2017).

In addition, encapsulation is the major technique allowing the use of alginate in biotechnological, pharmaceutical, and biomedical fields. Alginate gels can be administrated or injected in a least invasive manner, and hence it is the most widely used biopolymer for cell encapsulation. Alginate polymers with different characteristics are satisfactorily applied in the immobilization of different types of enzymes, medication, biochemical compounds, and different kinds of microorganisms/cells. Encapsulation of probiotics within alginate has shown to protect probiotic bacteria through their passage in the upper digestive tract, and hence exert their positive effects in the host. In the stomach, and because of the low pH, the alginate capsule shrinks and produces a viscose acidic gel which does not release its encapsulated cells. Only at a higher pH milieu, in the intestinal tract, a disruption of the polymeric network causes drug/or cell dissolution and release. The encapsulation of therapeutically active cells is also used for drug delivery systems, which found various applications in the continuous production of hormones and growth factors (Abasalizadeh et al. 2020). Alginate-assisted cell therapy for the regeneration of diseased or damaged tissue is now widely recognized. Various studies were performed to deliver cells to a particular location, while maintaining their viability during the slow degradation of the polymer to release the cells into the surrounding tissue (Abasalizadeh et al. 2020). Hunt and coworkers studied the encapsulation of human embryonic stem cells in alginate hydrogels (Hunt and Grover 2010; Hunt et al. 2017). Their results indicated that the development of retinal tissue from human embryonic stem cells can be enhanced by culturing it in 0.5% alginate hydrogel modified with RGD peptide sequence (Arg-Gly-Asp). Hence, alginate gels have the potential to be used as 3D cell culture systems and as prosthetic materials for the regeneration of the cornea (Hunt et al. 2017).

Recently, alginates have been used in the 3D bioprinting and bioink technology (Abasalizadeh et al. 2020; Barrs et al. 2021; Gohl et al. 2018; Gonzalez-Fernandez et al. 2021; Jia et al. 2014; Markstedt et al. 2015). Bioink is commonly referred to the biomaterials which could encapsulate the cells and are printable into three dimensional scaffolds and tissue-like structures. This emerging technology is seen as an

alternative to the organ transplantation and expected to revolutionize the field of tissue engineering and regenerative medicine. Alginate-based bioinks demonstrated the therapeutic potential to fabricate living osteogenic tissue graft. Recently, bone marrow-derived mesenchymal stromal cells cultured in osteogenic media in alginate cross-linked with $CaCl_2$ constructs exhibited increased osteogenic differentiation compared to alg-$CaSO_4$, alginate-gelatin, and alginate-nanocellulose bioinks. This alg-$CaCl_2$ bioink was used to 3D print anatomically accurate cell-laden scaphoid bones that were capable of partial mineralization after 14 days of in vitro culture (Gonzalez-Fernandez et al. 2021). A 3D bioprint of human chondrocytes using a bioink consisting of cellulose nanofibrils, isolated from bacteria and alginate, was successfully done few years ago (Markstedt et al. 2015). Cell viability analysis of the 3D-printed bioink showed its compatibility and suitability for growth of cartilage tissue. Indeed, the bioprint technology points to a rapid development in the last few years. However, a major limitation of this technology is the development of hydrogels that provide the required fidelity for high-printing resolution while maintaining long-term biological performance like cell viability, proliferation, and differentiation under physiological conditions. Modification of the inert alginate polymer (with limited biodegradability) or combination of alginate with other natural and synthetic materials has resulted in bioinks with optimal viscosity and high cell viability postprinting (Jia et al. 2014; Gonzalez-Fernandez et al. 2021).

Furthermore, alginate-based drug delivery systems and their use as vehicles in cancer therapy have great potential (Abasalizadeh et al. 2020; Lopez-Mendez et al. 2020; Yallapu et al. 2011). The conventional cancer treatment using chemotherapy can mainly affect DNA synthesis and mitosis which results in the death of rapidly growing and dividing cancer cells. In fact, these severe side effects induced by the chemotherapeutics on normal tissue and organs are the major causes of the high mortality rate of cancer patients. In contrast to the conventional chemotherapeutic agents, nanoscale delivery systems using alginate hydrogel or "nanogel" have the potential to improve the treatment efficacy while avoiding systemic toxicity via enhanced permeability (Abasalizadeh et al. 2020; Yallapu et al. 2011). Recently, different hybrids of alginate and liposomes were tested as oral mucoadhesive drug delivery systems (Shtenberg et al. 2018). Alginate induces the adhesive property on the tongue and the local release of the drug, while liposome improves the absorption of the drug into the cells and preserves it from degradation. The hydrids tested were highly effective in promoting cancer cell death and, therefore, considered to have novel potential in oral cancer treatment (Shtenberg et al. 2018). Liu et al. (2017) designed an injectable thermoresponsive hydrogel hybrid of alginate and poly(N-isopropylacrylamide) as a drug delivery system that can be used in prostate cancer treatment. The system overcomes the multidrug resistance problem often observed in cancer treatment. Similarly, Sun et al. (2017) showed that keratin-alginate nanogels with a size of 120 nm loaded with doxorubicin hydrochloride, an anticancer drug, had a better antitumor effect and lower side effects compared to the free drug. It should be stressed here that the use of nanotechnology has added many advantages and will be able to improve the efficacy of therapeutic agents significantly further (Li et al. 2015). Inhalable alginate nanoparticles of a

size of 235 nm encapsulating antituberculosis drugs were used to treat mice with tuberculosis, and the chemotherapeutic efficacy of three doses of drug-loaded alginate nanoparticles nebulized 15 days apart was comparable with 45 daily doses of oral free drugs (Ahmad et al. 2005). Hence, nanogels loaded with various drugs have a broad range of applications in treating lung infections in the near future.

Moreover, alginate aerogels are recently trending and were used in the last few years in various biotechnological (Yang et al. 2021) and in pharmaceutical and biomedical research (Athamneh et al. 2021; Goncalves et al. 2016; Wu et al. 2021). Aerogels are derived from a gel where the liquid part of the gel has been replaced using gas. This replacement leads to the formation of a solid that has extremely low density and low-thermal conductivity. Unlike hydrogel, alginate-based aerogels are well known for their open-porous structure and the high surface area. Recently, Athamneh et al. (2021) were able to form aerogel microspheres, using either alginate or a blend of alginate-hyaluronic acid, in small diameter to reach the lower respiratory tract. These aerogel particles have potentials to be used in pulmonary drug delivery.

5 Conclusion

Alginate polymers have been traditionally used as thickeners, stabilizers, and viscosifiers in various food and cosmetics industries. These polymers have been processed in various forms as nanoparticles, nanotubes, microspheres, microcapsules, sponges, hydrogels, foams, elastomers, fibers, etc. Because of their nontoxicity, biocompatibility, biodegradability, and their unique physical properties, they have found their way into some advanced pharmaceutical and biomedical applications. Alginate polymers are used for healing wounds and bone injuries, for the regeneration of joint cartilage, scaffolds for cell growth, as carriers of drug, and other novel biomedical applications. Indeed, such high-value alginate polymers must be of unique physiochemical properties and thus defined compositions. In addition, the polymers used for biomedical applications must be of particularly pure quality, and the removal of impurities that trigger the immune response is critical here. In contrast to algal polymers, the modifiability of bacterial alginate through tailoring/chemical modifications and its production in controlled environment in bioreactors enables the production of high-value alginate polymers suitable for biomedical usage. Nevertheless, most of the novel applications mentioned above are algal alginate based, and bacterial alginates have remained largely unexplored for advanced biomedical uses. As shown in this review, there are still few steps ahead before an industrial process for microbial alginate becomes a reality. However, due to the growing market demand for high-value alginate polymers, the recent efforts in engineering robust industrial strains for alginate production in conjunction with the knowledge gained in recent years with alginate-modifying enzymes for the in vitro valorization of alginate are closing the gap toward a viable biotechnological process, which will likely emerge in the next few years.

References

Aarstad O, et al. Analysis of G-block distributions and their impact on gel properties of in vitro epimerized mannuronan. Biomacromolecules. 2013;14(10):3409–16.

Aarstad OA, et al. Biosynthesis and function of long guluronic acid-blocks in alginate produced by Azotobacter vinelandii. Biomacromolecules. 2019;20(4):1613–22.

Abasalizadeh F, et al. Alginate-based hydrogels as drug delivery vehicles in cancer treatment and their applications in wound dressing and 3D bioprinting. J Biol Eng. 2020;14:8.

Ahmad Z, Sharma S, Khuller GK. Inhalable alginate nanoparticles as antitubercular drug carriers against experimental tuberculosis. Int J Antimicrob Agents. 2005;26(4):298–303.

Athamneh T, et al. Pulmonary drug delivery with aerogels: engineering of alginate and alginate-hyaluronic acid microspheres. Pharm Dev Technol. 2021;26(5):509–21.

Barnett SE, Varley SJ. The effects of calcium alginate on wound healing. Ann R Coll Surg Engl. 1987;69(4):153–5.

Barrs RW, et al. Engineering a chemically defined hydrogel bioink for direct bioprinting of microvasculature. Biomacromolecules. 2021;22(2):275–88.

Bayer AS, et al. Oxygen-dependent up-regulation of mucoid exopolysaccharide (alginate) production in Pseudomonas aeruginosa. Infect Immun. 1990;58(5):1344–9.

Castillo T, Galindo E, Pena CF. The acetylation degree of alginates in Azotobacter vinelandii ATCC9046 is determined by dissolved oxygen and specific growth rate: studies in glucose-limited chemostat cultivations. J Ind Microbiol Biotechnol. 2013;40(7):715–23.

Castillo T, et al. Respiration in Azotobacter vinelandii and its relationship with the synthesis of biopolymers. Electron J Biotechnol. 2020;48:36–45.

Chang WS, et al. Alginate production by Pseudomonas putida creates a hydrated microenvironment and contributes to biofilm architecture and stress tolerance under water-limiting conditions. J Bacteriol. 2007;189(22):8290–9.

Diaz-Barrera A, et al. Production of alginate by Azotobacter vinelandii grown at two bioreactor scales under oxygen-limited conditions. Bioprocess Biosyst Eng. 2014;37(6):1133–40.

Ertesvag H. Alginate-modifying enzymes: biological roles and biotechnological uses. Front Microbiol. 2015;6:523.

Ertesvag H, et al. Identification of genes affecting alginate biosynthesis in Pseudomonas fluorescens by screening a transposon insertion library. BMC Genomics. 2017;18(1):11.

Fan X, et al. Enhanced synthesis of alginate oligosaccharides in Pseudomonas mendocina NK-01 by overexpressing MreB. 3 Biotech. 2019;9(9):344.

Fan X, et al. Genome reduction enhances production of polyhydroxyalkanoate and alginate oligosaccharide in Pseudomonas mendocina. Int J Biol Macromol. 2020;163:2023–31.

Flores C, et al. Role of oxygen in the polymerization and de-polymerization of alginate produced by Azotobacter vinelandii. J Chem Technol Biotechnol. 2015;90:356–65.

Galindo E, et al. Molecular and bioengineering strategies to improve alginate and polydydroxyalkanoate production by Azotobacter vinelandii. Microb Cell Factories. 2007;6:7.

Garcia A, et al. Molecular weight and viscosifying power of alginates produced by mutant strains of Azotobacter vinelandii under microaerophilic conditions. Biotechnol Rep (Amst). 2020;26:e00436.

Gawin A, et al. Functional characterization of three Azotobacter chroococcum alginate-modifying enzymes related to the Azotobacter vinelandii AlgE mannuronan C-5-epimerase family. Sci Rep. 2020;10(1):12470.

Gimmestad M, et al. The Pseudomonas fluorescens AlgG protein, but not its mannuronan C-5-epimerase activity, is needed for alginate polymer formation. J Bacteriol. 2003;185(12):3515–23.

Gimmestad M, et al. New mutant strains of Pseudomonas fluorescens and variants thereof, methods of production and use thereof for the production of alginate. European Patent. 2014:EP1543105.

Gohl J, et al. Simulations of 3D bioprinting: predicting bioprintability of nanofibrillar inks. Biofabrication. 2018;10(3):034105.

Goncalves VS, et al. Alginate-based hybrid aerogel microparticles for mucosal drug delivery. Eur J Pharm Biopharm. 2016;107:160–70.

Gonzalez-Fernandez T, et al. Alginate-based bioinks for 3D bioprinting and fabrication of anatomically accurate bone grafts. Tissue Eng Part A. 2021;27(17–18):1168–81.
Guo W, et al. Augmented production of alginate oligosaccharides by the Pseudomonas mendocina NK-01 mutant. Carbohydr Res. 2012;352:109–16.
Hay ID, et al. Microbial alginate production, modification and its applications. Microb Biotechnol. 2013;6(6):637–50.
Hay ID, et al. Genetics and regulation of bacterial alginate production. Environ Microbiol. 2014;16 (10):2997–3011.
Hunt NC, Grover LM. Cell encapsulation using biopolymer gels for regenerative medicine. Biotechnol Lett. 2010;32(6):733–42.
Hunt NC, et al. 3D culture of human pluripotent stem cells in RGD-alginate hydrogel improves retinal tissue development. Acta Biomater. 2017;49:329–43.
Jia J, et al. Engineering alginate as bioink for bioprinting. Acta Biomater. 2014;10(10):4323–31.
Leiman DA, et al. Alginate therapy is effective treatment for gastroesophageal reflux disease symptoms: a systematic review and meta-analysis. Dis Esophagus. 2017;30(2):1–8.
Leitao JH, Sa-Correia I. Oxygen-dependent upregulation of transcription of alginate genes algA, algC and algD in Pseudomonas aeruginosa. Res Microbiol. 1997;148(1):37–43.
Li Y, et al. Biodegradable polymer nanogels for drug/nucleic acid delivery. Chem Rev. 2015;115 (16):8564–608.
Liu M, et al. Injectable thermoresponsive hydrogel formed by alginate-g-poly(N-isopropylacrylamide) that releases doxorubicin-encapsulated micelles as a smart drug delivery system. ACS Appl Mater Interfaces. 2017;9(41):35673–82.
Lobas D, et al. Structure and physical properties of the extracellular polysaccharide PS-P4 produced by Sphingomonas paucimobilis P4 (DSM 6418). Carbohydr Res. 1994;251:303–13.
Longergan SM, Topel DG, Marple DN. Fresh and cured meat processing and preservation. In: Longergan SM, Topel DG, Marple DN, editors. The science of animal growth and meat technology. 2nd ed. Elsevier-Academic Press; 2019. p. 205–28.
Lopez-Mendez TB, et al. Cell microencapsulation technologies for sustained drug delivery: clinical trials and companies. Drug Discov Today. 2020;26(3):852–61.
Lotfy WA, et al. Expression of extracellular polysaccharides and proteins by clinical isolates of Pseudomonas aeruginosa in response to environmental conditions. Int Microbiol. 2018;21(3): 129–42.
Maleki S, et al. Alginate biosynthesis factories in Pseudomonas fluorescens: localization and correlation with alginate production level. Appl Environ Microbiol. 2016;82(4):1227–36.
Maleki S, et al. New insights into Pseudomonas fluorescens alginate biosynthesis relevant for the establishment of an efficient production process for microbial alginates. New Biotechnol. 2017;37(Pt A):2–8.
Markstedt K, et al. 3D bioprinting human chondrocytes with nanocellulose-alginate bioink for cartilage tissue engineering applications. Biomacromolecules. 2015;16(5):1489–96.
Müller JM, Alegre RM. Alginate production by Pseudomonas mendocina in a stirred draft fermenter. World J Microbiol Biotechnol. 2006;23(5):691.
Oelze J. Respiratory protection of nitrogenase in Azotobacter species: is a widely held hypothesis unequivocally supported by experimental evidence? FEMS Microbiol Rev. 2000;24(4):321–33.
Pacheco-Leyva I, Pezoa FG, Diaz-Barrera A. Alginate biosynthesis in Azotobacter vinelandii: overview of molecular mechanisms in connection with the oxygen availability. Int J Polym Sci. 2016;Article ID 2062360, 12.
Pandit P, Gayatri TN, Regubalan B. Alginates production, characterization and modification. In: AShakeel A, editor. Alginates: applications in the biomedical and food industries. Wiley Scrivener Publishing; 2019.
Peters HU, et al. The influence of agitation rate on xanthan production by Xanthomonas campestris. Biotechnol Bioeng. 1989;34(11):1393–7.
Qin Y, et al. Applications of alginate as a functional food ingredient. In: Grumezescu AM, Holban MA, editors. Biopolymers for food design. Academic Press; 2018. p. 409–29.
Rehm BH. In: Steinbüchel A, editor. Alginate: biology and applications, Microbiology monographs. Springer-Verlag Berlin Heidelberg; 2009.

Rehm BH, Moradali MF. In: Wang M, editor. Alginates and their biomedical applications, 11. Springer series in biomaterials science and engineering. Singapore: Springer Nature Singapore; 2018.

Rehm BH, Valla S. Bacterial alginates: biosynthesis and applications. Appl Microbiol Biotechnol. 1997;48:281–8.

Sabra W, Zeng AP. Microbial production of alginates: physiology and process aspects. In: Rehm BHA, editor. Alginates: biology and applications. Berlin: Springer Berlin Heidelberg; 2009. p. 153–73.

Sabra W, et al. Effect of phosphate and oxygen concentration on alginate production and stoichiometry of metabolism of Azotobacter vinelandii under microaerobic conditions. Appl Microbiol Biotechnol. 1999;52:773–80.

Sabra W, et al. Effect of oxygen on formation and structure of Azotobacter vinelandii alginate and its role in protecting nitrogenase. Appl Environ Microbiol. 2000;66(9):4037–44.

Sabra W, Zeng AP, Deckwer WD. Bacterial alginate: physiology, product quality and process aspects. Appl Microbiol Biotechnol. 2001;56(3–4):315–25.

Sabra W, Kim EJ, Zeng AP. Physiological responses of Pseudomonas aeruginosa PAO1 to oxidative stress in controlled microaerobic and aerobic cultures. Microbiology (Reading). 2002;148 (Pt 10):3195–202.

Sabra W, Haddad AM, Zeng AP. Comparative physiological study of the wild type and the small colony variant of Pseudomonas aeruginosa 20265 under controlled growth conditions. World J Microbiol Biotechnol. 2014;30(3):1027–36.

Shakeel A. In: Ahmed S, editor. Alginates: applications in the biomedical and food industries. Beverly: Wiley Scrivener Publishing; 2019.

Shtenberg Y, et al. Mucoadhesive alginate pastes with embedded liposomes for local oral drug delivery. Int J Biol Macromol. 2018;111:62–9.

Smidsrod O, Haug A. Estimation of the relative stiffness of the molecular chain in polyelectrolytes from measurements of viscosity at different ionic strengths. Biopolymers. 1971;10(7):1213–27.

Stanisci A, et al. Identification of a pivotal residue for determining the block structure-forming properties of alginate C-5 epimerases. ACS Omega. 2020;5(8):4352–61.

Sun Z, et al. Bio-responsive alginate-keratin composite nanogels with enhanced drug loading efficiency for cancer therapy. Carbohydr Polym. 2017;175:159–69.

Szekalska M, et al. Alginate: current use and future perspectives in pharmaceutical and biomedical applications. Int J Polym Sci. 2016;8:1–17.

Tonnesen HH, Karlsen J. Alginate in drug delivery systems. Drug Dev Ind Pharm. 2002;28(6):621–30.

Urtuvia V, et al. Bacterial alginate production: an overview of its biosynthesis and potential industrial production. World J Microbiol Biotechnol. 2017;33:198.

Wu XX, et al. Long-term antibacterial composite via alginate aerogel sustained release of antibiotics and Cu used for bone tissue bacteria infection. Int J Biol Macromol. 2021;167:1211–20.

Yallapu MM, Jaggi M, Xhauhan SC. Design and engineering of nanogels for cancer treatment. Drug Discov Today. 2011;16(9/10):457–63.

Yang Y, et al. Construction of a superhydrophobic sodium alginate aerogel for efficient oil absorption and emulsion separation. Langmuir. 2021;37(2):882–93.

Kefiran

6

N. Simonelli, N. Gagliarini, M. Medrano, J. A. Piermaria, and A. G. Abraham

Contents

N. Simonelli · N. Gagliarini · M. Medrano
Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA); Facultad de Ciencias Exactas, Universidad Nacional de La Plata – CONICET CCT La Plata – CIC, La Plata, Argentina

J. A. Piermaria · A. G. Abraham (✉)
Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA); Facultad de Ciencias Exactas, Universidad Nacional de La Plata – CONICET CCT La Plata – CIC, La Plata, Argentina

Área Bioquímica y Control de Alimentos – Facultad de Ciencias Exactas, Universidad Nacional de La Plata, La Plata, Argentina
e-mail: aga@biol.unlp.edu.ar

J. M. Oliveira et al. (eds.), *Polysaccharides of Microbial Origin*,
https://doi.org/10.1007/978-3-030-42215-8_6

Abstract

Kefiran, a high-molecular weight exopolysaccharide produced by microorganisms present in the kefir grains or in the fermented milk prepared with them, has displayed an increased attention on account of its physicochemical properties and biological activity. Several health-promoting properties of kefiran such as epithelium protection against toxigenic factors of *Bacillus cereus*, antitumoral activity, or bifidogenic activity have been reported. Furthermore, antimicrobial, antioxidant, or wound healing activities were also reported. Kefiran has a Newtonian behavior in diluted solutions but pseudoplastic at high concentrations and has promising technological application since it has the ability to increase viscosity and improve viscoelastic properties of acid milk gels, forms viscoelastic gels at low temperature, and is able to form transparent films with good mechanical properties as well as can contribute to emulsion stability. Kefiran or kefiran-whey proteins-based films are interesting matrix as carriers of probiotics. Kefiran cryogels were also considered as scaffold for tissue engineering or skin repair. Therefore, kefiran arise as a novel biopolymer with a wide range of applications in food and biomedical industries.

Keywords

Kefiran · Kefir · *Lactobacillus* · Health-promoting properties · Techno-functional properties

1 Introduction

Several polysaccharides obtained from food grade microorganisms are used in food industry as hydrocolloids to improve mouthfeel and texture attributes of foods. Among them, an important natural novel source of exopolysaccharides (EPS) is lactic acid bacteria (LAB), since a wide diversity of polysaccharides with different monomers composition, molecular weight (MW), structure, and functionalities can be obtained from different LAB strains or by a single strain just modifying the culture conditions (growth media, temperature, sugar source, etc.). Polysaccharides produced by LAB can be tightly associated to the bacterial cell surface forming a capsule (CPS) or can be released to the media and then are called exopolysaccharides (EPS). These extracellular polysaccharides can contribute to the organoleptic characteristics of the product and at the same time could have a beneficial health effect on their own (Abraham et al. 2010).

Lactic acid bacteria are able to produce EPS "in situ" during fermentation process. EPS from LAB application in the dairy food industry is well documented (fermented milks and cheeses, as well as the application in cereal-based products) although the use of these polymers as additive or ingredient in food formulation is still limited due to their low production as compared to other microbial gums. The use of LAB exopolysaccharides as an additive arises as a challenge to develop dairy

and nondairy foods systems with technically desirable functional characteristics and which in turn has a beneficial effect on health (Lynch et al. 2018).

Among bacterial EPS, an interesting alternative is the employment of kefiran, the polymer produced by LAB included in kefir grains. Kefiran is a water-soluble branched glucogalactan containing approximately equal amounts of D-glucose and D-galactose residues. It is present in kefir grain but also in kefir, the beverage obtained by fermentation of milk with them. Kefir grain can be considered a natural immobilized starter where, in a matrix composed of 4–6% protein and 8–10% polysaccharide, coexist in symbiotic association a complex community of lactic acid bacteria, acetic acid bacteria, and yeast (Bengoa et al. 2019). These grains are whitish globular structures, of variable size (0.1–4.0 cm), similar to cauliflower, which are traditionally used in the production of fermented milk called kefir. Kefir is an acid, slightly carbonated drink and has variable viscous characteristics depending on the fermentation conditions. Kefiran is also present in kefir (fermented milk) in concentration ranging from 100 to 300 mg/L contributing to the health-promoting properties ascribed to this ancient fermented milk (Enikeev 2012; Bengoa et al. 2019).

Kefir grains represent a natural source of a food grade polysaccharide that can be used as food additive since kefiran can be obtained from grains with methods compatible to the food industry; it has good techno-functional properties but also kefiran intake has claimed to have health-promoting properties. Moreover, application in biomedical industries is also considered (Abraham et al. 2010; Moradi and Kalanpour 2019).

In the present chapter, a travel from the first reports on kefiran production, structure, and the microorganisms that are responsible for its production, to the novel techno-functional and healthy properties will be conducted. Along the chapter, it is expected to discover why kefiran became an interesting candidate as a novel EPS not only applicable to food but also to biomedical uses.

2 Kefiran from Kefir Grains and Kefir

Kefiran, the polysaccharide present in kefir grains, is a water-soluble branched glucogalactan containing equal amounts of D-glucose and D-galactose. It is produced by the complex microbiota included into kefir grains and turns into one of the main components of the grain matrix. Kefir grain is composed of protein, water, and kefiran (La Rivière et al. 1967). Kefiran content in the grain depends on the growth media used to obtain biomass being higher in milk or whey than in soymilk (Rimada and Abraham 2001; Bengoa et al. 2019).

Kefiran is also released to the milk during fermentation contributing to kefir viscosity (Bengoa et al. 2019). Kefiran concentration in milk is about 250 mg/L, being the production affected by time of fermentation, growth temperature, agitation rate, and the nutrient sources (Zajšek et al. 2013; Blandón et al. 2018). Otherwise, kefiran is also produced during whey fermentation in a temperature-depending manner (Rimada and Abraham 2003; Londero et al. 2012). The optimum temperature for kefiran production by kefir grains was about 30 °C, while the rise of

temperature increased soluble EPS in culturing media by means of decrease in kefir grain biomass yields (Rimada and Abraham 2001; Ghasemlou et al. 2012).

The knowledge of the conditions to improve kefiran production in kefir or fermented whey is relevant since its contents would determine the rheological properties and contribute to health benefits of the product. The amount of kefiran in the fermented products is low compared to lactose or protein content. Selection of an adequate EPS isolation method is a critical step for accurate EPS quantification since the value obtained strongly depends on the methodology used. The highest recovery of kefiran from fermented milk or whey is obtained when samples are heated as a first step of isolation procedure. Protein precipitation with TCA (trichloroacetic acid) is not recommended since EPS may co-precipitate with protein leading to lower EPS concentration. Methods that contained one or two steps of ethanol precipitation followed by dialysis or direct dialysis gave the highest values of polysaccharide concentration (Rimada and Abraham 2003) as well as pre-hydrolysis of lactose in the samples previous EPS precipitation was proposed in order to avoid dialysis (Enikeev 2012).

3 Kefiran Structure

The first report on kefiran chemical structure obtained from kefir grain was in 1967 by La Rivière et al. (1967). They studied kefir grain matrix composition and informed that the polysaccharide which has a negative iodine reaction dissolved slowly in cold water and rapidly in hot water, forming a highly viscous solution and after hydrolysis lead to obtaining equimolar amounts of D-glucose and D-galactose. Kooiman (1968) by using a β-D-(1→6) glucanase was able to fragment the polysaccharide, into glucose residues and a pentasaccharide. The structure of the repeating unit (kefirose) was elucidated by hydrolysis and methylation analysis. It is composed of a pentasaccharide lineal unit (β D Glup(1–2(6)) β Dgalp (1–4) α Dgalp(1–3)β D galp (1–4)D glu) to which one or two sugar residues are linked to the first β D gal by (1–6 (2)) glycosidic linkage (Kooiman 1968). Additional methylation/hydrolysis studies showed that kefiran presents a more complex structure indicating microheterogeneity suggesting that 6-O-substituted galactose in kefiran may be present while the grain is growing (Mukai et al. 1988). It is important to note that kefiran obtained from grains grown in whey has the same structure as the one proposed by Koimann (Ghasemlou et al. 2012).

4 Kefiran from Microorganisms Isolated from Kefir Grains

Several efforts have been directed towards the isolation and identification of kefiran-producing microorganisms from kefir grains since the first report in 1967. Kefiran biosynthesis was first ascribed to *Levilactobacillus brevis* (formerly *Lactobacillus brevis*) (La Rivière et al. 1967) and later to *Lentilactobacillus kefiri* (formerly *Lactobacillus kefiri*) (Toba et al. 1987) both species taxonomically closely related. Later it was demonstrated that *L. kefiri* was not a kefiran producer and that

polysaccharides were produced by homofermentative lactobacilli which were classified as *Lactobacillus kefiranofaciens* (Yokoi et al. 1991; Wang et al. 2008). Nowadays, kefiran production is associated to *Lactobacillus kefiranofaciens* which was reclassified as *L. kefiranofaciens* subsp *kefiranofaciens* (Vancanneyt et al. 2004).

Methylation analysis and ^{13}C-NMR (nuclear magnetic resonance) methodologies have been used to verify if the EPS produced by isolated bacterial strains was kefiran. The polysaccharides produced by *Lactobacillus* sp. KPB-167B (Yokoi et al. 1991), *L. kefiranofaciens* ZW3 (Wang et al. 2008), or *L. kefiranofaciens* WT-2BT in a liquid medium containing a rice hydrolysate (Maeda et al. 2004a) were found to be similar to kefiran. However, nonkefiran-producing *L. kefiranofaciens* strains were also reported such as *L. kefiranofaciens* 1P3, isolated from Brazilian kefir grain that produces an α-glucan instead of kefiran in the presence of sucrose (de Paiva et al. 2016) or *L. kefiranofaciens* DN1 that produces an EPS composed of mannose, arabinose, glucose, galactose, and rhamnose when the strain was grown in MRS broth (Jeong et al. 2017).

Other EPS-producing microorganisms were also isolated from kefir and they were described to produce EPS similar to kefiran such as *Lactobacillus* LM17 related to *L. casei* group (Micheli et al. 1999), while other microorganisms from kefir produce EPS with different structures such as *Lactiplantibacillus plantarum* (formerly *Lactobacillusplantarum*) CIDCA 8327 that produces an α-glucan in milk (Gangoiti et al. 2017) or *L. plantarum* YW11 that produces an EPS composed of glucose and galactose with possible presence of *N*-acetylated sugar residues (Wang et al. 2015). Though *L. delbrueckii* subsp. *bulgaricus* (Frengova et al. 2002) and *Lacticaseibacillus paracasei* (formerly *Lactobacillus paracasei*) (Bengoa et al. 2018) isolated from kefir grains produce EPS whose structure is not still elucidated.

Improvement of kefiran yields was also subject of research. Whey-based media have been found to be optimum for kefiran production as well as medium containing wine or alcohol or sago starch (Yokoi et al. 1990). Kefiran production is associated to growth and to high concentration of lactic acid production. As lactic acid inhibits the growth of *L. kefiranofaciens*, controlling this variable allows *L. kefiranofaciens* to consume sugars and increment kefiran production (Cheirsilp et al. 2018). One of the strategies used to improve kefiran production is to grow *L. kefiranofaciens* strains with yeast. The stimulation of kefiran production was successfully performed by the mixed culture with *Saccharomyces* sp. since the lactic acid produced by the *Lactobacillus* is consumed by *S. cerevisiae* and also provides nitrogen source to the *Lactobacillus* (Cheirsilp et al. 2003). Co-culture of *L. kefiranofaciens* with the yeast *Torulaspora delbrueckii* also leads to an improvement on kefiran production (Mitsue et al. 1999).

5 Kefiran Production for Industrial Application

From a technological point of view, an important requirement for the large-scale production of a microbial polysaccharide is to produce high polysaccharide yields in an inexpensive culture medium. In this direction, it is observed that kefiran obtaining

from kefir grain is an alternative to industrial production since it can be extracted/purified with a method compatible to food industry with high yields. The method applied to obtain kefiran from grain is based on its solubility properties by using heat treatment and ethanol precipitation where kefiran is obtained with high purity (greater that 99%) (Piermaria et al. 2008) or by simple heating in boiling water, sterilization at 121 °C, and centrifugation where the obtained solution contains kefiran with purity higher than 90% (Piermaria et al. 2015). Furthermore, kefir biomass can be produced in whey obtaining a growth similar to the growth obtained in milk (Rimada and Abraham 2001; Londero et al. 2012) being an interesting alternative for kefiran production for food/industrial application.

In order to improve kefiran production for industrial application by isolated microorganisms, Dailin et al. (2016) focused their research on maximizing the production of kefiran in semi-industrial scale. They optimized the growth condition in shake flask level and then the process was scaled up to a pilot scale 16-L stirred tank bioreactor under uncontrolled pH conditions. Kefiran production in the bioreactor was higher (1.91 g/L) than flask cultivation (1.29 g/L) being a first approach to industrial kefiran production from *L. kefiranofaciens.*

6 Biological Activity of Kefiran

Several studies on the biological and medical activities of kefiran have been published. Kefiran has been shown to exhibit antimicrobial, bifidogenic, immunomodulatory, antitumoral, and other different health-promoting features that will be discussed herein. The main biological activities are summarized in Fig. 1.

6.1 Antimicrobial Activity

Regarding its effect against pathogens, kefiran is capable of inhibiting various bacterial strains that are common inducers of human disease. An in vitro susceptibility study conducted with kefiran extracted from Portuguese kefir grains demonstrated that it was capable of inhibiting the growth of seven bacteria and yeast, these being *Streptococcus pyogenes, Staphylococcus aureus, Streptococcus salivarius, Salmonella typhimurium, Candida albicans and Listeria monocytogenes, Pseudomonas aeruginosa,* and *Escherichia coli* (Rodrigues et al. 2005a). According to Hasheminya and Dehghannya (2020), kefiran presented considerable inhibition capacity against gram positive bacteria (*Staphylococcus aureus* and *Streptococcus faecalis*) as well as gram negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*); however, antimicrobial mechanism of action has not yet been described. Some findings on this subject proposed that several possible mechanisms could explain EPS antibacterial activity, such as cytoplasmic-membrane permeabilization due to pore formation and cell wall disruption, leading to protein dissolution and leakage of molecules essential for cell survival (Barbosa et al. 2011).

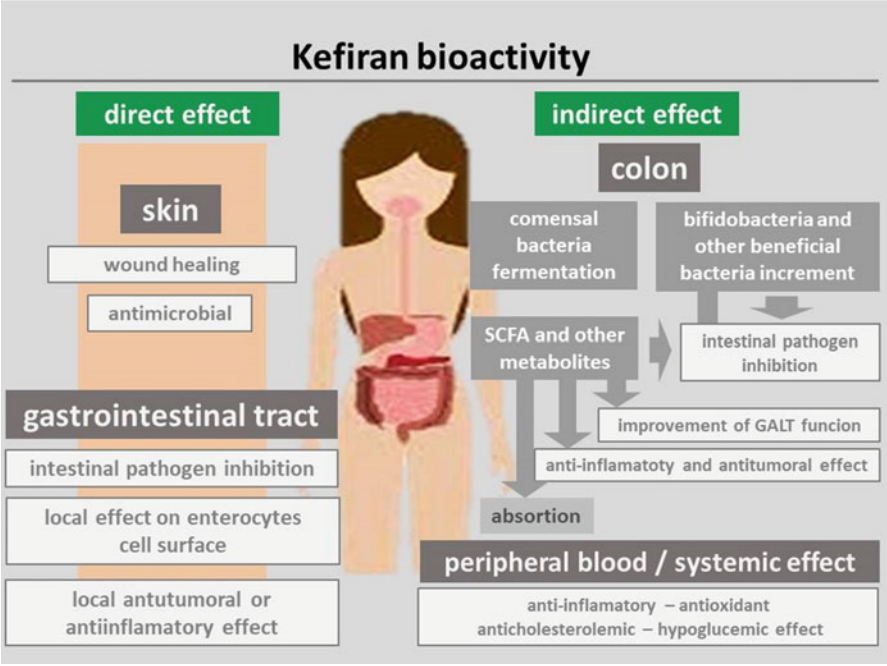


Fig. 1 Health-promoting properties of kefiran. Kefiran can exert biological effect by direct interaction with epithelial cells (gut or skin) or by modifying gut environment leading to bioactive metabolites that cross talk with the host cells

Moreover, it was demonstrated that kefiran can antagonize intestinal pathogens action. Interaction between kefiran and pathogenic *Bacillus cereus* was studied using a Caco-2 cells in vitro model for human enterocytes. Main cytopathic effects induced by *B. cereus* are necrosis, cytoskeleton disorganization, and microvilli effacement. At a concentration of 500 mg/L, kefiran (obtained from kefir grains) was able to diminish cell damage triggered by *B. cereus* extracellular factors to Caco-2 cells, by lowering cytotoxicity, preserving mitochondrial activity, reducing cell death processes, and diminishing pore formation, both induced by vegetative cells (Medrano et al. 2009) or by their extracellular factors (Medrano et al. 2008).

6.2 Bifidogenic Activity

Bifidobacterium represents, along with *Lactobacillus*, one of the best-known health-promoting genus that inhabit the human gut. Kefiran also has proved to enhance the number of intestinal bifidobacteria in orally fed mice after 2 and 7 days of exopolysaccharide consumption (Hamet et al. 2016). Balb/c mice were administered with kefiran at a concentration of 300 mg/L in their drinking water (daily intake 0.75–1 mg kefiran per animal per day). Bifidobacteria relative abundance in colon

and fecal samples showed an increase compared to the control group, which is an important finding regarding the potential kefiran prebiotic effect. An in vitro approach to the ability of the *Bifidobacterium bifidum* PRL2010 strain to grow in a culture medium containing kefiran was also performed (Serafini et al. 2014). Several studies have shown the relevance of bifidobacteria in the barrier effect against pathogens, in the regulation of immune response and in general intestinal homeostasis (O'Callaghan and van Sinderen 2016).

6.3 Modulation of Immune and Inflammatory Response

Kefiran has played an important role in inflammatory regulatory processes. Furuno and Nakanishi (2012) studied the anti-inflammatory effect of kefiran on bone marrow-derived mast cells (BMMCs) from BALB/c mice, analyzing its ability to suppress antigen-induced mast cell activation. They found that kefiran has a suppressive effect on mast cell function, by inhibiting degranulation and cytokine production. The exact molecular mechanisms by which kefiran diminishes mast cell activation remain unclear, but kefiran is likely to suppress intracellular signaling pathways, including PI3K-Akt and ERKs, by inhibiting their phosphorylation.

The molecular mechanism of chronic inflammatory processes involves the interplay of a number of proinflammatory cytokines, chemokines, adhesion molecules, inflammatory mediators, and oxygen free radicals (Bhattacharyya et al. 2014). In this sense, finding ways to prevent oxidative stress is highly relevant in current studies. Radhouani et al. (2018a) assessed antioxidant properties of kefiran in concentrations ranging between 0.5% and 1% w/v and discovered that this EPS presents a remarkable reducing power, as well as significant hydroxyl radical scavenging activity. Kefiran can play an interesting anti-inflammatory role by removing nitric oxide which, in pathological processes, behaves like a cytotoxic mediator, in particular when it involves an inflammatory disease (Radhouani et al. 2018a). When compared to the gold standard biomaterial hyaluronic acid (HA), kefiran demonstrated to have an even higher NO scavenging power than HA, which is greatly relevant due to its potential pharmaceutical application.

Jenab et al. (2020) studied cytokine (IL-6) production of peripheral blood mononuclear cells (PBMC) treated with kefiran. They found that kefiran upregulated IL-6 secretion at concentrations of 5 and 1 mg/mL. This is in agreement with previous results, such as Vinderola et al. (2006), who followed the production of cytokines and changes in the immunoglobulin profile after oral administration of kefiran at a concentration of 100 mg/kg of body weight/day for 2, 5, or 7 consecutive days. Kefiran reported an increase in IgA+ cells in the lamina propria of the small and large intestine, with no change in the number of IgG+ cells in the small intestine. Overall, it was observed that the exopolysaccharide not only induced a gut mucosal response but was also able to up- and downregulate it for protective immunity, maintaining intestinal homeostasis, increasing IgA production both at the level of small and large intestine and influencing systemic immunity through cytokines released to circulating blood. Consistent with the above results, kefiran was shown to induce changes in

the balance of immune cells in a murine model (Medrano et al. 2011). Balb/c mice were administered with kefiran at 300 mg/L ad libitum in drinking water for 2 or 7 consecutive days. An increase in goblet cells was found in the villus of the kefiran-treated mice, indicating a stimulation of mucin-producing cell proliferation and crypt cell differentiation that could lead to a mucus-rich environment. Along with the augmentation of IgA+ cells, this could represent a great contribution to the defense of the epithelium. As regards to the increase of peritoneal macrophages, it reveals that the induction of the immune response by kefiran in the intestinal environment leads to a systemic effect that modulates the response in different locations.

Also in reference to immunological pathologies, Kwon et al. (2008) made an interesting finding on the effect of kefiran on one of the best-known allergic conditions, asthma. In the study cited, intragastric administration of kefiran (50 mg/kg) in a murine asthma model effectively reduced ovalbumin-induced cytokine production, pulmonary eosinophilia, mucus hypersecretion, and airway hyper-responsiveness (AHR). These findings suggest that kefiran may have the potential to block the inflammation and remodeling of the airways in patients with allergic asthma.

6.4 Antitumoral

Antitumoral activity was, perhaps, one of the first biological properties studied for kefiran. Shiomi et al. (1982) studied the inhibition of allogenic solid tumor growth (Ehrlich carcinoma, EC, and sarcoma, S-180) in ddY mice and SLC-ICR mice. They administered a two-molecular-weight kefiran mixture obtained from kefir grains to mice in drinking water and intraperitoneally, from day 7 until tumor excision. They found that the growth of EC and S-180 solid tumor was inhibited by 40–59% and 21–81%, respectively, by oral and intraperitoneal administration of kefiran. As the performed in vitro controls showed no cytotoxicity of kefiran on both mesodermal tumoral cell lines, these authors concluded that the antitumoral effect of kefiran could be host-mediated. Another approach to antitumoral activity of kefiran was conducted by Murofushi et al. (1983) who demonstrated that kefiran treatment (drinking water or by intragastric administration) caused an increase in the response of delayed-type hypersensitivity (DTH) induced by picryl chloride in intact mice and also tumor-bearing mice. A significant correlation between DTH response and antitumor activity was observed in intact mice. However, up to the moment, mechanism of action of kefiran has not been elucidated and further studies are required to understand its effect.

For many years, the antitumoral activity of kefiran was not approached again until recently, when some in vitro studies regained interest in this under-explored bioactivity. The cytotoxic activity (MTT assay) of kefiran produced by the strain *L. kefiranofaciens* ATCC 8007 was studied in cervical cancer (HeLa) and liver cancer (HepG2) cell cultures (Elsayed et al. 2017). Kefiran significantly decreased cell viability, in a concentration dependent way. More recently, the antiproliferative effect on breast cancer cells (MCF-7 breast cancer cell line) was studied by

mitochondrial activity measurement (MTT assay) by Jenab et al. (2020). These authors used nanofibers from kefiran obtained from grain and found a diminishment of mitochondrial activity after 48 h of cell treatment, in all the concentrations assayed (0.5–4 mg/mL).

6.5 Other Biological Activities

Other systemic and healthy effects were studied in different in vivo models. Maeda et al. (2004a) studied the effect of kefiran on hypertension, lipid and glucose content in the blood (cholesterol and diabetes models), and constipation. Using a model of stroke-prone spontaneously hypertensive rats (SHRSP), the kefiran-treated group showed a diminishment of arterial blood pressure and a lower concentration of lipids in the liver and blood, compared to control rats. In genetically diabetic mice (KKAy), after 30 days of kefiran treatment, blood glucose levels were significantly lower than the control group. In Sprague-Dawley (SD) rats with induced constipation, humidity and fecal weight were increased in a dose-dependent kefiran consumption. Additionally, some experiments with rabbits suggested that kefiran could prevent the onset and increase of atherosclerosis, due to its anti-inflammatory and antioxidant properties (Uchida et al. 2010). In such study, it was observed that the animals treated with kefiran showed less aortic atherosclerotic lesions and lower levels of cholesterol in the liver.

The regenerative activities were described by Rodrigues et al. (2005b), who found that kefiran was able to reduce the inflammatory process of granuloma formation in Wistar male rats, administered orally with 1 g/L kefiran. Elsayed et al. (2017) proved that morphological and developmental characteristics of kefiran-treated wild and transgenic zebrafish embryos showed no abnormalities. In recent studies, kefiran obtained from Portuguese kefir grains improved the viability and also the metabolic activity of human adipose-derived stem cells and presented no cytotoxic effect on them (Radhouani et al. 2018a). Moreover, the same research group evaluated cell growth, proliferation, and damage analysis in a mouse L929 fibroblastic cell line, in the presence of kefiran (10 g/L). L929 cells showed to be metabolic active for the duration of the experiment and, more interestingly, the cells proliferated, as indicated by an increase in levels of metabolic activity over time (Radhouani et al. 2018b).

7 Physicochemical and Functional Properties of Kefiran

When a polysaccharide is going to be included in a food matrix, it is not only important to know its biological effects, but it is also important to evaluate its functional properties, since it will modify the characteristics of the food into which it is incorporated, affecting its texture, palatability, flavor. Kefiran techno-functional properties will be discussed and are resumed in Fig. 2.

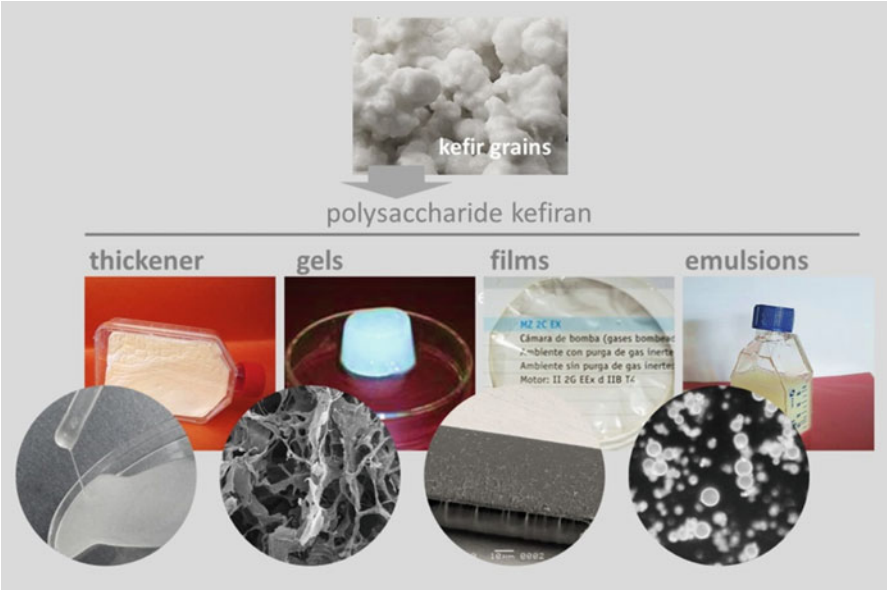


Fig. 2 Techno-functional properties of kefiran. The aspect of lyophilized kefiran, viscosity of kefiran solution, as well as self-supporting cryogels and edible transparent films can be observed

7.1 Kefiran as Thickener Agent

Kefiran can be solubilized or dispersed in water, leading the system to an increase in its viscosity. Kefiran solution containing 8 g/L has a viscosity up to 20 times higher than water. At concentrations below 1 g/L, a Newtonian behavior is observed, and at higher concentrations, a shear thinning flow is obtained similar than other representative neutral gums. A decreasing flow index is observed with the increase in polysaccharides concentration without presenting hysteresis area in the respective curves (Piermaria et al. 2009, 2016; Pop Rodica et al. 2016). In concordance, Exarhopoulos et al. (2018) studied flow properties of kefiran aqueous solutions with concentrations ranging from 2.5 to 40 g/L and found that the flow behavior of kefiran systems in solution is that of a pseudoplastic fluid at low shear rates, whereas at high shear rates is that of a Newtonian one.

The relationship of this polysaccharide with other macromolecules in its role as a thickener was evidenced in acid milk gels. Kefiran can contribute to the viscosity of acidified milk matrices when it is independently incorporated as well as when it is produced in situ by the microorganism responsible for acidification. Kefiran enhances the rheological properties of chemically acidified skim milk gels increasing their apparent viscosity and the elastic (G') and viscous (G'') modulus. The addition of 0.5 g/L of kefiran led to viscosities five times higher (50 mPa.s) than those obtained without the addition of EPS and was improved by the previous heat treatment usually applied for yogurts manufacture (Rimada and Abraham 2006).

7.2 Kefiran Gels

Kefiran produced a marked impact on the rheological characteristics of aqueous solutions when the cryo-freezing process is carried out. Freezing cause a forced alignment of kefiran molecules that may induce side-by-side associations of polymer chains, which then remain intact on thawing. After freeze-thaw treatment, the rheological behavior changes from a liquid entangled network system to a gel structure forming a cryogel. Kefiran can form cryogels from aqueous solutions containing about 5 g/L or more of the polysaccharide after only one freeze–thaw cycle. Higher polysaccharide concentrations lead to gels with a greater number of interactions, increasing the elastic character of the matrix. The high molecular weight of kefiran may be one of the chemical properties related to the ability to form cryogels. Kefiran cryogels lose their structure at above 37 °C determining its ability to melt at mouth temperature, being relevant for its application in food formulations (Piermaria et al. 2008). An interesting aspect about kefiran gels is that they are formed in one freeze–thaw cycle and at a concentration as low as 10 g/L (1%); meanwhile β-glucan cryogels, at the same concentration, need at least three freeze–thaw cycles to obtain gels with similar viscoelastic characteristics (Lazaridou et al. 2004).

The microstructure of kefiran gels can be modified by including mono- or disaccharides in their formulation. Kefiran maintain its ability to form gels in the presence of sucrose or fructose at a concentration up to 50.0% using only a single cryogenic treatment. The molecules of sucrose and fructose contribute to water retention of gels and impact differentially on their rheological characteristics. Since these sugars provide easily interchangeable linkages, they would act as a type of plasticizer to interfere with the formation of hydrogen bonds between the polysaccharide molecules, thus modifying the viscoelasticity of the gel (Zavala et al. 2015). Kefiran can also form weak gels when added to k-carrageenan and can form gels in aqueous solutions containing ethanol (see review Abraham et al. 2010).

Incorporation of kefiran to whey protein gels lead to important changes in their rheological and microstructural properties increasing gel strength and producing a more compact microstructure (Kazazi et al. 2017).

7.3 Kefiran-Based Films

In recent years, interest in edible film matrix has been growing, especially those which are capable of transporting drugs, nutrients, or probiotic microorganisms. The main challenge of current developments is to achieve matrices capable of protecting drugs or microorganisms during their passage through the gastrointestinal tract and their controlled release. Kefiran can form edible transparent films that are brittle and rigid. The addition of glycerol allows obtaining matrix with lower water vapor permeability, and extraordinary flexibility, even higher than those corresponding to low density polyethylene (Piermaria et al. 2009). Sugars and polyols are compatible with kefiran and can be used as plasticizers. According to Fourier-Transform infrared (FT-IR) spectroscopy studies, these molecules remain within kefiran structure disrupting its architecture. Glycerol allowed obtaining films with good mechanical

Table 1 Summary of various combinations of macromolecules with kefiran for their incorporation in edible films and their applications

Main film components	Applications	References
Starch/kefiran/ZnO	UV-protective packaging film	Babaei-Ghazvini et al. (2018)
Kefiran/Al_2O_3	Bionanocomposite film for food packaging applications	Moradi et al. (2019)
Kefiran/oleic acid	Improve barrier properties of kefiran films	Ghasemlou et al. (2011)
Kefiran/WPI/clay		Zolfi et al. (2015)
Kefiran/WPI/TiO_2	Food packaging and drug-delivery system	Zolfi et al. (2014)
Kefiran/chitosan	Antioxidant activity in food packaging	Sabaghi et al. (2015)
Kefiran/nano ZnO	UV protection	Shahabi-Ghahfarrokhi et al. (2015a)
Kefiran/corn starch		Motedayen et al. (2013)
Kefiran/nanocellulose	Use industrial residues to make an ecofriendly bionanocomposite film for food packaging	Shahabi-Ghahfarrokhi et al. (2015b)

properties and glucose, which leads to films with lower water vapor permeability (Piermaria et al. 2011).

Kefiran-based films have demonstrated being an effective vehicle for the delivery of probiotic microorganisms, both as the main component of the matrix (Piermaria et al. 2015) and in combination with others macromolecules like proteins (Gagliarini et al. 2019).

Otherwise, kefiran composite films were formulated in order to widen the range of functionalities (Table 1).

7.4 Kefiran in Emulsions

Kefiran was tested in oil-in-water emulsions in which whey proteins were used as surfactants. In these emulsions, the thickener character of this polysaccharide was observed. As the content of kefiran in the emulsion increased, a higher flow resistance was found that led to higher viscosity values for all the evaluated shear rates. Furthermore, the incorporation of kefiran in the emulsions produced a transition from a predominantly viscous behavior to a predominantly elastic behavior and it increased with increasing kefiran content. The possibility of incorporating this polysaccharide in emulsified systems represents an interesting potential, considering also its prebiotic effect and its health-promoting actions (Piermaria et al. 2020).

8 Biomedical Application of Kefiran

The most studied field of application of kefiran was those related to food applications such as a food ingredient or additive or to include it in coating and films to extend shelf life and increase quality of food reducing environmental impact. However, the

amazing heath promoting and techno-functional properties of this EPS as well as its biocompatibility and biodegradability gained attention for biomedical and nanotechnology applications (Moradi and Kalanpour 2019).

As was previously described, antimicrobial and healing activity of kefiran suggested that kefiran could be used for skin protection. Cicatrizing experiments in animal model using 70% kefir gel had a protective effect on skin connective tissue enhancing wound healing (Rodrigues et al. 2005a). Kefiran has also antioxidant performance against reactive oxygen species suggesting that would provide cell protection to oxidative stress (Radhouani et al. 2018a). Kefiran properties such as adhesive performance accompanied by a pseudoplastic behavior, resistance to hyaluronidase, and no cytotoxic response in vitro are adequate to be considered for application in tissue engineering and regenerative medicine (Radhouani et al. 2018b).

The ability of kefiran to form cryogels was relevant for biomedical application (Piermaria et al. 2008; Zavala et al. 2015). Kefiran alginate gels were used to oral delivery of ciprofloxacin but also to drug delivery in scaffold. Kefiran scaffold degradation is slow and allows sustained diclofenac release over 2 weeks (Radhouani et al. 2019). Kefiran 3D porous supports (scaffold) were also obtained via thermally induced phase separation (TIPS). Porous structure can be modulated by modifying the thermal path of the solution during the phase separation (Toscano et al. 2018) Kefiran or polyacrylonitrile (PAN)/kefiran nanofiber were also prepared by electrospun and proved to be adequate as scaffolds in the cell culture (Jenab et al. 2020). In addition, injectable, in situ forming kefiran gels have been developed for potential applications as implantable drug delivery devices or scaffolds for tissue regeneration (Sabatino et al. 2020).

9 Conclusion

After first finding on the EPS produced by microorganism included into kefir grains named kefiran, this EPS gained attention to scientist that were interested in its chemical structure, microorganisms that are responsible of its biosynthesis as well as its physicochemical and health-promoting properties.

Kefiran production is one of the main subjects to solve for industrial production. Grains are a good source of kefiran. However, the search of kefiran-producing microorganisms continued up to our days and it is a hard task due to the rigorous growth conditions required by the lactobacilli strains since for industrial production low-cost medium is required.

The technological properties of kefiran include its ability to act as a thickening agent, its contribution to the stabilization of emulsions, and its ability to form gels and films. Kefiran films are also an alternative to include probiotics or bioactive molecules in food matrices or are good carriers for drug delivery. Along with the evidence of kefiran's health-promoting activities such as anti-inflammatory, antioxidant, bifidogenic, and antitumoral, it can be concluded that it is a promising

microbial polysaccharide for application in functional foods and biomedical industries such as tissue engineering and regenerative medicine.

Acknowledgments NS and NG are recipient of fellowships from CONICET. MM, JP and AGA are members of Scientific Career of CONICET. This work was supported by the ANPCyT, MINCyT (PICT 2016-0639); and by the UNLP X813.

References

Abraham AG, Medrano M, Piermaria JA, Mozzi F. Novel applications of polysaccharides from lactic acid Bacteria: a focus on kefiran. In: Hollingworth CE, editor. Food hydrocolloids: characteristics, properties and structures. New York: Nova Science Publishers; 2010. p. 253–71.

Babaei-Ghazvini A, Shahabi-Ghahfarrokhi I, Goudarzi V. Preparation of UV-protective starch/kefiran/ZnO nanocomposite as a packaging film: characterization. Food Packag Shelf Life. 2018;16:103–11.

Barbosa AF, Santos PG, Lucho AMS, Schneedorf JM. Kefiran can disrupt the cell membrane through induced pore formation. J Electroanal Chem. 2011;653(1–2):61–6.

Bengoa AA, Llamas MG, Iraporda C, Dueñas MT, Abraham AG, Garrote GL. Impact of growth temperature on exopolysaccharide production and probiotic properties of *Lactobacillus paracasei* strains isolated from kefir grains. Food Microbiol. 2018;69:212–8.

Bengoa AA, Iraporda C, Garrote GL, Abraham AG. Kefir micro-organisms: their role in grain assembly and health properties of fermented milk. J Appl Microbiol. 2019;126(3):686–700.

Bhattacharyya A, Chattopadhyay R, Mitra S, Crowe SE. Oxidative stress: an essential factor in the pathogenesis of gastrointestinal mucosal diseases. Physiol Rev. 2014;94(2):329–354.

Blandón LM, Noseda MD, Islan GA, Castro GR, de Melo Pereira GV, Thomaz-Soccol V, Soccol CR. Optimization of culture conditions for kefiran production in whey: the structural and biocidal properties of the resulting polysaccharide. Bioact Carbohydr Diet Fibre. 2018;16:14–21.

Cheirsilp B, Shimizu H, Shioya S. Enhanced kefiran production by mixed culture of *Lactobacillus kefiranofaciens* and *Saccharomyces cerevisiae*. J Biotechnol. 2003;100(1):43–53.

Cheirsilp B, Suksawang S, Yeesang J, Boonsawang P. Co-production of functional exopolysaccharides and lactic acid by *Lactobacillus kefiranofaciens* originated from fermented milk, kefir. J Food Sci Technol. 2018;55(1):331–40.

Dailin DJ, Elsayed EA, Othman NZ, Malek R, Phin HS, Aziz R, Wadaan M, El Enshasy HA. Bioprocess development for kefiran production by *Lactobacillus kefiranofaciens* in semi industrial scale bioreactor. Saudi J Biol Sci. 2016;23(4):495–502.

de Paiva IM, da Silva Steinberg R, Lula IS, de Souza-Fagunde EM, de Oliveira Mendes T, Bell MJV, Nicoli JR, Nunes AC, Neumann E. *Lactobacillus kefiranofaciens* and *Lactobacillus satsumensis* isolated from Brazilian kefir grains produce alpha-glucans that are potentially suitable for food applications. LWT-Food Sci Technol. 2016;72:390–8.

Elsayed EA, Farooq M, Dailin D, El-Enshasy HA, Othman NZ, Malek R, Danial E, Wadaan M. In vitro and in vivo biological screening of kefiran polysaccharide produced by *Lactobacillus kefiranofaciens*. Biomed Res. 2017;28(2):594–600.

Enikeev R. Development of a new method for determination of exopolysaccharide quantity in fermented milk products and its application in technology of kefir production. Food Chem. 2012;134(4):2437–41.

Exarhopoulos S, Raphaelides SN, Kontominas MG. Flow behavior studies of kefiran systems. Food Hydrocoll. 2018;79:282–90.

Frengova GI, Simova ED, Beshkova DM, Simov ZI. Exopolysaccharides produced by lactic acid bacteria of kefir grains. Z Naturforsch. 2002;57:805–10.

Furuno T, Nakanishi M. Kefiran suppresses antigen-induced mast cell activation. Biol Pharm Bull. 2012;35(2):178–83.

Gagliarini N, Diosma G, Garrote G, Abraham AG, Piermaria JA. Whey protein-kefiran films as driver of probiotics to the gut. LWT. 2019;105:321–8.

Gangoiti MV, Puertas AI, Hamet MF, Peruzzo PJ, Llamas MG, Medrano M, Prieto A, Dueñas MT, Abraham AG. *Lactobacillus plantarum* CIDCA 8327: an α-glucan producing-strain isolated from kefir grains. Carbohydr Polym. 2017;170:52–9.

Ghasemlou M, Khodaiyan F, Oromiehie A, Yarmand MS. Characterization of edible emulsified films with low affinity to water based on kefiran and oleic acid. Int J Biol Macromol. 2011;49(3): 378–84.

Ghasemlou M, Khodaiyan F, Jahanbin K, Gharibzahedi SMT, Taheri S. Structural investigation and response surface optimisation for improvement of kefiran production yield from a low-cost culture medium. Food Chem. 2012;133(2):383–9.

Hamet MF, Medrano M, Pérez PF, Abraham AG. Oral administration of kefiran exerts a bifidogenic effect on BALB/c mice intestinal microbiota. Benefic Microbes. 2016;7(2):237–46.

Hasheminya SM, Dehghannya J. Novel ultrasound-assisted extraction of kefiran biomaterial, a prebiotic exopolysaccharide, and investigation of its physicochemical, antioxidant and antimicrobial properties. Mater Chem Phys. 2020;243:122645.

Jenab A, Roghanian R, Ghorbani N, Ghaedi K, Emtiazi G. The efficacy of electrospun PAN/kefiran nanofiber and kefir in mammalian cell culture: promotion of PC12 cell growth, anti-MCF7 breast cancer cells activities, and cytokine production of PBMC. Int J Nanomedicine. 2020;15: 717–28.

Jeong D, Kim DH, Kang IB, Kim H, Song KY, Kim HS, Seo KH. Characterization and antibacterial activity of a novel exopolysaccharide produced by *Lactobacillus kefiranofacien*s DN1 isolated from kefir. Food Control. 2017;78:436–42.

Kazazi H, Khodaiyan F, Rezaei K, Pishvaei M, Mohammadifar MA, Moieni S. Rheology and microstructure of kefiran and whey protein mixed gels. J Food Sci Technol. 2017;54(5):1168–74.

Kooiman P. The chemical structure of kefiran, the water-soluble polysaccharide of the kefir grain. Carbohydr Res. 1968;7(2):200–11.

Kwon OK, Ahn KS, Lee MY, Kim SY, Park BY, Kim MK, Lee IY, Oh SR, Lee HK. Inhibitory effect of kefiran on ovalbumin-induced lung inflammation in a murine model of asthma. Arch Pharm Res. 2008;31(12):1590–6.

La Rivière JW, Kooiman P, Schmidt K. Kefiran, a novel polysaccharide produced in the kefir grain by *Lactobacillus brevis*. Arch Microbiol. 1967;59:269–78.

Lazaridou A, Biliaderis CG, Micha-Screttas M, Steele BR. A comparative study on structure–function relations of mixed-linkage (1→3),(1→4) linear β-d-glucans. Food Hydrocoll. 2004;18 (5):837–55.

Londero A, Hamet MF, De Antoni GL, Garrote GL, Abraham AG. Kefir grains as a starter for whey fermentation at different temperatures: chemical and microbiological characterisation. J Dairy Res. 2012;79(3):262–71.

Lynch KM, Zannini E, Coffey A, Arendt EK. Lactic acid bacteria exopolysaccharides in foods and beverages: isolation, properties, characterization, and health benefits. Annu Rev Food Sci Technol. 2018;9:155–76.

Maeda H, Zhu X, Suzuki S, Suzuki K, Kitamura S. Structural characterization and biological activities of an exopolysaccharide kefiran produced by *Lactobacillus kefiranofaciens* WT-2B (T). J Agric Food Chem. 2004a;52:5533–8.

Maeda H, Zhu X, Omura K, Suzuki S, Kitamura S. Effects of an exopolysaccharide (kefiran) on lipids, blood pressure, blood glucose, and constipation. Biofactors. 2004b;22(1–4):197–200.

Medrano M, Pérez PF, Abraham AG. Kefiran antagonizes cytopathic effects of *Bacillus cereus* extracellular factors. Int J Food Microbiol. 2008;122(1–2):1–7.

Medrano M, Hamet MF, Abraham AG, Pérez PF. Kefiran protects Caco-2 cells from cytopathic effects induced by Bacillus cereus infection. Antonie Van Leeuwenhoek. 2009;96(4):505.

Medrano M, Racedo SM, Rolny IS, Abraham AG, Pérez PF. Oral administration of kefiran induces changes in the balance of immune cells in a murine model. J Agric Food Chem. 2011;59(10): 5299–304.

Micheli L, Uccelletti D, Palleschi C, Crescenzi V. Isolation and characterisation of a ropy *Lactobacillus* strain producing the exopolysaccharide kefiran. Appl Microbiol Biotechnol. 1999;53:69–74.
Mitsue T, Tachibana K, Fujio Y. Efficient kefiran production by a mixed culture of *Lactobacillus kefiranofaciens* KF-75 and yeast strains. J Biosci Bioeng. 1999;3(87):400.
Moradi Z, Kalanpour N. Kefiran, a branched polysaccharide: preparation, properties and applications: a review. Carbohydr Polym. 2019;223(1):115100.
Moradi Z, Esmaiili M, Almasi H. Development and characterization of kefiran-Al2O3 nanocomposite films: Morphological, physical and mechanical properties. Int J Biol Macromol 2019;122:603–609.
Motedayen AA, Khodaiyan F, Salehi EA. Development and characterisation of composite films made of kefiran and starch. Food Chem. 2013;136(3–4):1231–8.
Mukai T, Toba T, Itoh T, Adachi S. Structural microheterogeneity of kefiran from Kefir grains. Jap J Zootechn Sci. 1988;9:167–76.
Murofushi M, Shiomi M, Aibara K. Effect of orally administered polysaccharide from kefir grain on delayed-type hypersensitivity and tumor growth in mice. Jpn J Med Sci Biol. 1983;36(1):49–53.
O'Callaghan A, van Sinderen D. Bifidobacteria and their role as members of the human gut microbiota. Front Microbiol. 2016;7:925. https://doi.org/10.3389/fmicb.2016.00925.
Piermaria JA, de la Canal ML, Abraham AG. Gelling properties of kefiran, a food-grade polysaccharide obtained from kefir grain. Food Hydrocoll. 2008;22(8):1520–7.
Piermaria JA, Pinotti A, Garcia MA, Abraham AG. Films based on kefiran, an exopolysaccharide obtained from kefir grain: development and characterization. Food Hydrocoll. 2009;23(3):684–90.
Piermaria JA, Bosch A, Pinotti A, Yantorno O, Garcia MA, Abraham AG. Kefiran films plasticized with sugars and polyols: water vapor barrier and mechanical properties in relation to their microstructure analyzed by ATR/FT-IR spectroscopy. Food Hydrocoll. 2011;25:1261–9.
Piermaria J, Diosma G, Aquino C, Garrote G, Abraham AG. Edible kefiran films as vehicle for probiotic microorganisms. Innovative Food Sci Emerg Technol. 2015;32:193–9.
Piermaria J, Bengoechea C, Abraham AG, Guerrero A. Shear and extensional properties of kefiran. Carbohydr Polym. 2016;152:97–104.
Piermaria JA, López-Castejón ML, Bengoechea C, Guerrero A, Abraham AG. Prebiotic emulsions stabilised by whey protein and kefiran. Int J Food Sci Technol. 2020. https://doi.org/10.1111/ijfs.14601.
Pop Rodica C, Salanţă L, Rotar AM, Semeniuc CA, Socaciu C, Sindic M. Influence of extraction conditions on characteristics of microbial polysaccharide kefiran isolated from kefir grains biomass. J Food Nutr Res. 2016;55(2):121–30.
Radhouani H, Gonçalves C, Maia FR, Oliveira JM, Reis RL. Biological performance of a promising kefiran-biopolymer with potential in regenerative medicine applications: a comparative study with hyaluronic acid. J Mater Sci Mater Med. 2018a;29(8):124.
Radhouani H, Gonçalves C, Maia FR, Oliveira JM, Reis RL. Kefiran biopolymer: evaluation of its physicochemical and biological properties. J Bioact Compat Polym. 2018b;33(5):461–78.
Radhouani H, Bicho D, Gonçalves C, Maia FR, Reis RL, Oliveira JM. Kefiran cryogels as potential scaffolds for drug delivery and tissue engineering applications. Mater Today Commun. 2019;20:100554.
Rimada PS, Abraham AG. Polysaccharide production by kefir grains during whey fermentation. J Dairy Res. 2001;68:653–61.
Rimada PS, Abraham AG. Comparative study of different methodologies to determine the exopolysaccharide produced by kefir grains in milk and whey. Lait. 2003;83:79–87.
Rimada PS, Abraham AG. Kefiran improves rheological properties of glucono-δ-lactone induced skim milk gels. Int Dairy J. 2006;16(1):33–39.
Rodrigues KL, Caputo LRG, Carvalho JCT, Evangelista J, Schneedorf JM. Antimicrobial and healing activity of kefir and kefiran extract. Int J Antimicrob Agents. 2005a;25(5):404–8.
Rodrigues KL, Carvalho JCT, Schneedorf JM. Anti-inflammatory properties of kefir and its polysaccharide extract. Inflammopharmacology. 2005b;13(5–6):485–92.

Sabaghi M, Maghsoudlou Y, Habibi P. Enhancing structural properties and antioxidant activity of kefiran films by chitosan addition. Food Struct. 2015;5:66–71

Sabatino MA, Pavia FC, Rigogliuso S, Giacomazza D, Ghersi G, La Carrubba V, Dispenza C. Development of injectable and durable kefiran hydro-alcoholic gels. Int J Biol Macromol. 2020;149:309–19.

Serafini F, Turroni F, Ruas-Madiedo P, Lugli GA, Milani C, Duranti S, Zamboni N, Bottacini F, van Sinderen D, Margolles A, Ventura M. Kefir fermented milk and kefiran promote growth of Bifidobacterium bifidum PRL2010 and modulate its gene expression. Int J Food Microbiol. 2014;178:50–9.

Shahabi-Ghahfarrokhi I, Khodaiyan F, Mousavi M, Yousefi H. Preparation of UV-protective kefiran/nano-ZnO nanocomposites: physical and mechanical properties. Int J Biol Macromol. 2015a;72:41–6.

Shahabi-Ghahfarrokhi I, Khodaiyan F, Mousavi M, Yousefi H. Green bionanocomposite based on kefiran and cellulose nanocrystals produced from beer industrial residues. Int J Biol Macromol. 2015b;77:85–91.

Shiomi M, Sasaki K, Murofushi M, Aibara K. Antitumor activity in mice of orally administered polysaccharide from kefir grain. Jpn J Med Sci Biol. 1982;35(2):75–80.

Toba T, Arihara K, Adachi S. Comparative study of polysaccharides from kefir grains, an encapsulated homofermentative *Lactobacillus* species and *Lactobacillus kefir*. Milchwissenschaft. 1987;42(9):565–8.

Toscano M, Pavia FC, Conoscenti G, Sabatino MA, Carrubba VL, Dispenza C, Maria A. Kefiran-based scaffolds for biomedical applications. Chem Eng Trans. 2018;64:181–6.

Uchida M, Ishii I, Inoue C, Akisato Y, Watanabe K, Hosoyama S, Toida T, Ariyoshi N, Kitada M. Kefiran reduces atherosclerosis in rabbits fed a high cholesterol diet. J Atheroscler Thromb. 2010;17(9):980–8.

Vancanneyt M, Mengaud J, Cleenwerck I, Vanhonacker K, Hoste B, Dawyndt P, Degivry MC, Ringuet D, Janssens D, Swings J. Reclassification of *Lactobacillus kefirgranum* Takizawa et al. 1994 as *Lactobacillus kefiranofaciens* subsp. *kefirgranum* subsp. nov. and emended description of *L. kefiranofaciens* Fujisawa et al. 1988. Int J Syst Evol Microbiol. 2004;54(2):551–6.

Vinderola G, Perdigón G, Duarte J, Farnworth E, Matar C. Effects of the oral administration of the exopolysaccharide produced by *Lactobacillus kefiranofaciens* on the gut mucosal immunity. Cytokine. 2006;36(5–6):254–60.

Wang Y, Ahmed Z, Feng W, Li C, Song S. Physicochemical properties of exopolysaccharide produced by *Lactobacillus kefiranofaciens* ZW3 isolated from Tibet kefir. Int J Biol Macromol. 2008;43:283–8.

Wang J, Zhao X, Tian Z, Yang Y, Yang Z. Characterization of an exopolysaccharide produced by *Lactobacillus plantarum* YW11 isolated from Tibet Kefir. Carbohydr Polym. 2015;125:16–25.

Yokoi H, Watanabe T, Fuji Y, Toba T, Adachi, S. Isolation and characterization of polysaccharide-producing bacteria from kefir grains. J Dairy Sci. 1990;73(7):1684–1689.

Yokoi H, Fuji Y, Mukai T, Toba T, Adachi S. Some taxonomical characteristic of encapsulated *Lactobacillus* sp. KPB-167B isolated from kefir grains and characterisation of its extracellular polysaccharide. Int J Food Microbiol. 1991;13:257–64.

Zajšek K, Goršek A, Kolar M. Cultivating conditions effects on kefiran production by the mixed culture of lactic acid bacteria imbedded within kefir grains. Food Chem. 2013;139(1–4):970–7.

Zavala L, Roberti P, Piermaria JA, Abraham AG. Gelling ability of kefiran in the presence of sucrose and fructose and physicochemical characterization of the resulting cryogels. J Food Sci Technol. 2015;52(8):5039–47.

Zolfi M, Khodaiyan F, Mousavi M, Hashemi M. Development and characterization of the kefiran-whey protein isolate-TiO_2 nanocomposite films. Int J Biol Macromol. 2014;65:340–5.

Zolfi M, Khodaiyan F, Mousavi M, Hashemi M. Characterization of the new biodegradable WPI/clay nanocomposite films based on kefiran exopolysaccharide. J Food Sci Technol. 2015;52(6):3485–93.

Microbial Glucuronans and Succinoglycans

7

Structures, Properties, and Enzymes Acting About Them

P. Dubessay, P. Andhare, D. Kavitake, P. H. Shetty, A. V. Ursu, C. Delattre, G. Pierre, and P. Michaud

Contents

P. Dubessay · A. V. Ursu
Université Clermont Auvergne, CNRS, SIGMA Clermont, Institut Pascal, Clermont-Ferrand, France

P. Andhare
PDPIAS, CHARUSAT University, Anand, India

D. Kavitake · P. H. Shetty
Department of food Science and Technology, Pondicherry University, Pondicherry, India

C. Delattre
Université Clermont Auvergne, Clermont Auvergne INP, CNRS, Institut Pascal, Clermont-Ferrand, France

Institut Universitaire de France (IUF), Paris, France

G. Pierre · P. Michaud (✉)
Université Clermont Auvergne, Clermont Auvergne INP, CNRS, Institut Pascal, Clermont-Ferrand, France
e-mail: philippe.michaud@uca.fr

J. M. Oliveira et al. (eds.), *Polysaccharides of Microbial Origin*,
https://doi.org/10.1007/978-3-030-42215-8_8

Abstract

Glucuronan and succinoglycan are two anionic exopoysaccharides produced by some soil bacteria and abundantly published by the community working on high valuable hydrocolloids. The glucuronan is a linear homopolymer of β-(1,4)-D-glucuronic acids sometimes subsituted by *O*-acetyl, whereas succinoglycan is a ramified heteropolymer of β-(1,6) and β-(1,4) and β-(1,3)-linked *O*-pyruvylated, *O*-succinylated and sometimes *O*-acetylated D-glucoses and D-galactoses. These unique structural features give to these two polysaccharides original physicochemical and rheological properties investigated in 1980s and 1990s. However, contrary to some other microbial polysaccharides, they have known a poor commercial success as texturing agent. The reasons of this "defeat" is difficult to analyze but is probably linked to the technical and economic competitions of other plant, seaweeds, and microbial hydrocolloids. This chapter aims to do the state of the art on structures, production, and rheological properties of these two polysaccharides but also on enzymes acting about them.

Keywords

Glucuronan · Succinoglycan · Polysaccharide · Lyase · Hydrolase

1 Introduction

Glucuronan and succinoglycan are two unlucky microbial polysaccharides in an applicative point of view, sharing to be produced as exopolysaccharides (EPS) by, among others, some bacteria belonging to Rhizobiaceae family. Indeed an acidic extracellular heteropolysaccharide isolated from the culture medium of *Alcaligenes faecalis* var *myxogenes* and called succinoglycan, as it contained succinate, was firstly described in 1965 (Harada et al. 1965). Its full structure was elucidated in 1994 (Reinhold et al. 1994). The identification of glucuronan, an homopolymer of glucuronic acid, excreted by a mutant strain of *Sinorhizobium meliloti* is more recent and date of 1993 (Courtois et al. 1993). Just after these first publications focusing on these two EPSs, they received consideration of the academic and industrial communities working on polysaccharides for their original, biological, physicochemical, and rheological properties (Elboutachfaiti et al. 2011; Halder et al. 2017). The terms "glucuronan" and "succinoglycan" generate currently 150 and 1053 references, respectively, including a significative number of patents (32 for the glucuronan and 529 for the succinoglycan) using SciFinder (https://scifinder.cas.org). The lower scientific production for glucuronan is mainly linked to the no availability of the producing strain, *Sinorhizobium meliloti* M5N1CS (NCIMB 40472). However the development of the TEMPO chemistry applied to cellulose modification in the 1990s led to similar structures generally called "anionic cellulose," "acidic cellulose," or "cellouronic acid" rather than "glucuronan" (3415 references using SciFinder). The interest for these two bacterial polysaccharides was linked to their potential of applications. Succinoglycan, which was the first EPS identified as an acting macromolecule (signal molecule) in the formation of nitrogen fixing nodules on the leguminous roots, exhibits also rheological properties competitive with those of other

commercial texturing agents such as xanthan or hydroxyethycellulose. For that, it was described and patented for numerous applications such as stabilizing, thickening, fluid loss controlling, gel forming, and precipitating agents notably by companies such as Rhodia, Shell, Pfizer, and others. However at this time, Solvay Novecare is the sole supplier of industrial succinoglycan with the commercial name of Rheozan. This texturing agent is obtained after fermentation of *Agrobacterium tumefaciens* and extracted by alcoholic isopropanol precipitation. Rheozan is commercialized to display pseudoplastic nonthixotropic behavior of aqueous solution not affected by temperature. This low commercial success can be attributed to the competition of xanthan or some plant polysaccharide introduced on the market earlier. This view is shared by numerous other bacterial polysaccharides which are industrialized only if they are economically competitive with those already on the market and/or if their specific properties led them to occupy a free application niche. The bacterial glucuronan was in this case the first natural β-(1,4) homopolymer of glucuronic acids as it was described in the literature even if polyglucuronic acids segments have been previously published in some polysaccharidic structures. This original biopolymer, similar to an acidic cellulose, gave some opportunities as bioactive agent in the field of cosmetic but not as gelifying or chelating agents due to the presence in the commercial polysaccharides arena of other low-cost polyuronic acids such as alginate or polygalacturonic acid (Elboutachfaiti et al. 2011). However the recent development of new high values applications in the fields of biosourced materials including hydrogels, adhesives, or bioactive agents could offer a second opportunity for these polysaccharides which have failed to introduce the market as texturing agents. This review do the state of the art of recent knowledges on succinoglycan and glucuronan.

2 Glucuronans

2.1 Sources and Structures of Microbial Glucuronans (GP)

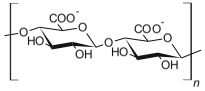
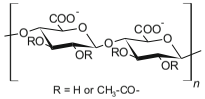
Natural polyglucuronic acids can be isolated from various kind of microorganisms but in fact from very few families and mostly mutant strains. As for other polyuronides, changes of the structural features and mass yield have been reported in the literature regarding the type of strains (and involved mutation(s)), culture medium including the C-source substrate (e.g., fructose, glucose, and saccharose) and ions concentration (e.g., magnesium salt), culture growth, way of biosynthesis, and excretion (Elboutachfaiti et al. 2011). As briefly described in the introduction section, the main backbone is still described as a linear homopolymer composed of β-(1,4)-D-polyglucuronic acid residues, which can be *O*-acetylated at *C*-2 and/or *C*-3 positions. The final yields, glucuronan purity, ratio, and position of acetyl groups, patterns of alternating linkages (α- and β-(1,4)), and also the molar mass (distribution and size) are key parameters to apprehend the structural variability inside the family of microbial glucuronan, knowing that macroalgae (i.e., ulvan) glucuronan are excluded from this reasoning in the present chapter.

The cell wall of Mucorales has been described decades ago as a source of extracellular mucoric acid of low molecular weight (30–60 residues) (de Ruiter et al. 1992). In 1986, McNeil et al. published sequences of β-(1,4)-linked Glc*p*A

residues which were from a new exopolysaccharide (EPS) produced by *Rhizobium leguminosarum*. Few years later, a mutant called *Sinorhizobium melitoti* M5N1CS (NCIMB 40472) obtained from *Rhizobium meliloti* M5N1 by using *N*-methyl-*N*-′-nitro-*N*-nitrosoguanidine was identified as one strain capable of producing during fermentation an extracellular β-(1,4)-D-glucuronan, partially acetylated in *O*-2 and/or *O*-3 positions (Heyraud et al. 1993). From this point and during the following years, many papers appeared in the literature regarding the identification of new (i) strain sources (Khan et al. 2007), (ii) structural characteristics (Courtois et al. 1993), (iii) rheological behavior and uses (Tai et al. 2019), (iv) biological properties (Elboutachfaiti et al. 2011), (v) depolymerizing enzymes to degrade glucuronan (which lead to the discovery of glucuronan lyase, EC 4.2.2.14, see Sect. 2.3) (Da Costa et al. 2001; Konno et al. 2008), and (vi) "synthetic" glucuronan, especially thanks to the TEMPO chemistry for producing new uronate (Delattre et al. 2006). In 2020 and with the benefit of hindsight, this short overview clearly shows no breakpoint in terms of structural features or strain families (Table 1). Polyglucuronic acids can thus be isolated from the Rhizobiaceae family, e.g., driven by *Sinorhizobium melitoti* M5N1CS (Courtois et al. 1993), bacterial strains producing alternative glucuronan, e.g., *Gluconacetobacter hansenii* PJK (KCTC 10505BP) which produces a α-(1,4)-oligoglucuronic acid (Khan et al. 2007) or the alkalophilic *Bacillus* strain C-125 which syntheses in its cell walls an alternating α- and β-(1,4)-glucuronan (Aono 1990), the mycelia wall of fungal strain such as *Mucor rouxii* (Dow et al. 1983), or from the well wall of oleaginous yeast *Trichosporon cutaneum* (Depree et al. 1993). Finally, designing new poly- and oligoglucuronan from abundant sources and easy chemo-enzymatic methodologies (Pierre et al. 2017) obviously seems the better option to sustain both molecular/enzymatic (e.g., glucuronan lyase family) or biological potential (antiviral, antitumor, antimicrobial, antioxidant, elicitor, etc.) investigations for the following decade. Few tips should be given for newcomers interested to determine the structural features of glucuronan. Size-exclusion chromatography coupled to a refractometer, if possible coupled to multi-angle laser light scattering (SEC-MALLS), is often used to describe the molecular weight of native, highly acetylated and deacetylated glucuronan, ranging from 6.10^4 to around 10^6 g/mol (Heyraud et al. 1993).

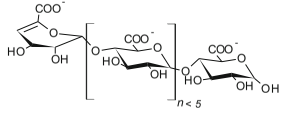
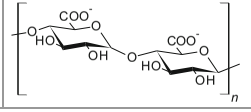
The intrinsic pK value is reported around to 3 which is in accordance with the values published for pectin or alginate. Gas chromatography (coupled to mass spectrometry) GC(/MS), high-performance liquid chromatography (HPLC), and/or high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) can be used for determining the monosaccharide compositions (using GlcA as standard) but also estimating the degree of acetylation (DA) after saponification, for example, by using a C18 reverse phase column in HPLC to quantify acetic acid. DA can obviously be estimated by Fourier transform infrared spectroscopy (FT-IR) even if nuclear magnetic resonance (NMR) has to be used to determine the positions of acetyl groups throughout the main backbone. FT-IR can be used for comparing footprint spectra since many data can be obtained. These spectra are often useful to identify the type and source of glucuronan, without performing more complicated, expensive, and time-consuming analyses, like NMR.

Table 1 Main natural glucuronan extracted from bacteria and fungi

Name	Source	Structural features	Physicochemical overview	References
Fungal β-(1,4)-glucuronan Mucoric acid	*Mucor rouxii* *Trichosporon cutaneum*	β-(1,4)-D-polyglucuronic acid 	From the cell wall, can be soluble in water C-source: Glucose Two types of polymers (I and II) from Mucor: Polymer I: D-GlcA, D-Man, D-Gal, L-Fuc Polymer II: Mucoric acid (D-GlcA) Molar mass ranging from 19–35,000 g/mol	Depree et al. (1993)
Glucuronan	*Sinorhizobium meliloti* M5N1CS	β-(1,4)-D-polyglucuronic acid, acetylated in *O*-2 and/or *O*-3 positions 	EPS, water soluble C-source: glucose, fructose, saccharose DA ranging from 50 to >70% depending on Mg^{2+} ions supplementation Molar mass ranging from 6×10^4 to 8×10^5 g/mol Alternative Ac groups on *C*-2/*C*-3 positions Gelling properties with Na^+, Ca^{2+}, Cu^{2+}, or Ba^{2+} ions	Heyraud et al. (1993)

(continued)

Table 1 (continued)

Name	Source	Structural features	Physicochemical overview	References
Glucuronan oligosaccharides Glucuronic acid oligomers	*Gluconacetobacter hansenii* PJK	α-(1,4)-D-oligoglucuronic acid, can included nonreducing ending (4-deoxy-L-erythro-hex-4-enopyrnosylruonic acid) 	EPS, water soluble C-source: Glucose, waste of beer fermentation broth Molar mass less than 1050 g/mol (oligomers) Unsaturated GlcA (nonreducing terminal unit) Thermal stability Emulsifying profile Ac and/or Me groups in the terminal unit	Khan et al. (2007)
Alternative glucuronan from teichuronopeptide (TUP)	*Bacillus* strain C-125	Alternating α- and β-(1,4)-D-polyglucuronic acid 	From the cell wall C-source: Glucose Equimolar mixture of α- and β-Glc*p*A	Aono (1990)

Ac Acetyl, *EPS* Exopolysaccharide, *DA* Degree of acetylation, *Me* Methyl

The glycosidic linkages can be easily obtained by one-dimensional ^{1}H and ^{13}C NMR since the attributions are quiet easy to make and should be preferred to derivatization methodology, e.g., making permethylated acetate alditol (PMMA) residues for GC/MS-electron impact (EI) analyses (Heyraud et al. 1993). This method generates multiple derivatives, including obviously the one corresponding to 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methylglucitol, i.e., 1,4-linked monomers, making unnecessarily "complex" the total ion chromatograms (TIC), because of the quite resistance of glucuronan to acidic hydrolysis (Heyraud et al. 1993). This observation is not new since Timell et al. (1965) showed the stability of uronosidyl linkages to acidic hydrolysis. Overall, FT-IR and NMR should be always preferred, and some details are briefly given above about the structural characterization of glucuronan using these techniques.

FT-IR can be used for efficiently determining the type of acetylated glucuronan and the degree of acetylation even if the results should be always confirmed by ^{1}H NMR. FT-IR spectra of glucuronan show various characteristic peaks in the 3600–3000, 3000–2800, and 1800–1200 cm^{-1} areas. The first one corresponds to -OH stretching vibrations from GlcA residues but also residual water inside the purified polysaccharides. The second area is dedicated to -CH stretching vibrations but also O-H stretching vibrations of -COOH groups. The last one, which is rich in information, includes deformation vibration of groups possessing local symmetry. The bands closed to 1598 and 1410 cm^{-1} correspond to asymmetric and symmetric stretching modes of planar -COOH groups, respectively. For acetylated glucuronan, the bands around 1726 and 1249 cm^{-1} correspond, respectively, to C=O and antisymmetric C-O-C stretching vibrations of ester groups. This data can be used to show if the functional group in *C*-6 position is only a carboxylate. Symmetric vibration of C-H groups from methoxy groups (O-CH_3) can be associated to the band around 1374 cm^{-1}. The intense peak around 1037 cm^{-1} correspond to the valence vibration C-O of polysaccharides. After deconvolution (Levenberg-Marquardt or others), the degree of substitution (DS %) (at both 1726 and 1249 cm^{-1}) can still be determined by IRTF using, for example, the method of Chatjigakis et al. (1998). Generally, estimating the degree of acetylation (DA %) is done by only focusing on the band around 1726 cm^{-1} since no parasite band corresponding to other functional groups in glucuronan can be found here (Stefke et al. 2008). The simple following equation (Eq. 1) can thus be used:

$$\mathrm{DA}(\%) = \frac{\mathrm{A}_{1726}}{\mathrm{A}_{1037}} \times 100 \tag{1}$$

where A corresponds to the areas calculated after deconvolution for both peaks at 1726 and 1037 cm^{-1}.

^{1}H NMR and ^{13}C one-dimensional NMR can be monitored for accurately identifying the structural characteristics of glucuronan. Note that both native and deacetylated glucuronan have to be done to complete the structural determination. Signals from the ring-proton 1.9–2.2 ppm region are usually attributed to acetyl groups on ^{1}H spectra. The up- and downfield regions ranging from 4.3 to 5.1 and 3.1

to 4 ppm, respectively, can be also used to determine the DA but also the distribution of acetyl groups (*C*-2 and/or *C*-3 positions). The chemical shift at 4.41 ppm ($J_{1,2}$ of 7.7 Hz) can be attributed to a β-linkage. On ^{13}C spectra, six main peaks (around 74.9 ppm for *C*-2, 76.4 ppm for *C*-3, 77.5 ppm for *C*-5, 83.1 ppm for *C*-4, 104.5 ppm for *C*-1, and 176.5 ppm for COOH in C6) are in general assigned for deacetylated glucuronan which corresponds to a β-(1,4)-linked uronic acid (Heyraud et al. 1993). Finally, assignments should be made by two-dimensional NMR techniques.

X-ray diffraction, molecular modeling, and crystallography can also be performed to access the conformational and configurational features of both de- and acetylated glucuronan (Braccini et al. 1998). Bacterial glucuronan are structurally more regular and seem to adopt a ribbon-like twofold conformation like numerous other β-(1,4)-linked polysaccharides (Heyraud et al. 1993). Braccini et al. (1998) have studied four diglucuronic acid layouts corresponding to both extended (A, B) and folded conformations (C, D). The A conformation gave the best results since it allowed the formation of a hydrogen bond between *O*-5′ and *O*-3. Introducing an acetyl group in *C*-2 position had no effect on hydrogen bond on the contrary to the *C*-3 position. Overall, the presence of acetylation resulted each time in steric conflicts. Modeling similar structures showed that a twofold helix and a left-handed threefold helix allowed to obtain the best (and similar) calculation energies. Regarding to X-ray diffraction experiments, the first one seemed the most favorable in solid state especially for acetyl-free glucuronan. For acetylated glucuronan, the position and distribution of acetyl groups were involved in the stabilization of the helix due to the formation and/or losses of hydrogen bonds. Acetylation in position 3 gave a better stabilization of the 3_2 helices whereas acetylation in position 2 increased the stability of 2_1 conformations. Overall, it was concluded that glucuronan generally adopt a twofold conformation with local defects depending on the position and numbers of acetyl groups (*C*-2 and/or *C*-3 positions), as reported previously by Heyraud et al. (1993). Finally, the role of calcium ions is often reported both for the stability of ordered conformations which it is of primary importance during investigations of the rheological behavior (e.g., gelling properties depending on the ionic strength) of glucuronan (see Sect. 4).

2.2 Enzymatically and Chemically Oxidized Glucans

From more than 20 years, synthetic glucuronan have been generated by regioselective oxidation of glucans and more especially cellulose to produce β-(1,4)-polyglucuronic acid as mimetic of microbial glucuronan. As related by lot of authors, a very important bioprocess concept was to develop interesting high value-added and cost-effective valorization of cellulosic fiber from paper and wood industries and more especially bleached softwood kraft pulp (Isogai et al. 2011; Pierre et al. 2017). For this purpose, advanced chemical strategies using nitrogen oxides, sodium nitrite, and phosphoric acid have been largely performed in literature on microfibrillar celluloses (Elboutachfaiti et al. 2011; Pierre et al. 2017). However, these hazardous chemical methods were quickly discarded due to

their high toxicity, dangerousness, uncontrolled time reactions, and high oxidative depolymerization (with elevated by-products made up of dicarboxylic compounds) limiting the final industrial uses of oxidized cellulose fractions. In this context, one of the most important innovative process was definitely the chemical regioselective oxidation at C6-hydroxyl position of glucose residue from cellulose carbohydrate backbone using TEMPO/NaOCl/NaBr chemistry (Elboutachfaiti et al. 2011; Pierre et al. 2017). In fact, in the field of chemical synthesis, a very efficient catalyst of organic oxidation such as the nitroxyl radical 2,2,6,6-tetramethylpyperidine-1-oxyl (TEMPO) combined with NaOCl/NaBr/NaOH system have been widely described in literature for the regioselective oxidation of primary hydroxyl group (C6-hydroxyl position) of numerous natural glycans (Fig. 1) such as starch, mannan, agarose, inulin, chitin, chitosan, gellan, pullulan, curdlan, hyaluronan, galactomannan, amylose, cellulose (De Nooy et al. 1995; Elboutachfaiti et al. 2011; Pierre et al. 2017).

As a general rule, the oxidation process was performed at pH 9–11 (by adding sodium hydroxide) and 0–4 °C to reduce depolymerization and control the molecular weight of anionic polysaccharides (polyuronides). As shown in Fig. 1, chemical oxidation of polysaccharides is a complex mechanism where TEMPO is used at low catalytic concentration (2 mol of radical TEMPO to convert 1 mol of primary C6 hydroxyl group into carboxyl group) and NaOCl (sodium hypochlorite) is commonly used as co-oxidant while NaBr (sodium bromide) is added to increase oxidation process.

As mentioned in a very interesting recent review focusing on TEMPO oxidation advances, Pierre et al. (2017) gave an overview of the main advantages to use the nitroxyl radical 2,2,6,6-tetramethylpyperidine-1-oxyl to produce anionic polysaccharides, such as the high regioselectivity (on C6-hydroxyl position), the low-cost process, and the high oxidation level.

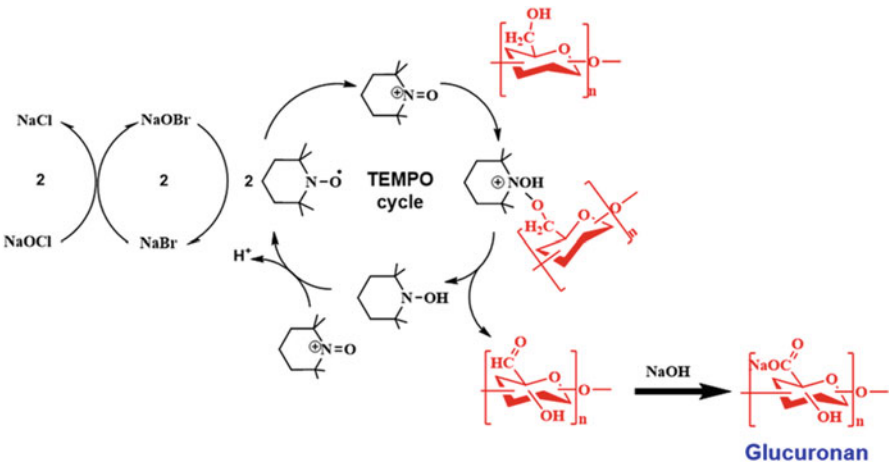


Fig. 1 TEMPO/NaOCl/NaBr-mediated oxidation of glucans for the chemical synthesis of glucuronan

In the area of glucuronan synthesis, cellulose have been extensively investigated as substrate model to generate β-(1,4)-polyglucuronic acid sodium salt (Pierre et al. 2017). However, it is important to mention that cellulose have to be previously treated by chemical, physical, and/or mechanical treatment in order to produce regenerated cellulose for the synthesis of high-yielding water-soluble glucuronan by increasing the level of carboxyl group using TEMPO oxidation chemistry (Elboutachfaiti et al. 2011). In fact, several experimental investigations well established the physicochemical obstacles detected during TEMPO oxidation of native cellulose essentially due to the full water insolubility and the high crystallinity of backbone microfibrillar cellulose leading to hydrogen bonding establishment which drastically reduced the ideal accessibility of primary C6 hydroxyl groups of glucose residues toward nitroxyl radical 2,2,6,6-tetramethylpyperidine-1-oxy (Elboutachfaiti et al. 2011). Subsequently, for the production of good yield of pure polyglucuronic acid sodium salt (glucuronan), detailed pretreatments of cellulose performed to generate pseudo-amorphous polysaccharides have been mostly proposed such as: use of high concentration of NaOH (sodium hydroxide) for mercerization, chemical acetylation, and sonication or high-pressure mechanical treatment (Pierre et al. 2017). In these conditions, all the water-soluble oxidized celluloses obtained by using TEMPO chemistry could be employed for thickening or gelling application for pharmaceutical, cosmetic, and food topic.

Note to mention that lot of studies have shown that non-water-soluble glucuronan fractions produced from native cellulose could be applied as additive biobased nanomaterials for papermaking market and others high-tech disciplines (Isogai et al. 2011; Pierre et al. 2017). As for example, in their works, Isogai et al. (2011) converted native wood celluloses by TEMPO (2,2,6,6-tetramethylpiperidine-1-oxyl radical)-mediated oxidation to give individual partially oxidized nanofibers of cellulose. These news glucuronan fractions provided flexible and transparent films with good mechanical, gas barrier, water resistance, and permeability properties.

It is important to notice that very recently, Delattre et al. (2015) described for the first time the production of synthetic glucuronan called xanthouronan with β-(1,4)-polyglucuronic acid sodium salt as main backbone chain using TEMPO oxidation of xanthan as polysaccharides substrate extracted from *Xanthomonas campestris* (Fig. 2). The main advantage of this innovative process was to use a soluble polysaccharide (xanthan) which is constituted of a cellulose backbone chain. The regioselective chemical oxidation of this water-soluble pseudo-cellulose allowed then to generate a very high yield of pure glucuronan fraction with degree of polymerization more than 600 and a molecular weight estimated at around 585 kilodaltons. This new promising synthetic glucuronan could be an industrial alternative to oxidized cellulose with good rheological properties and interesting antioxidant activities able to be used for the development of active ingredient for food packaging and biomaterial for tissue engineering applications.

Nevertheless, as mentioned by lot of authors, numerous disadvantages are described when TEMPO/NaOCl/NaBr system was performed for specific regioselective oxidation of polysaccharides such as cellulose, xanthan, starch, galactomannan, glucomannan, galactan (Elboutachfaiti et al. 2011; Pierre et al.

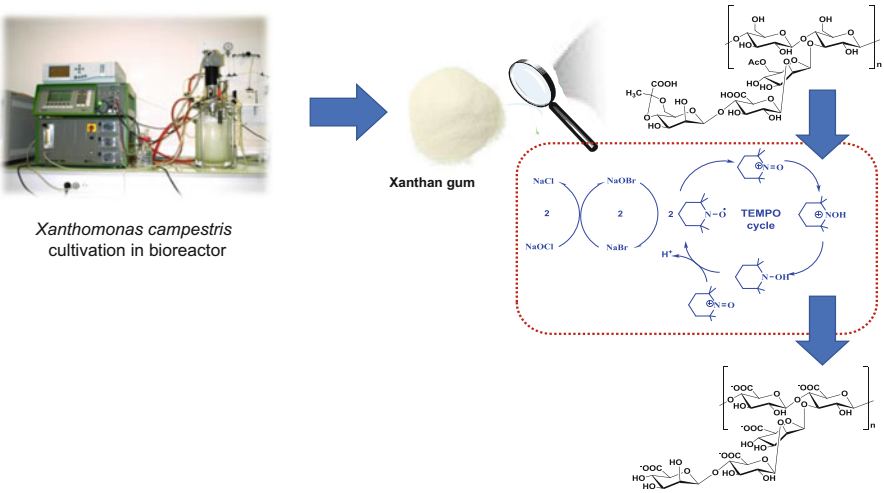


Fig. 2 TEMPO/NaOCl/NaBr-mediated oxidation of xanthan from *Xanthomonas campestris* for the chemical synthesis of microbial glucuronan mimetic

2017). In fact, one of the main drawbacks occurred during the chemical oxidation process is without a doubt the high depolymerization of the backbone polymeric chain for the final anionic polysaccharides. This degradation is essentially due to the drastic alkaline condition (NaOCl/NaOH at pH 9–11) which lead to a β-elimination process resulting in glycosidic linkage breaking. To limiting the anionic polysaccharides depolymerization, original alternatives to chemical processes have been looking for. Therefore, during the last decades, some enzymatic strategies have been developed in order to generate anionic polysaccharides such as polyglucuronic acid sodium salt to increase techno-functional properties of neutral polysaccharide as in the case of cellulose and lignocellulose fractions (Elboutachfaiti et al. 2011; Pierre et al. 2017) without decreasing the initial molecular weight of polymeric chain. For this reason, lot of studies used green processes with laccase as biocatalyst which were proposed as sustainable and innovative biochemical reactions for enzyme anionic modifications of natural polysaccharides from plant, algae, and other biotopes in order to suggest new applications in food, environmental, and pharmaceutical fields for packaging and active material (Pierre et al. 2017; Slagman et al. 2018). It was principally shown that laccase (EC 1.10.3.2.) which is an enzyme belonging to the oxyreductase family could be easily applied as green biocatalyst combined with TEMPO and derivatives such as 4-Amino-TEMPO or 4-acetamide-TEMPO for sugar derivatives and polysaccharides oxidation optimization in slightly acidic environmentally friendly conditions with major advantages to restrain and/or minimize depolymerization of oxidized polysaccharides by stopping β-elimination reaction (Mathew and Adlercreutz 2009; Elboutachfaiti et al. 2011; Pierre et al. 2017). Generally, laccases are produced from *Trametes versicolor*, *Aspergillus oryzae*, *Trametes pubescens*, or *Trametes villosa* (Pierre et al. 2017; Slagman et al. 2018). Lately, in a very interesting review, authors focused on the mains synthetic pathway of

polysaccharides mediated by laccases (Slagman et al. 2018). As a general rule, authors described the main mode of action of enzymatic process onto polysaccharides by a laccase/TEMPO mediator system assisted regioselective oxidation of primary hydroxyl group of polysaccharides (such as C6 hydroxyl group) to carboxylic acids (COOH) or aldehydes functions (R-HC=O). As proposed in Fig. 3, the first step is the conversion of TEMPO to the oxoammonium ion by laccase one-electron oxidation mechanism followed by the regioselective (C6 hydroxyl group) two-electron oxidation of polysaccharides.

As commonly observed, this enzymatic oxidation method is similar to the TEMPO/NaOCl/NaBr system approach (Fig. 1) where in alkaline condition oxoammonium ion was first generated to directly convert C6 hydroxyl group of polysaccharides into carboxylic function (Elboutachfaiti et al. 2011; Pierre et al. 2017). Hence, there are a multitude of examples in literature regarding laccase/TEMPO-mediated oxidation of glucans such as galactomannan, starch, arabinoxylan, glucomannan, chitosan (Mathew and Adlercreutz 2009; Botelho da Silva et al. 2018; Pierre et al. 2017), and more especially β-(1,4)-glucans such as cellulose (nano- and microfibrillar) from bleached softwood kraft pulp and paper industry which were efficiently oxidized by laccase/O_2/TEMPO system to generate mimetics of microbial glucuronan fractions made up of β-(1,4)-polyglucuronic acid sodium salt blocks (Elboutachfaiti et al. 2011; Jausovec et al. 2015; Pierre et al. 2017; Jiang et al. 2017; Slagman et al. 2018).

Nonetheless, by using this enzymatic system mediated by laccase/TEMPO process, the reaction time have to be important to obtain high oxidation level of glucans and the ratio of carbonyl/carboxyl group is closely related to experimental conditions such as temperatures, laccase, and TEMPO concentrations (Elboutachfaiti et al.

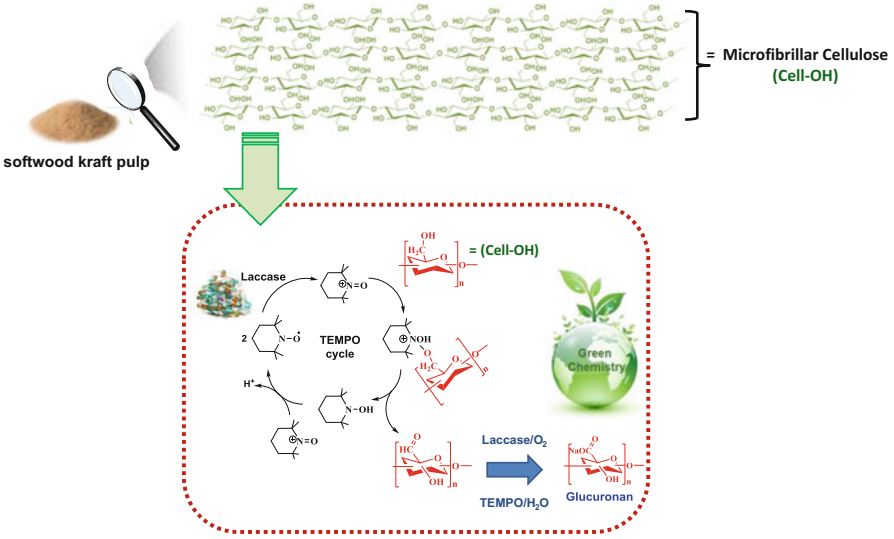


Fig. 3 Laccase/TEMPO-mediated oxidation of microfibrillar cellulose for the enzymatic production of β-(1,4)-glucuronan

2011; Pierre et al. 2017; Slagman et al. 2018). Consequently, to be sure to obtain highly structural mimetic of microbial β-(1,4)-glucuronan it appeared preferable to use the controlled chemical glucan oxidation with TEMPO/NaOCl/NaBr system which allowed the full C6 carboxylation of cellulosic fractions.

2.3 Enzymes Acting on Glucuronans

In the natural world, glucuronan is considered as a low abundant polysaccharide, compared with other existing polyuronates. However, the presence of β-(1-4)-linked polyglucuronate or related glucuronan-rich motifs in natural polymers (constituting exopolysaccharides and/or cell wall structure), described in some bacterial (Heyraud et al. 1993; Elboutachfaiti et al. 2011), fungal (de Ruiter et al. 1992; Elboutachfaiti et al. 2011), and algal (Elboutachfaiti et al. 2011, 2009) species, led to explore and identify active enzymes promoting the depolymerization of glucuronan. These enzymes currently known belong to polysaccharide lyases (PLs), referenced in the carbohydrate active enzyme (CAZy) database (http://www.cazy.org) and classified according to amino acids sequence similarities in 40 families (PL1-PL40) (Lombart et al. 2010), among which PL 5, 7, 14, 20, 31, and 38 are assigned to β-(1-4)-glucuronan lyases (GL). PLs are specifically active on polysaccharides containing uronic acids and cleave the O-C4 bond to the uronic acid via a β elimination reaction, leading to a 4-deoxy-*L- erythro*-hex-4-enopyranosyluronate at the new nonreducing end of the product. The catalytic mechanism has been suggested to required three steps consisting in: (i) the neutralization of negative charge on the C6 carboxyl group, (ii) the abstraction by a general base of the C5 proton at subsite +1; and (iii) the β-elimination of the O-C4 glycosidic bond with simultaneous formation of C4-C5 double bond at the nonreducing end (Yip and Withers 2006).

Currently, the identification of glucuronan lyases activities or enzymes remains very limited and restricted to some organisms such as bacteria, fungi, and virus (Table 2). An endopolyglucuronan lyase was firstly purified by anion-exchange chromatography from *Sinorhizobium meliloti* bacterial strain M5N1CS, producing a high- and low-molecular-weight (1,4)-β-D-polyglucuronic acid partially acetylated at C-2 and/or C-3 position (Da Costa et al. 2001; Heyraud et al. 1993). This enzyme, identified as a monomer with a molecular mass of 20 kDa, was demonstrated specifically active on deacetylated and mainly 3-*O*-acetylated glucuronans, with a randomly endolytic activity on glucuronan substrate, resulting in oligoglucuronans with degree of polymerization (DP) of 2 to >7 from glucuronans degradation (Da Costa et al. 2001). On the same way, two cellouronate lyases were isolated from the bacterial strain *Brevundimonas* sp. SH203. The CUL-I and CUL-II enzymes synergistically perform, with respectively an endo- and exolytic mode, the depolymerization of the cellouronate, a (1,4)-β-D-glucuronan obtained by TEMPO-mediated oxidation from regenerated cellulose (Konno et al. 2006, 2008). Among fungi, the *Trichoderma* sp.GL2 strain was identified as producing an extracellular glucuronan lyase (TrGL), when cultivated on minimal medium as sole source of carbon (Delattre et al. 2006). The purified enzyme of 27 kDa, requiring

Table 2 Glucuronan lyases (GL) enzymes characterized in various species

PL Family	Organism/Strain	GL activitiy mode	Protein identification number	References
PL5	*Stenotrophomonas maltophilia* K279a, mutant H208F	Exo	*GenBank: SNW08053.1*	Mac Donald et al. (2016)
PL7	*Catenulispora acidiphila* DSM 44,928	ND	*GenBank: ACU70527.1*	Helbert et al. (2019)
PL14	Chlorovirus CVK2	Endo	*DDBJ accession number 044791*	Sugimoto et al. (2004)
PL20	*Trichoderma reesei*	Endo	*GenBank: BAG80639.1*	Konno et al. (2009a)
PL31	*Saccharophagus degradans* 2–40 *Streptomyces hygroscopicus*	Endo	*GenBank: ABD82242.1* *GenBank: AGF62897.1*	Helbert et al. (2019)
PL38	*Brevundimonas* sp. SH203	Endo and exo	*GenBank: GAW41138.1*	Kikuchi et al. (2020)
ND	*Sinorhizobium meliloti M5N1CS*	Endo	*ND*	Da Costa et al. (2001)

ND Non-determinated

bivalents ions (Ca^{2+}, Mg^{2+}, Li^{2+}, Mn^{2+}) as cofactors for its enzyme activity, was shown functionally active on all glucuronans, with a variable efficiency depending to the acetylation rate of the substrate, and also on ulvan, a glucuronorhamnoxyloglucan extracted from *Ulva lactuca* (Delattre et al. 2006). The production of glucuronan lyase by virus was also reported, notably with the example of the chlorovirus CVK2 expressing the vAL-1 enzyme, in part involved in the cell wall degradation of the *Chlorella* host cells (Sugimoto et al. 2000, 2004).

The identification of the genes encoding glucuronan lyases in some organisms has considerably contributed to acquire knowledge of structural features and families classification of these enzymes. Based on amino acids sequence similarities, the glucuronan lyases have been distributed within six families of PLs: PL5, PL7, PL14 (vAL-1), PL20 (TrGL), PL31, and PL38 (CUL-1) (CAZy database, http://www.cazy.org) (Sugimoto et al. 2004; Konno et al. 2009a; Helbert et al. 2019; Kikuchi et al. 2020). Although the amino acids sequences may be highly divergent between the different families, the catalytic domains adopt a classical β-jelly roll-type structural scaffold, except for PL5 having an α/α-barrel organization. Interestingly, several studies underlined some convergences between the glucuronan lyases with the alginate lyases that may suggest a relatively close evolutionary process of these enzymes. For example, the alignment of β-jelly roll structures of the glucuronan lyase TrGL and PL7-A1 alginate lyase revealed the overlap of secondary features and the conservation of some amino acids catalytic residues (Konno et al. 2009b). In addition, the mutation (H208F) of an alginate lyase (PL5) from the *Stenotrophomonas maltophilia* K279a (Smlt2602) conferred the acquirement of a significant exolytic poly-β-D-glucuronic acid activity by the mutant strain (Mac Donald et al. 2016).

Currently, the genomic and metagenomic data related to polysaccharide lyases allowed to identify and list at CAZy server many PLs for several organisms, which remained unexplored functionally. The contribution of research in activity and structural definition of "non classified" enzymes should lead to discover new PLs, among which glucuronan lyases, and could contribute to a better understanding of the large diversity of theses enzymes.

3 Succinoglycans

3.1 Sources and Structures of Microbial Succinoglycans

Succinoglycan is a high-molecular-weight ($>1 \times 10^6$ Da) heterogeneous polysaccharide with a branched repeating unit consisting of seven (1,3), (1,4), (1,6), and (1,4; 1,6)-linked β-D-glucoses and one (1,3)-linked β-D-galactose residues, associated to succinic and pyruvic acids adducts (Reinhold et al. 1994). Its basic structure is shown in Fig. 4. It is produced by bacteria belonging to *Agrobacterium*, *Sinorhizobium*, *Rhizobium*, *Pseudomonas*, and *Alcaligenes* genera when carbohydrates are used as carbon source. These bacteria often produce other EPSs. In several rhizobia an acetate substitution is also present on the main chain but not in agrobacteria. Pyruvate is always present in a stochiometric ratio whereas succinyl and acetyl ratios depend on culture conditions and bacterial species.

The backbone of succinoglycan is composed of a tetrasaccharididic β-(1,4/3)-D-galactoglucan. The side chain is linked to it by a β-(1,6) glycosidic linkage to the terminal glucose unit and has the following structure: [β-(1,3)-D-Glc-β-(1,3)-D-Glc-β-(1,6)-D-Glc-β-(1,6)-D-Glc]. The terminal glucose of the side chain contains the pyruvate acetal O4 and O6 linked whereas the other β-(1,3)-linked D-Glc of the side chain is O6 connected with the succinate. The O-acetyl is present on the main chain of some suvccinoglycans O6 linked to a Glc unit. This structure was fully elucidated using methylation analysis,$^{1}H/^{13}C$ NMR spectroscopy but also electrospray ionization and collision-induced dissociation and tandem mass spectrometry after methylation and deuteriomethylation of the octamer. ^{1}H NMR spectroscopy gave access to to chemical shifts of CH_3 protons of pyruvate and acetyl groups but also to methylene protons from succinyl one. ^{13}C NMR revealed the chemical shift

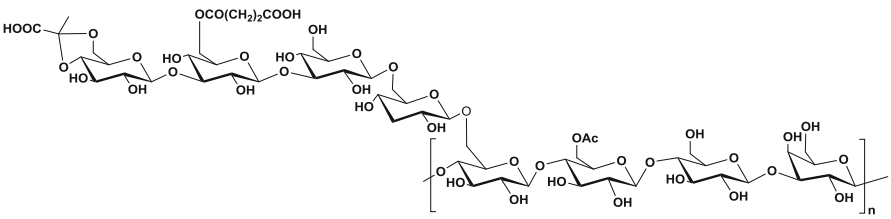


Fig. 4 Chemical structure of succinoglycan

of CH_3 carbon of pyruvate (25.7 ppm), CH_2 carbons of succinate (32.4 and 31.2 ppm), and acetyl carbon (102.2 ppm).

Contrary to glucuronan, the biosynthesis pathway of succinoglycan is well known as it was abundantly studied in *Sinorhizobium meliloti* in 1990s. It consists of the assembly of nucleotide sugars (UDP-glucose formed from glucose-1-phosphate by a phosphoglucomutase being the precursor) in an octasaccharide repeating unit and the addition of acetate, succinate, and pyruvate before its polymerization and exportation. A large *exo*/*exs* cluster located on megaplasmid pSymB is responsible of the succinoglycan biosynthesis (Halder et al. 2017). Succinoglycan is the first EPSs identified as a molecule signal for numerous *Rhizobium* and *Sinorhizobium* invasions of leguminous nodules. The biological role of succinoglycan for other producers such as *Pseudomonas* or *Agrobacterium* is unknown but seems to be not implicated in interactions with plants.

The production of succinoglycan has been abundantly documented in regards to its potential as texturing agent. Classical strategies of industrial microbiology have been applied to its production, including fed batch, as well as continuous and batch fermentations (Stredansky 2002). As observed with other production of soluble microbial EPSs, the rheology of broths limited the mixing of culture media but also the heat and gaz transfer with a negative impact on succinoglycan yields. Indeed oxygen availability in the reactor depend mostly on agitation and unlimited oxygen cultures giving the higher succinoglycan yields. This problem has been successfully overcame at the laboratory scale using solid-state fermentation (Stredansky and Conti 1999). The culture media for succinoglycan production in reactor by strains of *Agrobacterium*, *Pseudomonas*, or *Rhizobium* are not complex and with high C/N substrates ratio. They include a salt buffer, a low level of other mineral such as Mg^{2+} source, nitrogen, and carbohydrate sources. The nitrogen sources in industrial production are nitrates or ammonium salts even if other sources such as yeast extract, lysine, or glutamate are used. The carbohydrate sources are mainly sucrose even if glucose, maltose, and polyols such as mannitol at concentrations between 1% and 5% (w/v) give good yields. The pH has to be maintained around 7 and the optimal temperature of culture is 30 °C.

Batch processes are the more commonly used to produce succioglycan as they are much either to implement in industry with cheap substrates. They give also the higher final concentration in a time of culture of 3–4 days. Other processes such as continuous or fed batch ones have been also published but not really developed for large-scales productions. Indeed, even if continuous or fed batch processes have major advantages (productivity, for example), they have some disadvantages such as stability of strains, risk of contamination, and control of the dilution rate. Whatever the fermentation process, the extraction procedure is commun to other microbial hydrocolloids. Firstly the biomass is extracted from the broths using speraration technics such as filtration or centrifugation. The cell-free broths are then concentrated (or not) before to be dried to obtain low-grade succinoglycan or can be precipitated using polar solvent and mainly ethanol or isopropanol befor to be collected and dried leading to high-grade succinoglycan.

3.2 Enzymes Acting on Succinoglycan

The identification of the succinoglycan degrading enzymes was impaired by the relatively complex structure of this polysaccharide and its resistance to several β-D-glucanases (Amemura et al. 1974). The first successful approach was performed by the screening of the microorganisms able to grow on medium containing succinoglycan as the sole source of carbon, and thus likely producing succinoglycan depolymerizing enzymes (Oyaizu et al. 1982). Such an approach allowed the isolation and purification of an extracellular β-D-glucanase of 180 kDa (succinoglycan depolymerase) from the *Flavobacterium* sp. M64 strain (Amemura et al. 1974). The succinoglycan depolymerase, whose production is induced by the presence of succinoglycan or desuccinylated succinoglycan (D-SG) as sole carbon source, was reported to depolymerize both D-SG and succinoglycan to yield a polymer with a degree of polymerization, firstly estimated to 12 and finally considered as 8 (Amemura et al. 1974; Hisamatsu et al. 1978). The high specificity of the enzyme was clearly demonstrated for succinoglycan and D-SG, when no depolymerization activity was observed for others oligo- and polysaccharides, containing β-(1,3), β-(1,4) or β-(1,6) glycosidic bonds (Amemura et al. 1974). Due to its specificity, the succinoglycan depolymerase was used to elucidate the structure of polysaccharides related to succinoglycan, and has notably led to define octasaccharide as repeating units forming the polymer chain of polysaccharides elaborated by *Rhizobium*, *Alacaligenes*, and *Agrobacterium*, (Reinhold et al. 1994). In parallel to the action of the succinoglycan depolymerase, an intracellular endo-(1,6)-β-D-glucanase was isolated from *Flavobacterium* M64, and was shown to be involved in the hydrolyzing of desuccinylated octasaccharide to two tetrasaccharides, one composed of D-glucose and pyruvic acid in a molar ratio 4:1 and the other composed of D-glucose and D-galactose in a molar ratio 3:1 (Abe et al. 1980). The combination of the succinoglycan depolymerase and endo-(1,6)-β-D-glucanase enzymes on different succinoglycans from diverse microorganisms has led to the same tetrasaccharide hydrolysis products, confirming the synergistic action of these enzymes (Fig. 5) (Abe et al. 1980; Reinhold et al. 1994).

The ability for some microorganisms to regulate the size of their exopolysaccharides has suggested the hypothesis of the production of endogene glycanases acting on the depolymerization of these polymers. *Rhizobium meliloti* was described to produce in culture both a high-molecular-weight (HMG) succinoglycan (Wang et al. 1999) and low-molecular-weight (LMG) forms, including monomers, trimers, and tetramers of the octasaccharide-repeating unit (Battisti et al. 1992; Leigh and Lee 1988). Two endo-(1,3)-(1,4)-β-glycanases, ExoK and ExsH, were identified functionally active for the cleavage of succinoglycan from *R.meliloti*, only during the ongoing synthesis of succinoglycan by bacteria, but not efficient on succinoglycan present in cell-free medium (Glucksmann et al. 1993; York and Walker 1998). The physiologically role of ExoK and ExsH, specifically acting on nascent succinoglycan is clearly different from the succinoglycan depolymerase from *Flavobacterium.*

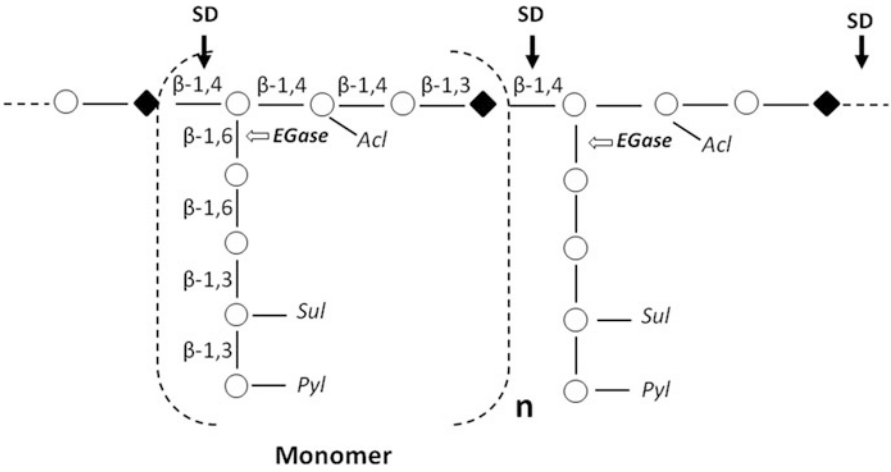


Fig. 5 Action of glucanases from *Flavobacterium* in the hydrolysis of succinoglycan. *SD* succinoglycan depolymerase, *EGase* Endo-(1,6)-β-D-glucanase, *Acl* Acetyl, *Sul* Succinyl, *Pyl* Pyruvyl, O Glucose, ♦ Galactose

In conclusion, succinoglycan is a relatively complex structure which requires specific enzymes, remaining still little known, to ensure the degradation of this polysaccharide.

4 Rheology of Glucuronan and Succinoglycan

One of the main advantages of microbial extracellular polysaccharides over plant and seaweed polysaccharides is the possibility to induce constant chemical and physical properties (Manivasagan and Kim 2014) providing desirable and stable characteristics for high-value technological applications. Many authors have underlined the theoretical and practical value of the knowledges related to the rheological behavior of polysaccharides aqueous solutions through relations between viscoelastic flow properties, the chain structural conformation, and other physicochemical properties crucial for establishing potential applications area for these polymers.

Microbial glucuronan and succinoglycan are water-soluble biopolymers at pH close to neutral and at room temperature and could have a significant commercial value related to interesting and specific features such as viscous, thickening, and gelling property. However, only a few studies detailed the rheological behavior of these polymer solutions. The main factors that affect the viscoelastic properties are classified as relative to the polymer-solvent system (as the polymer concentration, size and shape, the polydispersity index, the nature and the concentration of the solvent cations, and the interactions between polymer chains and between polymer and solvent) and relative to the operating parameters (shear rate, temperature, etc.).

Heyraud et al. (1993) reported about the thickening properties of low concentrated (1 $g.L^{-1}$) solution of partially acetylated (16% of O-acetyl groups (w/w))

extracellular (1,4)-β-D-glucuronan produced by fermentation by a mutant strain of *Rhizobium meliloti* M5Nl. At high concentrations, the polymer produced a thermoreversible gel in monovalent cation media and a thermally stable gel in presence of divalent cation (as Ca^{2+}) for temperatures lower than 100 °C (Heyraud et al. 1993). The gel strength increased when the degree of acetylation of the polymer decreased. The same authors proposed an empirical relationship between the intrinsic viscosity and the molecular weight close to models proposed for sodium alginate. The acetylation degree of glucuronan also influences the polymer solubility as high acetyl content promotes interchain associations and aggregate formation. Hence, Elboutachfaiti et al. (2011) suggested that the solubility is higher for less acetylated exocellular β-(1,4)-D-glucuronan from *Rhizobium meliloti* M5N1CS strain.

Navarini et al. (1992) studied the viscoelastic properties of a mixture of succinoglycan and galactoglucan produced by *Rhizobium meliloti YE-2* grown under different osmolarity conditions. The proportion of succinoglycan increases at high osmolarity (0.4 and 0.6 M) till 85% and as this polymer adopts an ordered conformation in solution, the mixture forms a "weak gel" instead of an entangled system as at low osmolarity (0.0 and 0.2 M) galactoglucan is dominant. The viscoelastic behavior of polymer mixture recovered from the culture media of 0.4 M osmolarity is similar to that obtained for xanthan/guar mixture and the shear-rate-dependent behavior is shear thinning for all osmolarities. Succinoglycan produced by *Pseudomonas* sp. NCIB 11592 exhibits reversible pseudoplastic behavior in dilute and semi-dilute solutions (Gravanis et al. 1990). Viscosity is sensitive to the ionic strength since a decrease was observed when ionic strength increases. This is explained by the worm-like chain behavior of the polymer as a result of electrostatic interactions when cations are present in the solvent.

The overlap parameter C*[η] for succinoglycan solubilized in 0.1 M NaCl was estimated at 1.3. In comparision to xanthan, the succinoglycan exhibites a sharper conformational transition with a greater decrease in the viscosity explained by the greater flexibility of the chain conformation.

Simsek et al. (2009) correlate physical, chemical, and rheological properties of succinoglycans of different molecular weights produced by four different strains of *Sinorhizobium meliloti* strains. The rheograms show typical shear thinning behavior following a power law model for all samples and a flow index between 0.61 and 0.79. Oscillatory dynamic tests have shown a more elastic behavior at high frequencies and various degrees of entanglement of these molecules. A low abundance of succinyl group favors the elasticity and viscosity of succinoglycans.

Andhare et al. (2017) proved that succinoglycan produced by *Rhizobium radiobacter* strain CAS has a high industrial significance due to its higher viscosity at the same concentration (1% aqueous solution) regarding widespread commercial biopolymers such as xanthan, guar gum, and sodium alginate. The rheological analysis shows that the polymer solution exhibits high stability at several thermal cycles, high temperatures, large pH range, and wide electrolytes concentrations (mono- and divalent cations) and presents succinoglycan as a great rheological modifier/stabilizer in food and pharmaceutical industries.

5 Conclusions

The developments of succinoglycan and glucuronan applications is clearly limited by the market competition of other microbial and above all terrestrial plants or seaweeds polysaccharides. Indeed, these two anionic and water-soluble rhizobacterial EPSs with varied biological and physicochemical properties should be more investigated for their commercialization and for their costs of production. Process dévelopments to increase the yield of production and metabolic engineering could be two levers to increase their marketization. Process developments should focus on improvement of fermentation techniques with the aim to a better oxygen diffusion in the culture medium and a limited foam formation in the reactor. The oxygen and nutrients diffusion being currently the main limiting factor implements the mixing of systems. Moreover, the extraction and purification processes avoiding high water and/or alcohol consumptions could be also a part of the production process to implement. However the metabolic engineering of the production strains is probably the largest potential of innovation for the production of these two polysaccharides. The metabolic engineering should deregulate the biosyntheis pathways of these two EPs but also to inactivate polysaccharide hydrolases and polysaccharide lyases expressed by the producing strains probably to regulate the molecular weights of these biopolymers. For that a better understanding of biosyntheis pathway of glucuronan should be of first importance. Moreover, majority of patents focusing on succinoglycan and glucuronan applications are older than 15 years and will have shortly no more patented protections.

References

Abe J, Amemura A, Harada T. An endo-(1,6)-β-D-glucanase of Flavobacterium M64 hydrolyzing the octasaccharide repeating unit of succinoglycan to two tetrasaccharides. Agric Biol Chem. 1980;44:1877–84.

Amemura A, Moori K, Harada T. Purification and properties of a specific, inducible β-D-glucanase, succinoglycan depolymerase from *Flavobacterium*. Biochim Biophys Acta. 1974;334:398–409.

Andhare P, Delattre C, Pierre G, et al. Characterization and rheological behaviour analysis of the succinoglycan produced by Rhizobium radiobacter strain CAS from curd sample. Food Hydrocoll. 2017;64:1–8.

Aono R. The poly-a- and -b-1,4-glucuronic acid moiety of teichuronopeptide from the cell wall of the alkalophilic Bacillus strain C-125. Biochem J. 1990;270:363–7.

Battisti L, Lara J, Leigh J. Specific oligosaccharide form of the *Rhizobium meliloti* exopolysaccharide promotes nodule invasion in alfalfa. Proc Natl Acad Sci U S A. 1992;89:5625–9.

Botelho da Silva S, Krolicka M, Van Den Broek LAM, et al. Water-soluble chitosan derivatives and pH-responsive hydrogels by selective C-6 oxidation mediated by TEMPO-laccase redox system. Carbohydr Polym. 2018;186:299–309.

Braccini I, Heyraud A, Perez S. Three dimensional features of bacterial polysaccharide (1→4)-β-D-glucuronan: a molecular modeling study. Biopolymers. 1998;45:165–75.

Chatjigakis AK, Pappas C, Proxenia N, et al. FT-IR spectroscopic determination of the degree of esterification of cell wall pectins from stored peaches and correlation to textural changes. Carbohydr Polym. 1998;37:395–408.

Courtois J, Seguin JP, Declosmesnil S, et al. A (1→4)-β-D-glucuronan excreted by a mutant of *Rhizobium meliloti* M5N1 strain. J Carbohydr Chem. 1993;12:441–8.
Da Costa A, Michaud P, Petit E, et al. Purification and properties of a glucuronan lyase from *Sinorhizobium meliloti* M5N1CS (NCIMB 40472). Appl Environ Microbial. 2001;67:5197–203.
De Nooy AEJ, Besemer AC, Van Bekkum H. Highly selective nitroxyl radical-mediated oxidation of primary alcohol groups in water-soluble glucans. Carbohydr Res. 1995;269:89–98.
de Ruiter GA, Josso SL, Colquhoun IJ, Voragen AGJ, Rombouts FM. Isolation and characterization of β-(1–4)-D-glucuronans from extracellular polysaccharides of moulds belonging to Mucorales. Carbohydr Polym. 1992;18:1–7.
Delattre C, Michaud P, Elboutachfaiti R, et al. Production of oligocellouronates by biodegradation of oxidized cellulose. Cellulose. 2006;13:63–71.
Delattre C, Pierre G, Gardarin C, et al. Antioxidant activities of a polyglucuronic acid sodium salt obtained from TEMPO-mediated oxidation of xanthan. Carbohydr Polym. 2015;116:34–41.
Depree J, Emerson GW, Sullivan PA. The cell wall of the oleaginous yeast *Trichosporon cutaneum*. J Gen Microbiol. 1993;139:2123–33.
Dow JM, Darnall DW, Villa VD. Two distinct classes of polyuronide from the cell walls of a dimorphic fungus, Mucor rouxii. J Bacteriol. 1983;155:1088–93.
Elboutachfaiti R, Delattre C, Petit E, et al. Improved isolation of glucuronan from algae and the production of glucuronic acid oligosaccharides using a glucuronan lyase. Carbohydr Res. 2009;344:1670–5.
Elboutachfaiti R, Delattre C, Petit E, et al. Polyglucuronic acids: structures, functions and degrading enzymes. Carbohydr Polym. 2011;84:1–13.
Glucksmann MA, Reuber TL, Walker G. Family of glycosyl transferases needed for the synthesis of succinoglycan by *Rhizobium meliloti*. J Bacteriol. 1993;175:7033–44.
Gravanis G, Milas M, Rinaudo M. Rheological behaviour of a succinoglycan polysaccharide in dilute and semi-dilute solutions. Int J Biol Macromol. 1990;12:201–6.
Halder U, Banerjee A, Bandopadhyay R. Structural and functional properties, biosynthesis and patenting trends of bacterial succinoglycan: a review. Indian J Microbiol. 2017;57:278–84.
Harada T, Yoshimura T, Hidaka H, et al. Production of new acidic polysaccharide, succinoglycan, by *Alcaligenes faecalis* var. *myxogenes*. Agric Biol Chem. 1965;29:757.
Helbert W, Poulet L, Drouillard S, et al. Discovery of novel carbohydrate-active enzymes through the rational exploration of the protein sequences space. Proc Natl Acad Sci U S A. 2019;116:6063–8.
Heyraud A, Courtois J, Dantas L, et al. Structural characterization and rheological properties of an extracellular glucuronan produced by a *Rhizobium meliloti* M5N1 mutant strain. Carbohydr Res. 1993;240:71–8.
Hisamatsu M, Sano K, Amemura A, Harada T. Acid polysaccharides containing succinic acid in various strains of Agrobacterium. Carbohydr Res. 1978;61:89–96.
Isogai A, Saito T, Fukuzumi H. TEMPO-oxidized cellulose nanofibers. Nanoscale. 2011;3:71–85.
Jausovec D, Vogrincic R, Kokol V. Introduction of aldehyde vs. carboxylic groups to cellulose nanofibers using laccase/TEMPO mediated oxidation. Carbohydr Polym. 2015;116:74–85.
Jiang J, Ye W, Liu L, et al. Cellulose nanofibers prepared using the TEMPO/laccase/O_2 system. Biomacromolecules. 2017;18(1):288–94.
Khan T, Hyun SH, Park JK. Production of glucuronan oligosaccharides using the waste beer fermentation broth as a basal medium. Enzym Microb Technol. 2007;42:89–92.
Kikuchi M, Konno N, Suzuki T, et al. A bacterial endo-1,4-β-glucuronan lyase, CUL-I from *Brevundimonas* sp. SH203, belonging to a novel polysaccharide lyase family. Protein Expr Purif. 2020;166:105502.
Konno N, Habu N, Maeda I, Azuma N, et al. Cellouronate (β-1,4-linked polyglucuronate) lyase from *Brevundimonas* sp. SH203: purification and characterization. Carbohydr Polym. 2006;64:589–96.
Konno N, Habu N, Lihashi N, et al. Purification and characterization of exotype cellouronate lyase. Cellulose. 2008;15:453–63.
Konno N, Igarashi K, Habu N, et al. Cloning of the *Trichoderma reesei* cDNA encoding a glucuronan lyase belonging to a novel polysaccharide lyase family. Appl Environ Microbiol. 2009a;75:101–7.

Konno N, Ishida T, Igarashi K, et al. Crystal structure of polysaccharide lyase family 20 endo--β-1,4-glucuronan lyase from filamentous fungus *Trichoderma reesei*. FEBS Lett. 2009b;583:1323–6.

Leigh JA, Lee CC. Characterization of polysaccharides of *Rhizobium meliloti exo* mutants that form ineffective nodules. J Bacteriol. 1988;170:3327–32.

Lombart V, Bernard T, Rancurel C, Brumer H, Coutinho PM, Henrissat B. A hierarchical classification of polysaccharide lyases for glycogenomics. Biochem J. 2010;432:437–44.

Mac Donald LC, Weiler EB, Berger BW. Engineering broad-spectrum digestion of polyuronides from an exolytic polysaccharide lyase. Biotechnol Biofuels. 2016;9:43–55.

Manivasagan P, Kim SK. Extracellular polysaccharides produced by marine bacteria. Adv Food Nutr Res. 2014;72:79–94.

Mathew S, Adlercreutz P. Mediator facilitated, laccase catalysed oxidation of granular potato starch and the physico-chemical characterization of the oxidized products. Bioresour Technol. 2009;100:3576–84.

McNeil M, Darvill J, Darvill AG, Albersheim P, van Veen R, Hooykaas P, Schilperoort R, Dell A. The discernible, structural features of the acidic polysaccharides secreted by different *Rhizobium* species are the same. Carbohydr Res. 1986;146:307–26.

Navarini L, Cesdro A, Ross-Murphy SB. Exopolysaccharides from *Rhizobium meliloti YE-2* grown under different osmolarity conditions: viscoelastic properties. Carbohydr Res. 1992;223:227–34.

Oyaizu H, Komagata K, Amemura A, et al. A succinoglycan-decomposing bacterium, *Cytophaga arvensicola* sp. Nov. J Gen Appl Microbiol. 1982;28:369–88.

Pierre G, Punta C, Delattre C, et al. TEMPO-mediated oxidation of polysaccharides: an ongoing story. Carbohydr Polym. 2017;165:71–85.

Reinhold B, Chan S, Reuber L, et al. Detailed structure characterization of succinoglycan, the major exopolysaccharide of *Rhizobium meliloti* Rm 1020. J Bacteriol. 1994;176:2997–002.

Simsek S, Mert B, Campanella OH, et al. Chemical and rheological properties of bacterial succinoglycan with distinct structural characteristics. Carbohydr Polym. 2009;76:320–4.

Slagman S, Zuihof H, Franssen MCR. Laccase-mediated grafting on biopolymers and synthetic polymers: a critical review. Chem Bio Chem. 2018;19(4):288–311.

Stefke B, Windeisen E, Schwanninger M, et al. Determination of the weight percentage gain and of the acetyl group content of acetylated wood by means of different infrared spectroscopic methods. Anal Chem. 2008;4:1272–9.

Stredansky M, Conti E. Succinoglycan production by solid-state fermentation with agrobacterium tumefaciens. App Microbiol Biotechnol. 1999;52:332–7.

Stredansky M. Succinoglycan. Biopolymers. 2002;5:159–77.

Sugimoto I, Hiramatsu S, Murakami D, Usami S, Yamada T. Algal-lytic activities encoded by *Chlorella* virus CVK2. Virology. 2000;277:119–26.

Sugimoto I, Onimatsu H, Fujie M, Usami S, Yamada T. vAL-1, a novel polysaccharide lyase encoded by chlorovirus CVK2. FEBS Lett. 2004;559:51–6.

Tai C, Bouissil S, Gantumur E, et al. Use of anionic polysaccharides in the development of 3D bioprinting technology. Appl Sci. 2019;9(13):2596. https://doi.org/10.3390/app9132596.

Timell TE, Enterman W, Spencer F, et al. The acid hydrolysis of glycosides. II. Effect of substituents at C-5. Can J Chem. 1965;43:2296–305.

Wang LX, Wang Y, Pellock B, et al. Structural characterization of the symbiotically important low-molecular-weight succinoglycan of *Sinorhizobium meliloti*. J Bacteriol. 1999;181:6788–96.

Yip VLY, Withers SG. Breakdown of oligosaccharides by the process of elimination. Curr Opin Chem Biol. 2006;10:147–55.

York G, Walker G. The *Rhizobium meliloti* ExoK and ExsH glycanases specifically depolymerase nascent succinoglycan chains. Proc Natl Acad Sci U S A. 1998;95:4912–7.

Cyanobacterial Extracellular Polymeric Substances (EPS)

8

Rita Mota, Carlos Flores, and Paula Tamagnini

Contents

R. Mota
i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal

IBMC – Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal
e-mail: rita.mota@ibmc.up.pt

C. Flores
i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal

IBMC – Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal

Centre for Urological Biology, Department of Renal Medicine, University College London, London, UK
e-mail: c.flores@ucl.ac.uk

P. Tamagnini (✉)
i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal

IBMC – Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal

Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Porto, Portugal
e-mail: pmtamagn@ibmc.up.pt

J. M. Oliveira et al. (eds.), *Polysaccharides of Microbial Origin*,
https://doi.org/10.1007/978-3-030-42215-8_11

Abstract

Cyanobacteria are a very particular group of photoautotrophic prokaryotic organisms with major ecological significance in the carbon and nitrogen cycles. Most cyanobacterial strains produce extracellular polymeric substances (EPS), mainly heteropolysaccharides that can remain attached to cell surface or be released into the extracellular environment (RPS). These EPS play prominent roles on cell protection and biofilms/colonies formation, and have distinct features compared to other bacterial EPS. Here, we review the cyanobacterial EPS biological roles, what is known/can be inferred about their biosynthetic pathways, and describe their particularly complex composition/structure. In addition, we address how to optimize the production and yield, and how to tailor the polymers either by genetic manipulation or by chemical modification. At last, already implemented applications as well as promising ones are discussed foreseeing the commercialization of cyanobacterial EPS-based products.

Keywords

Cyanobacteria · Extracellular polymeric substances (EPS) · EPS applications · EPS biosynthesis · EPS characteristics

1 Introduction

Cyanobacteria are an ancient group of gram-negative prokaryotes with the ability to perform oxygenic photosynthesis, and many strains can also fix atmospheric nitrogen, making these organisms prominent players in the carbon and nitrogen cycles. Moreover, cyanobacteria are highly diverse morphologically, comprising species that are unicellular, form multicellular filaments, or grow in colonies. Some filamentous strains undergo cell differentiation, forming heterocysts, specialized cells involved in nitrogen fixation, and akinetes, resting cells that are able to endure environmental stress. Some have also the ability to produce hormogonia, small chains with different size, shape, and motility compared to the parent filament, essential for short-distance dispersal and host infection. The cyanobacteria long evolutionary history, autophototrophy, and diazotrophy are regarded as the main reasons for their success in modern habitats, allowing these microorganisms to thrive from fresh to salt water, soils, and extreme environments. Additionally, cyanobacteria managed to adapt their living styles according to the metabolic necessities, being able to live as planktonic free-living cells, in biofilms, aggregates, and complex mats, or even in symbiosis with a wide range of eukaryotic hosts (providing them combined nitrogen and/or carbon).

Most of the cyanobacterial strains are also able to produce extracellular polymeric substances (EPS) that can remain attached to the cell surface or be released into the surrounding environment – RPS (Rossi and De Philippis 2016). The development

and biotechnological application of added value products based on cyanobacterial EPS is increasingly attractive, since there is a constant implementation of restrictive regulation in the synthetic polymer industry to promote environmental preservation. This led to an increase in the global market demand for products with a lower ecological footprint ("green materials"), even considering the production costs, downstream processing, and some batch inconsistency in quantity/quality of the final product (Singh et al. 2019). The natural polymers are derived from renewable resources and by definition, they present several competitive advantages compared to the recalcitrant petroleum/oil-based products, such as biodegradability, eco-friendliness, and often biocompatibility. Furthermore, production of bacterial EPS at the industrial scale presents important advantages compared to the production of biopolymers from other natural sources: (i) they are generally actively secreted by the cells, being easy to extract and usually do not need a challenging purification process reducing the production costs; (ii) are not ruled by the stringent environmental measures applied in the production of polymers using animals or plants (e.g., ethics and animal protection or deforestation preventive measures); (iii) the strains used have usually fast growth rates accelerating the production process; and (iv) in some cases, the polymers and/or the producing strain can be easily functionalized/engineered to obtain a product with the desired properties and/or enhanced performance. Additionally, EPS production by cyanobacteria brings also two other major advantages, even compared to the production of bacterial EPS that are currently being commercially exploited: (i) cyanobacteria have extremely simple nutritional requirements, since their growth and EPS production do not depend on the addition of expensive EPS precursors or substrates, and (ii) the cyanobacterial EPS are more complex, which make them more versatile and with a broader spectrum of potential application fields, mainly in high-value market niches, such as cosmetic, pharma, and biomedicine (Singh et al. 2019). However, despite all the already available data, the cultivation, extraction, and purification of these polymers is not yet implemented at industrial level allowing the control of fundamental requirements for clinical applications such as purity, stability, and safety. In addition, a detailed knowledge on the cyanobacterial EPS biosynthetic pathways is still required in order to enhance bespoke production of target EPS, and to engineer structural and compositional variants tailored for a given application. Therefore, the main aim of this chapter is to provide the state of the art on cyanobacterial EPS biosynthesis, characteristics, and putative biotechnological applications, seeking to contribute to the implementation of cyanobacterial polymers or products based on cyanobacteria polymers on the market.

2 Cyanobacterial Extracellular Polymeric Substances (EPS) and Their Biological Roles

Cyanobacteria can produce EPS, mainly composed by complex heteropolysaccharides (but also containing proteins, nucleic acids, and lipids) that can remain attached to the cell surface as capsular polysaccharides (CPS), or be released into the surrounding environment (RPS). According to their thickness and

consistency, the EPS attached to the cell surface can be designated as sheaths (usually a thin, dense layer loosely covering cells or groups of cells); capsules (a thick and structurally coherent layer tightly associated with the cell surface); or slimes (a mucilaginous material dispersed around the cells but not reflecting their distinct shape) (Fig. 1) (Rossi and De Philippis 2016). The RPS biosynthetic process seems to diverge from the synthesis of CPS, and this hypothesis is mainly supported by differences in their composition, namely the presence of different monosaccharidic residues and sulfur content, *Synechocystis* sp. PCC 6803, and some of its EPS-related mutants (Pereira et al. 2019a).

The cyanobacterial EPS can have distinct biological functions, depending on their structure and complexity and, thus, their physicochemical properties. The most acclaimed EPS role is cell protection, since they constitute a physicochemical barrier surrounding the cell that protects it against several environmental and stress factors, such as desiccation, heat, ultraviolet radiation, high salts concentration, predation, infection, and other external agents (e.g., antibiotics and toxic metal cations) (Kehr and Dittmann 2015). EPS can also contribute to the concentration/sequestration of nutrients, namely of essential metal cations and minerals, in particular in environments

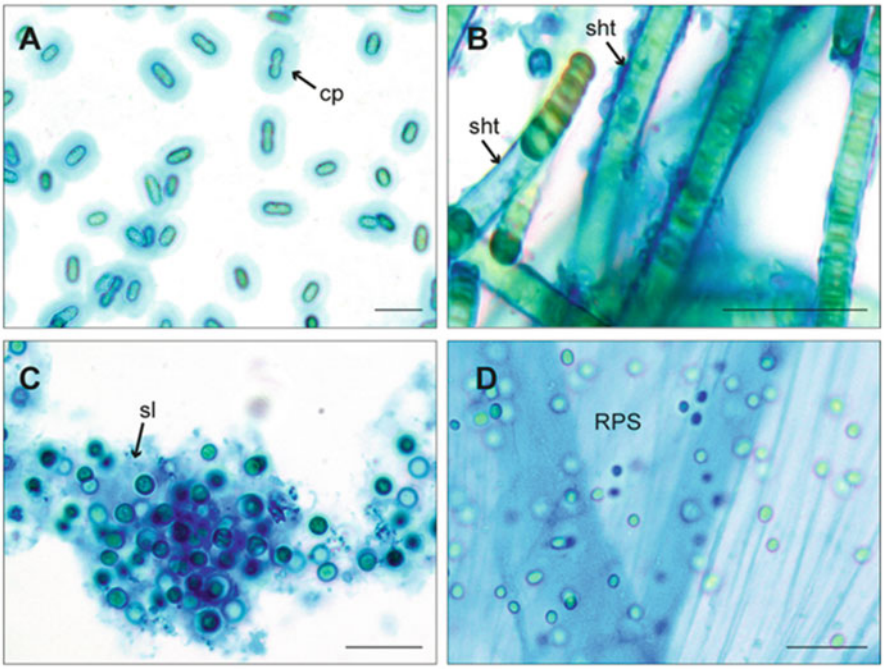


Fig. 1 Light micrographs highlighting extracellular polymeric substances (EPS) from several cyanobacteria stained with Alcian blue. (**a**) *Cyanothece* sp. VI 22, (**b**) *Calenema singularis* LEGE 06188, (**c**) *Geminobacterium atlanticum* LEGE 07459, and (**d**) *Cyanothece* sp. CCY 0110. *cp* capsule, *sht* sheath, *sl* slime, *RPS* released polysaccharides. (Images **b** and **c** were kindly provided by Ângela Brito. Scale bars: 15 μm)

where the availability is limited, e.g., marine. Metabolic imbalances can also represent a challenge for cyanobacteria, and EPS can serve as a sink for excess of energy if the carbon/nitrogen balance is shifted toward excess carbon (Pereira et al. 2019b). Moreover, it is widely known that the establishment of symbiosis between cyanobacterial symbionts and their hosts is mediated by glycans and lectins (Kehr and Dittmann 2015), and that EPS play a role in the gliding motility mechanism of hormogonia by slime extrusion through junctional pore complexes (Khayatan et al. 2015). Moreover, EPS can be involved in biofilm formation, having a major role in the mats complex microbial community structure. Furthermore, EPS can mediate cell adhesion to surfaces by a cohesive three-dimensional polymer network that allows cell-cell communication (either as vehicles of compounds or as quorum sensing molecules) and retention of extracellular enzymes (Rossi and De Philippis 2016). Likewise, they can contribute to cell aggregation and adhesion to solid surfaces and particles leading to the formation of biological soil crusts (Rossi et al. 2018). The contribution of cyanobacterial EPS to the establishment of colonies is also evident, and in the case of *Microcystis* they are involved in the initial steps of the formation of cell aggregates that can lead to the formation of toxic mucilaginous blooms with serious environmental and ecological hazard (Kehr and Dittmann 2015).

3 Biosynthesis

The intricate machinery underlying bacterial EPS biosynthesis have been extensively studied over the last two decades, mainly in pathogenic gram-negative and lactic acid gram-positive bacteria (Low and Howell 2018; Schmid 2018). In general, the biosynthetic process relies on three major steps (Fig. 2): (i) in the cytoplasm, energy-rich metabolites, mainly from glycolysis, react with monosaccharides to form nucleotide sugar precursors; (ii) glycosyltransferases catalyze the formation of glycosidic bonds by transferring sugar residues from the nucleotide sugars onto acceptor molecules (e.g., lipid carrier at the plasma membrane or other sugars) defining the composition of the polysaccharide backbone; and (iii) proteins in the cell envelope continue the assembly and/or polymerization and export of the polymer (participating in the control of both EPS composition and chain length). The last steps are quite conserved and mainly occur via three different pathways, the so-called Wzy-, ABC transporter- or synthase-dependent pathways (see Sect. 3.2).

3.1 First Steps and Modification Enzymes

The group of enzymes working in the first steps of bacterial EPS biosynthesis is highly diverse and heterogeneous, varying significantly between strains. Furthermore, the majority of these proteins are not exclusively dedicated to EPS production, being important players in the general (carbohydrate) metabolism. After the synthesis of nucleotide sugar precursors, carbohydrate-active enzymes (well known as CAZymes) with functional domains that degrade, modify, or create glycosidic bonds

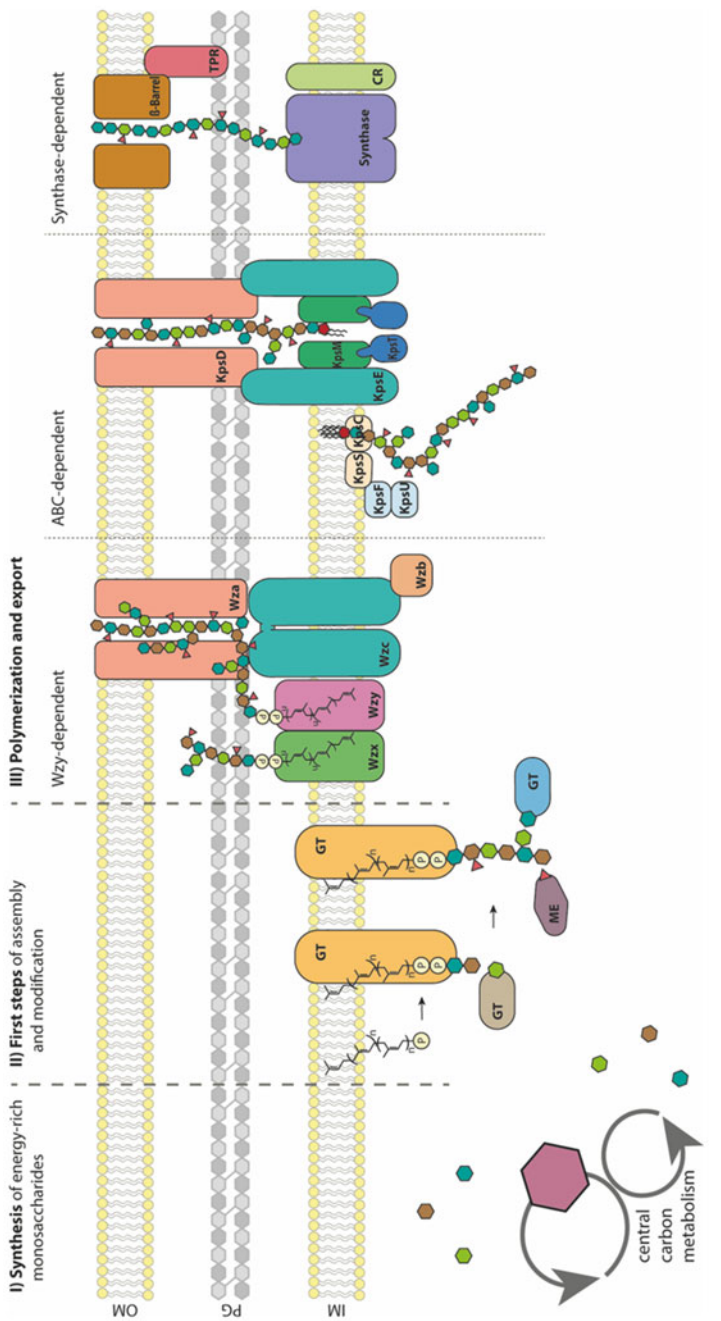


Fig. 2 Schematic representation of the main bacterial EPS biosynthetic pathways. (I) Nucleotide sugar precursors are formed in the cytoplasm; (II) the monosaccharides start to be assembled onto a lipid carrier by glycosyltransferases (GT) and modified by enzymes such as epimerases and other carbohydrate-modifying enzymes (ME); (III) the polymerization of the repeating units and export of the polymer through the cell envelope may occur by one of the three main mechanisms (Wzy-, ABC-, or synthase-dependent). In the synthase-dependent pathway, a lipid carrier may not be present, and the synthase accumulates the functions of GT and transporter. *IM* inner membrane, *PG* peptidoglycan, *OM* outer membrane, *TPR* tetratricopeptide repeat (TPR)-containing scaffold lipoprotein, *CR* c-di-GMP receptor

start the assembly and modification of the polymer or its repeating units (Lombard et al. 2014). Although a wide range of these enzymes have been identified in cyanobacteria, the majority is not biochemically characterized, being difficult to associate them specifically to EPS biosynthesis (Lombard et al. 2014).

The most studied group of CAZymes in the context of cyanobacterial EPS production are the glycosyltransferases (GTs). They have been mainly identified in filamentous strains as players involved in the production of the protective heterocyst envelope polysaccharide (HEP) layer, and in hormogonia contributing to their EPS-mediated gliding motility. For example, in *Anabaena* sp. PCC 7120, nine GTs encoded in a gene cluster, so-called "HEP island" (*alr2825-alr2841*) (Huang et al. 2005), and other three outside this cluster, *alr3698* (*hepB*), *alr3699* (*hepE*), and *all4160* (Wang et al. 2007), were shown to be essential for the HEP layer biosynthesis. In addition, other genes in the "HEP island" encode relevant proteins that act on the modification of the EPS, such as epimerases (*alr2827* and *alr2830*) and an oxireductase (*alr2831*) (Huang et al. 2005). The importance of 13 GTs-encoding genes for the production/secretion of hormogonia EPS was also reported for *Nostoc punctiforme* (Zuniga et al. 2020; Risser and Meeks 2013). These genes are either in EPS-related gene clusters (*hpsA-K*, *hpsL-P*, *hpsS-U*) or isolated, such as *hpsQ* and *hpsR*, and their deletion frequently leads to the loss of gliding motility by this strain (Zuniga et al. 2020; Risser and Meeks 2013). In unicellular strains, such as *Synechoccocus* species, GTs have been studied in the context of cellulose production, which can accumulate between the outer and inner membranes or be exported via synthase-dependent pathway (Zhao et al. 2015). These enzymes seem to be ancestral to the GTs involved in the plants cell wall formation. In *Synechocystis* sp. PCC 6803, five GTs encoded in the gene cluster *sll1722-sll1726* were also shown to be involved in EPS production (Foster et al. 2009). Moreover, Fisher et al. (2013) associated the gene cluster *slr0977-sll0575* to EPS biosynthesis, with genes encoding a GT (*slr0983*, *rfbF*) and carbohydrate-modifying enzymes, such as the epimerase (*slr0985*, *rfbB*) and the dehydratase (*slr0984*, *rfbG*). They also showed that the methyltransferase Slr1610 participates in *Synechocystis* EPS production, through the formation of carbohydrate-methylated residues that are most probably recognition sites for the end of the polymerization and/or EPS export. Moreover, another modification enzyme, the polysaccharide monooxygenase Sll1783 (glycoside hydrolase family), was shown to be involved in EPS degradation and uptake in this strain (Miranda et al. 2017). In addition, recent studies suggest that a wide range of carbohydrate-modifying enzymes might be involved in cyanobacterial EPS production, such as enzymes responsible for the addition of methyl, acetyl, pyruvyl, and/or sulfate groups (Rossi and De Philippis 2016), as well as extracellular enzymes responsible for the reutilization of EPS (Stuart et al. 2016).

3.2 Assembly and Export

In spite of the diversity of EPS producers and of the produced polymers, the proteins involved in the last steps of EPS assembly and export are relatively well conserved

among bacteria. This is a challenging process for the bacterium, since these large and usual hydrophilic molecules have to cross the cell envelope without compromising the critical barrier properties. In general, the EPS export follows one of the three main mechanisms (recently reviewed in Schmid 2018): the Wzy-, the ABC transporter-, or the synthase-dependent pathways (Fig. 2). The genes encoding proteins involved in these pathways are usually localized in large gene clusters in both gram-positive and gram-negative bacteria, nearby genes encoding sugar-activating/modifying enzymes, and glycosyltransferases. In the Wzy-dependent pathway, oligosaccharide repeating units linked to the lipid carrier are flipped across the plasma membrane by Wzx. Then, Wzy continues the polymerization task at the periplasmic face of the membrane. Both enzymes cooperate in order to control the polymer chain length. Subsequently, the translocation process of the polymer through the cell envelope is performed by the Wza/Wzc complex, which forms a transmembrane channel. Wzc also works as copolymerization unit in collaboration with Wzy, being implicated in the chain length control, and its activity is regulated by phosphorylation/dephosphorylation cycles, usually by a Wzb phosphatase.

In the ABC transporter-dependent pathway, the polysaccharide is fully polymerized at the cytoplasmic face of the plasma membrane. The KpsC/KpsS, KpsF, and KpsU are usually involved in the synthesis of the 3-deoxy-D-manno-oct-2-ulosonic acid (KDO) linker or its activated donor that connect the polysaccharide to a terminal lipid in the membrane. After that, the polymer is translocated across the plasma membrane by an ABC transporter complex formed by the KpsM and KpsT subunits. Finally, the export of the polymer is accomplished by the activity of the transmembrane complex KpsE/KpsD. KpsE and KpsD may form a transenvelope system analogous to Wzc and Wza, and they belong to the same protein families, the polysaccharide copolymerase (PCP) and the outer membrane polysaccharide export protein (OPX), respectively.

Regarding the molecular machinery involved in synthase-dependent pathways, it may differ significantly depending on the polymer produced (Low and Howell 2018). In general, a polysaccharide synthase embedded in the plasma membrane (e.g., Alg8 or BcsA) is responsible for the simultaneous polymerization and export of the polysaccharide. This process occurs in the presence or absence of a lipid acceptor molecule and it is controlled by the secondary messenger c-di-GMP. Meanwhile, other proteins in the periplasm may modify the polymer (e.g., AlgI, AlgF, and AlgG) or even degrade polymer surplus (e.g., AlgL or BscZ). The polymer is then guided and protected from degradation by a periplasmic tetratricopeptide repeat (TPR)-containing scaffold lipoprotein through a β-barrel porin, which will export it. This step can be performed by two independent proteins (e.g., AlgK and AlgE) or a single protein that combines both functions (e.g., BcsC).

In cyanobacteria, a phylum-wide analysis revealed homologs from the three aforementioned pathways among the 124 genomes analyzed, but often not the complete set defining one pathway (Pereira et al. 2015). In general, multiple gene copies were found in cyanobacterial strains with higher morphological complexity and larger genomes, while strains with reduced genomes, such as *Synechococcus* and *Prochlorococcus*, seem to have lost most of the EPS-related genes. This study

suggested that EPS production in cyanobacteria is much more complex compared to other bacteria, probably with more players involved, which may cooperate, cross talk, or display redundant functions. Recently, the involvement of some of these genes/proteins in EPS production has been elucidated, mainly using *Synechocystis* sp. PCC 6803 knockout mutants in homologs from the Wzy-dependent pathway: *sll1581* (*wza*), *sll0923* (*wzc*), and *sll0328* (*wzb*) (Pereira et al. 2019a; Jittawuttipoka et al. 2013), and the ABC-dependent pathway: *slr0977* (*kpsM*), *slr0982* (*kpsT*), *sll0574* (*kpsM*), and *sll0575* (*kpsT*) (Fisher et al. 2013). In general, these knockout mutants produce a lower amount of EPS that also vary in composition, compared to the polymer produced by the wild type. Moreover, and in agreement to what was previously observed for other bacteria, *slr0328* (*wzb*) encodes a phosphatase envisaged to be important for the regulation of Sll0923 (Wzc) activity, and consequently EPS export, but it might also interact with many other targets such as proteins involved in photosynthesis (Pereira et al. 2019a). In addition, *slr1875* (*exoD*) has also been associated to EPS assembly/export in *Synechocystis*, being a homolog of the *exoD* involved in the production of rhizobia EPS (Jittawuttipoka et al. 2013). However, it is still unclear whether ExoD cooperates with the conventional EPS assembly/export pathways.

In filamentous strains, mainly in *Anabaena* sp. PCC 7120, a few proteins responsible for the assembly/export of the HEP layer have been described, such as homologs from KpsT (Alr2835, HepA), Wzc (All1711, HepP), and Wzb (Alr5068) (Huang et al. 2005; Herrero et al. 2016). Furthermore, for the assembly and secretion of hormogonia EPS, two distinct mechanisms were proposed, either involving homologs to proteins of the Wzy-dependent pathway (Zuniga et al. 2020), or of the type IV secretion system (pili secretion) in cooperation with the well-known junctional pore complex (JPC) (Risser and Meeks 2013). In filamentous cyanobacteria, JPC components are encoded in the universally conserved hormogonia polysaccharide (*hps*) cluster, and homologs to these genes/proteins are rarely found in unicellular strains (Khayatan et al. 2015).

3.3 Regulatory Mechanisms

Up to now, very few cyanobacterial regulatory elements were undoubtedly associated to EPS production, and mainly in *Anabaena* sp. PCC 7120 and *Nostoc punctiforme*. In these filamentous cyanobacteria, the global nitrogen-control transcription factor NtcA was shown to be a key player in the regulation of the transport of glycosides, which has an indirect influence in the distribution of the building blocks for EPS biosynthesis (Herrero et al. 2016). Another global regulator, the alternative sigma factor SigJ was specifically associated with the heterocysts HEP layer formation, controlling the expression of genes involved in the first steps of EPS production (e.g., genes encoding glycosyltransferases), as well as in EPS assembly/export (Gonzalez et al. 2019). In addition, a two-component system involving the response regulator DevR (Huang et al. 2005), histidine kinases, such as HepK and HepN, and a serine/threonine kinase (HepS) (Fan et al. 2006) were also implicated in

the control of the synthesis of the HEP layer. Other crucial regulators for heterocysts differentiation and patterning also directly or indirectly influence the production of EPS by these cells, such as the Het and Pat regulators, sigma factors, and the noncoding RNA, NsiR1 (Herrero et al. 2016). For the production of hormogonia EPS, a few regulatory mechanisms have also been described, mainly for *Nostoc punctiforme*, such as the Hmp (hormogonium motility and polysaccharide) signal transduction system (Risser and Meeks 2013), and the partner-switching regulatory system involving the HmpU (phosphatase), the HmpV (with a STAS domain), and the HmpW (kinase) (Riley et al. 2018). These systems are controlled by a hierarchical sigma factor cascade, being primarily dependent on SigJ, and to a lesser extent on SigF, which interferes only with the regulation of the hormogonia polysaccharide (*hps)* gene cluster and the type IV secretion system (Gonzalez et al. 2019). This study also suggested that there is an overlap in the components involved in the synthesis of the HEP layer and hormogonia EPS. Moreover, and in agreement with the results from Gonzalez et al. (2019), the alternative group 3 sigma factor SigF was shown to influence several secretion pathways in *Synechocystis sp.* PCC 6803, including the production/export of EPS and the type IV secretion system (Flores et al. 2019a). The Δ*sigF* also exhibits cell envelope alterations, and a three- to fourfold increase in RPS production compared to the wild type.

The secondary messengers, cAMP and c-di-GMP, might also be involved in regulatory mechanisms of cyanobacterial EPS production, since they were already associated to other processes intrinsically related, such as cell motility, biofilm formation, and heterocyst differentiation (Agostoni and Montgomery 2014). In addition, the regulation by noncoding RNAs (ncRNAS) might also be important, similarly to what occurs in the control of the production of other cyanobacterial polysaccharidic polymers, such as glycogen (de Porcellinis et al. 2016). Most importantly, the abovementioned secondary messengers and several ncRNAS were also shown to be essential for EPS production in a wide range of bacteria (O'Toole and Wong 2016).

Overall, the knowledge about the regulatory network behind cyanobacterial EPS production is still very scarce, being the full picture far from elucidated.

4 Optimization of Production and Isolation Procedures

The use of cyanobacterial EPS for biotechnological applications depends on the knowledge of the physiological conditions that enhance the productivity, the desired characteristics (e.g., monosaccharidic composition, structure, and molecular mass), and consequently the functional properties of the polymer. This task is particularly difficult since there is a lack of systematic studies, and the influence of each factor is strongly strain dependent and, often, related to the cell growth (Mota et al. 2013; Pereira et al. 2009). In some strains, the EPS are produced under virtually all physiological conditions (possibly constitutively), while in others this depends on specific nutritional conditions, growth phase, or as a stress response (Delattre et al. 2016). For obvious reasons, and in contrast to their bacterial counterparts, light

(intensity and quality) is most probably the main factor influencing cyanobacterial EPS production (Fig. 3). For some strains, the culture exposure to continuous light or high-light intensities enhances EPS production (Mota et al. 2013). In other cases, specific light wavelengths stimulate production, e.g., UV radiation for *Nostoc punctiforme* (Soule et al. 2016) or red light for *Nostoc flagelliforme*, which also change significantly the monosaccharide composition of their CPS and RPS (Han et al. 2015). Light may also cause temperature fluctuations (e.g., due to heat generation), resulting in alterations on EPS productivity. Although only a few studies addressed the impact of temperature on cyanobacterial EPS production (and often in combination with changes in light intensity), it seems that the production tends to be higher under slightly higher temperatures than the ones used for optimal cell growth. However, in other cases, no effect or even slight reductions in EPS production were also detected (Pereira et al. 2009). Moreover, higher temperatures compared to the ones used for optimal cell growth also altered the monosaccharide composition of the CPS bulk from a biofilm formed by the cyanobacterium *Synechocystis* and the algae *Chlorococcum* (Di Pippo et al. 2012). Nutrient availability (namely carbon, nitrogen, phosphate, and sulfate) and its cell ratio (e.g., C:N) are also very important for cyanobacterial EPS production, thus selecting the most appropriate culture medium is vital (Rossi and De Philippis 2016). The presence of a combined nitrogen source, such as nitrate, ammonium, or urea, usually results in increased EPS production, probably by requiring less energy than nitrogen fixation. However, nitrogen starvation may also lead to higher production due to the increase of C:N

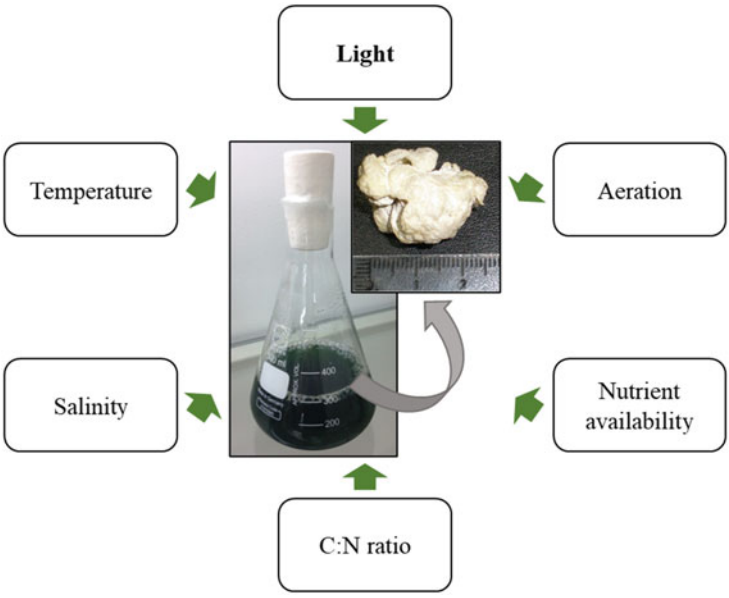


Fig. 3 Key parameters influencing the production of cyanobacterial extracellular polymeric substances (EPS)

ratio, and the availability of different nitrogen sources may affect EPS composition and/or cyanobacterial productivity. Furthermore, it is known that under low carbon dioxide mass-loading rates the conversion of CO_2 is mainly to biomass, while under high CO_2 mass-loading rates, the main routes are toward the synthesis of EPS and volatile organic compounds (Pereira et al. 2019b). Nutrient limitation/starvation, as depletion of phosphorous and sulfate sources, trace metals (e.g., calcium, magnesium, iron, and potassium), or vitamins (e.g., cyanocobalamin), is a common strategy used to trigger EPS synthesis and accumulation, however this can lead to cell growth impairment (Delattre et al. 2016). Therefore, this strategy is only used for batch culture systems, consisting in an initial growth phase with nutrients supply and then a production phase under nutrient limitation. Aeration can also be important for EPS production, by promoting a physical separation of cell bound EPS or simply a better stirring of the culture improving light penetration and nutrient availability (Pereira et al. 2009). In addition, increased salinity is a common stimulator of carbohydrate synthesis, mainly for compatible solutes production, but also for EPS production, and may also contribute for the alteration of their monosaccharide composition (Pereira et al. 2009).

Nowadays, most large-scale production of cyanobacteria occurs in outdoor open ponds that are relatively inexpensive, but several aspects, such as temperature, light distribution, and microbial contamination, are difficult to control. These raceway ponds are used worldwide to grow the most commercially exploited cyanobacterium *Arthrospira platensis* (Spirulina), where microbial contamination can be overcome through highly selective operating conditions, namely high pH (Takenaka and Yamaguchi 2014). However, other commercially interesting strains do not tolerate high pH, temperature, and salt concentration. Therefore, a variety of closed photobioreactor configurations have been developed to suit the particular characteristics of cyanobacteria, including flat panels reactors (vertical or alveolar), tubular reactors (horizontal or helical), cascade reactors, or bubble columns. The selection of the appropriate system should be based on several factors, such as the strain characteristics, the costs of land, energy, nutrients and labor required, the availability of water, the suitability of climate, and the specification of the final product (Takenaka and Yamaguchi 2014). In particular, the cultivation of EPS producers may result in some drawbacks, namely higher grazing susceptibility and slow growth due to redirection of nutrients and energy for EPS production. Furthermore, in an industrial context, it may lead to some problems of cultivation and cell harvesting, due to cell adhesion to the bioreactors and/or the impairment of culture mixing/aeration. Therefore, to overcome these obstacles, and after lab-scale optimization, several issues should be taken into consideration for each strain, such as the inoculum quality (age and concentration), the photobioreactor design, and the downstream processing (Pierre et al. 2019).

Following the optimization of the conditions to increase the EPS production, it is necessary to develop economic, effective, and environmentally friendly protocols for the isolation and purification of the polymers, depending on their cellular location. To detach the EPS from the cell surface it is necessary to apply physical methods, such as sonication, heating or cation exchange resin, and/or chemical methods, such

as the use of ethylenediaminetetraacetic acid (EDTA), formaldehyde, sodium hydroxide (NaOH), or glutaraldehyde as detaching agents (Pereira et al. 2019b). However, it is essential to cause minimal cell lysis and disruption in the EPS structure. The EPS are then separated from the cells by centrifugation or filtration, and the supernatant/filtrate is precipitated using an absolute alcohol, such as ethanol, methanol or isopropanol, or acetone (two–three volumes for one volume of supernatant/filtrate) (Flores and Tamagnini 2019). However, the polarity of the alcohol and the temperature of precipitation can influence the EPS yield and coprecipitate impurities (Delattre et al. 2016). Dialysis and membrane techniques can be used to remove the impurities, such as salts from the culture medium and nucleic acids (Flores and Tamagnini 2019). To increase the purification degree of EPS fractions it might be necessary to remove nonpolysaccharidic molecules, as proteins, pigments, or salts, with trichloroacetic acid treatment, tangential ultrafiltration, or selective alcoholic precipitation (Delattre et al. 2016). Finally, the EPS should be dried, usually by spray-, freeze-, or oven-drying. It should also be taken into account that the extraction protocol used can affect the quality and amount of EPS extracted, particularly the harsh procedures generally applied for removing EPS attached to the cells (Rossi and De Philippis 2016). Moreover, all the downstream processing need to be improved and optimized for high-efficient industrial scale-up to avoid expensive and time-consuming drawbacks, e.g., contamination, membrane clogging, and loss of yield of the final product. Therefore, alternative strategies for EPS extraction and purification are being proposed, such as nanofiltration using antifouling nanocomposite membranes, and ultrasound- and microwave-assisted extraction, but it is still necessary to consider the advantages and disadvantages for each specific strain/product (Delattre et al. 2016).

5 Composition and Structure

Cyanobacterial EPS consist of repeating units built from monosaccharides resulting in molecules several hundred kDa in size, with a molecular mass that can surpass 2 MDa (Pereira et al. 2009). These EPS possess a large number of different monosaccharides (up to 13), which is a unique feature, since bacterial EPS frequently contain up to four monosaccharide building blocks. Therefore, the cyanobacterial polymers have several repeating units and branching points, resulting in a complex structure that allow a wide range of possible conformations. Glucose is usually predominant, but they can also contain other hexoses (mannose, galactose, and fructose), deoxyhexoses (fucose and rhamnose), and pentoses (arabinose, ribose, and xylose), as well as acidic sugars (glucuronic and galacturonic acid) and amino sugars (glucosamine, galactosamine, and their N-acetyl derivatives) (Fig. 4). The presence of uronic acids (in ~90% of cyanobacterial EPS) results in polymers rich in negatively charged groups, but is rarely observed in the EPS of other gram-negative bacteria (Pereira et al. 2019b). Another unusual feature in bacterial EPS, but common among EPS from algae and some archaea, is the presence of sulfate groups (up to 20% of cyanobacterial EPS dry weight). Moreover, peptides and noncarbohydrate constituents (e.g., phosphate,

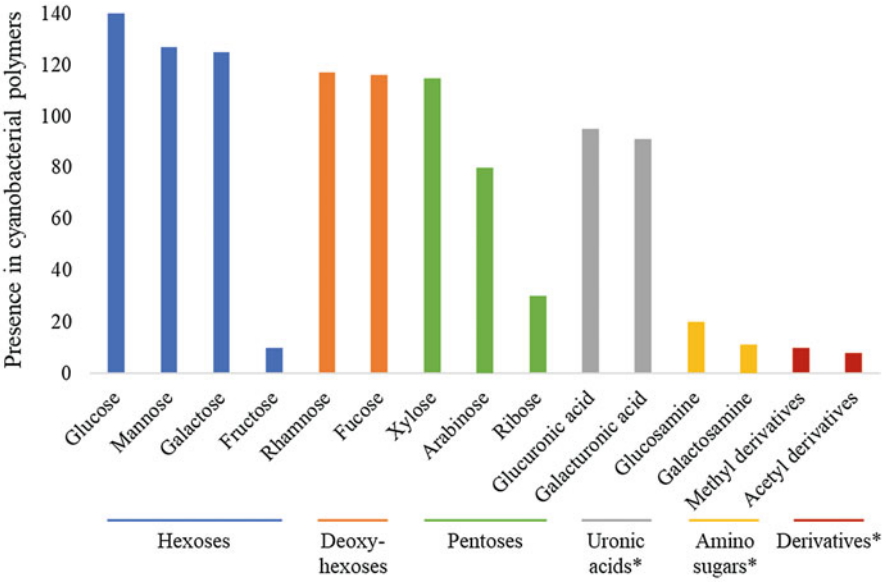


Fig. 4 Abundance of a given monosaccharide in cyanobacterial polymers. *Depending on the methodology, monomers in low amount or rare in cyanobacterial polymers might be underestimated. (Data from 145 cyanobacterial strains and adapted from Rossi and De Philippis (2016), Delattre et al. (2016), Flores et al. (2019a), and Mota et al. (2020))

pyruvate, lactate, acetate, and glycerol) have been reported. The presence of unusual EPS sugars, such as methyl, acetyl, and amino sugars, are expected to contribute to cyanobacterial polymers' biological activity, most probably involved in cell recognition, adhesion, and protection. The peptides, enriched in glycine, alanine, valine, leucine, isoleucine, and phenylalanine, but also with aspartic and glutamic acids in some cases, contribute to the stabilization of the cyanobacterial EPS through the creation of hydrogen bonds. Despite the complexity usually observed, there are some particular cases of cyanobacterial homopolymers, namely *Microcystis wesenbergii* RPS constituted by uronic acids only and *Cyanothece* sp. 113 EPS constituted by D-glucose only (Pereira et al. 2019b).

Cyanobacterial EPS can have an amphiphilic behavior, due to the presence of different polysaccharidic fractions with hydrophilic and/or hydrophobic properties, determining the rheological behavior and jellification ability of the polymer. The hydrophobic fractions (ester-linked acetyl groups, peptidic residues, and deoxyhexoses) enhance the capacity of cell adhesion and the polymers emulsifying capacity, while the hydrophilic ones (sulfate groups, uronic acids, and ketal-linked pyruvyl groups) confer a strong anionic charge and sticky behavior to the polymer, being involved in the entrapment of minerals, nutrients, and water (Rossi and De Philippis 2016).

The particular features and the complexity of the cyanobacterial EPS hinder the solving of their structures (reviewed in Pereira et al. 2009), but at the same time

make these polymers more versatile and particularly attractive for specific industrial and biotechnological application (see Sect. 6). To determine the EPS structure, it is required the identification of type, sequence, and branching patterns of monosaccharides, and the anomeric configuration and isomer position of each glycosidic bond. A previously depolymerization into oligo- or monosaccharides is usually necessary, most often by acidic hydrolysis or enzymatic cleavage, due to their complex structure and conformation (Delattre et al. 2016). The content of neutral carbohydrates and other constituents of the EPS as uronic acids, proteins, and sulfate groups are usually quantified by colorimetric assays. Other techniques are commonly used to analyze physicochemical properties of EPS, such as scanning electron microscopy coupled with energy-dispersive X-ray spectroscopy (SEM-EDX), thermogravimetry (TGA), differential scanning calorimetry (DSC), and rheology (Xiao and Zheng 2016). Moreover, the carbohydrate residues can be further analyzed qualitatively and quantitatively using several methods: (i) spectroscopic, such as Fourier transformed infrared (FTIR) to detect the functional groups, and X-ray photoelectron spectroscopy (XPS) to study the elemental composition; (ii) chromatographic, such as gas (GC), liquid (LC), and ion-exchange chromatography (IEC) to determine the monosaccharidic composition, and size exclusion chromatography (SEC) to analyze the molecular mass; and (iii) spectrometric techniques, such as nuclear magnetic resonance (NMR) and mass spectrometry (MS), to elucidate the branching patterns and molecular structure (Delattre et al. 2016). Since many different techniques can be used for the analysis of the EPS monosaccharidic composition and structure, the comparison of the results available in the literature is not straightforward.

5.1 Tailoring

The manipulation of cyanobacterial EPS has two main goals: the cost-effective maximization of productivity/polymer yields and the generation of tailor-made polymer variants with desirable properties. Recently, strategies to obtain designed polymers based on cyanobacterial EPS were proposed/reviewed by Pereira et al. (2019b). These authors also discuss approaches to improve the amount of polymers produced through metabolic engineering, and relevant advances for modification of EPS to generate biomaterials, such as hydrogels, coatings, or scaffolds, e.g., through chemical modification or combination of EPS with plasticizers.

Regarding the increase of cyanobacterial polymer yields, other procedures have also been shown to be very promising, such as the manipulation of global regulators (e.g., sigma factors) that are involved in the control of carbon metabolism and/or secretion mechanisms (Flores et al. 2019a), and the heterologous expression of genes involved in EPS biosynthesis. This strategy is still in an early stage regarding cyanobacteria, but successful examples using *Synechococcus* sp. PCC 7002 were reported like the overexpression of six genes related to the synthase-dependent pathway of *Gluconacetobacter xylinus* that resulted in a high-yield production of extracellular type-I cellulose (Zhao et al. 2015), and the expression of two hyaluronic

acid (HA) synthetases from the gram-negative *Pasteurella multocida* and the gram-positive *Streptococcus equisimilis* that allowed the production of ~80 mg/L of HA by this unicellular cyanobacterial strain (Zhang et al. 2019). Preliminary evidences also showed that the manipulation of genes encoding carbohydrate-modifying enzymes, e.g., methyltransferases (Fisher et al. 2013) or proteins involved in EPS assembly/export (Fisher et al. 2013; Pereira et al. 2019a), might be used to tailor cyanobacterial EPS length, molecular mass (MM), and composition. In agreement, Wzy and Wzx proteins are widely considered as preferential targets for the incorporation of desired sugars during EPS polymerization, due to their broad specificity observed among different bacterial genus (Schmid 2018).

Concerning the generation of tailor-made cyanobacterial EPS, many physiochemical modifications have been increasingly used to alter the isolated polymers, such as trifluoroacetic acid hydrolysis to reduce polymers' MM (Flamm and Blaschek 2014a), peptide removal by trichloroacetic acid precipitation (Delattre et al. 2016), or oversulfation with dicyclocarbodiimide-sulfuric acid (Lee et al. 2007). For desulfation, solvolysis remains the most common method used, but this method can have several side effects, such as polymer degradation, and reduction of polymer MM and yield (Flamm and Blaschek 2014a; Lee et al. 2007). Alternative, silylating agents have been used to efficiently desulfate cyanobacterial EPS (up to ~98% of desulfation), although often accompanied by modifications in the linkage pattern of some monosaccharides (Flamm and Blaschek 2014b).

Other strategies to achieve higher polymer yields and/or tailor-made EPS are still largely unexplored using cyanobacteria, such as protein engineering regarding enzymes involved in EPS biosynthesis. In this context, several approaches have been efficiently used in heterotrophic bacteria, such as the shuffling of glycosyltransferases domains, the alteration of active sites of proteins involved in carbohydrate modification (e.g., epimerases) and in the assembly/export pathways, and the use of platforms with immobilized and designed enzymes for EPS production/modification (Schmid 2018).

6 Biotechnological Applications

As cyanobacteria have been emerging as source for the development of novel, versatile, and tailor-made "green materials" based on EPS, their application broadens to different fields such as wastewater treatment and soil conditioning, food, cosmetic, textile and painting industries, biomedicine, tissue engineering, and pharmaceutics (Singh et al. 2019). To promote the commercial use of cyanobacterial EPS/EPS-based products, several patents have been filled, regarding the polymers/polymer products, their production, extraction, and/or downstream processing (Table 1). Interestingly, the same polymer frequently shows the potential to be used in completely distinct fields. For example, the EPS from *Nostoc flagelliforme* are promising emulsifying and flocculating agents that could be used in cosmetics and food industries (Han et al. 2014), but these have also potent antiviral activity that will allow its exploitation in biomedicine (Kanekiyo et al. 2007).

Table 1 Patents related to cyanobacterial polysaccharides published in the last 15 years

Patent number[a]	Title	Strains	Inventors (year)
WO2006078284A3	Methods and compositions related to antiviral therapy using algae and cyanobacteria	*Spirulina*	Teas J (2006)
US7205284B2	Potent immunostimulants from microalgae	*Aphanizomenon flos-aquae, Spirulina platensis*	Pasco DS, Pugh ND, Elsohly M, Ross S, ElSohly NM (2007)
WO2007044439A2	Microbial exopolymers useful for water demineralization	*Oscillatoria, Lyngbya, Schizothrix, Chroococcus, Calothrix*	Perry TD (2007)
US8350024B2	Sugar derivatives and application of same	*Aphanothece sacrum*	Kaneko M, Kaneko T (2013)
WO2013136029A1	Production of polysaccharides in mixotrophic mode using *Nostoc*	*Nostoc*	Romari K, Godart F, Calleja P (2013)
US20180256627A1	Cosmetic compositions comprising exopolysaccharides derived from microbial mats, and use thereof	*Phormidium, Scytonema, Schizothrix, Chlorococcales*	Loing E, Briatte S, Vayssier C, Beaulieu M, Dionne P, Richert L, Moppert X (2018)
WO2018042378A1	Cyanobacterium extracellular polymer, compositions, and uses thereof	*Cyanothece* sp. CCY 0110	Mota R, Tamagnini P, Gales L, Leite JP, Pereira SB (2018)
WO2019171344A1	Uses of cyanobacterium extracellular polymer, compositions, coated surfaces, or articles	*Cyanothece* sp. CCY 0110	Mota R, Tamagnini P, Costa F, Martins MCL (2019)

[a]Includes US patents and applications under the Patent Cooperation Treaty (PCT)

6.1 Water Treatment and Soil Conditioning

The use of EPS-producing cyanobacteria/isolated EPS in metal removal has been the subject of several studies in the last decade (Table 2), with some reports presenting insights on the mechanisms/functional groups involved in this process (De Philippis et al. 2011; Mota et al. 2016). Metal bioremediation by microorganisms comprises two processes: bioaccumulation, an active process that results in intracellular metal accumulation mediated by cell metabolism, and biosorption, a passive process generally involving several complex physicochemical mechanisms, such as ion exchange, complexation, and adsorption (De Philippis et al. 2011). Biosorption has been suggested to be the main mechanism by which the positively charged metal ions are removed from water solutions, being this due to the presence of a large

Table 2 Examples of cyanobacterial EPS and their applications in water treatment and soil conditioning

Cyanobacteria	Polymers	Applications	References
Water treatment			
Cyanothece sp. CCY 0110	Cyanoflan (RPS)	Heavy metal bioremediation	Mota et al. (2016)
Oscillatoria limnetica; *Anabaena spiroides*	Mucilage	Heavy metal bioremediation	Tien (2002)
Soil conditioning			
Leptolyngbya ohadii	EPS	Bare sandy substrates stabilization	Mugnai et al. (2018)
Microcoleus vaginatus	EPS	UV-B radiation protection	Chen et al. (2009)
Microcoleus vaginatus; Scytonema javanium	EPS	Water retention	Colica et al. (2014)
Nostoc commune	EPS	Desiccation and freezing protection	Tamaru et al. (2005)
Phormidium ambiguum	EPS	Soil physical stability	Chamizo et al. (2020)
Scytonema javanicum	EPS	Soil fertility	Chamizo et al. (2020)

number of negatively charged groups on the EPS attached to the cell and/or on the RPS, such as carboxyl, hydroxyl, phosphoryl, sulfhydryl, and amino functional groups. Various culture fractions of *Cyanothece* sp. CCY 0110 (living cells plus RPS, dead cells plus RPS, or isolated RPS only) were tested for their affinity toward different metal ions in aqueous solutions. The results obtained revealed that the RPS played the main role in the removal process due to the organic functional groups available, mainly carboxyl and hydroxyl (Mota et al. 2016). However, up to now, no industrial-scale process has been fully developed since the process is much more complex than under laboratory-controlled conditions due to, e.g., the variety of the chemicals present in wastewaters. Moreover, the implementation of a new process involves always significant upfront costs, usually considered too high for the treatment of wastewaters containing low valuable metals. Nonetheless, these disadvantages could, in the future, be counterbalanced with the revenue derived from the recovery of commercially valuable heavy metals, a process that is not efficient using the traditional physicochemical methods (Colica and De Philippis 2014).

Cyanobacteria have also been described as useful agents in the remediation and amelioration of soils, by contributing with organic matter and improving soil biological activity, since they are pioneers in the formation of biocrusts – complex communities composed by different proportions of cyanobacteria, microalgae, microfungi, lichens, and bryophyte – that are important for the establishment and growth of vascular plants (Rossi et al. 2018). In the biocrusts, the EPS matrix play a significant role in sediment cohesion, resistance to erosion, moisture maintenance, immobilization of nutrients, protection from external harmful factors, and enhancement of soil fertility. The inoculation of soil with EPS-producing cyanobacteria, such

as *Microcoleus* and *Nostoc* spp. (Table 2), improves the aggregation of the top coat, and increases the water retention capacity and the regeneration of the ecosystems through the in situ fixation of nitrogen and carbon (Colica and De Philippis 2014). Moreover, some cyanobacterial strains, in particular from soil and extremophilic habitats, can excrete a brown pigment deposited in EPS (particularly in the sheath), named scytonemin, which can protect the cells against long-wavelength ultraviolet A (UVA) radiation. Likewise, mycosporine-like amino acids (MAAs) are natural UV sunscreen compounds that can be found in the sheath of some cyanobacterial strains (Chen et al. 2009). Furthermore, the inoculation of EPS-producing cyanobacteria in desert areas promotes the formation of soil crusts, stabilizing sand dunes and driving ecological succession, which has been already successfully applied in large hyper arid areas in China (Colica et al. 2014). Lab-scale studies also revealed that the use of EPS-producing cyanobacteria in burned soils can decrease soil hydrophobicity and increase surface penetration resistance, resulting in higher stabilization of postfire soils (Chamizo et al. 2020). Fixing dunes to fight desertification and prevent sandstorms as well as recovering and stabilizing unconsolidated soils due to fires or floods are examples of the valuable use of EPS-producing cyanobacteria.

6.2 Food, Personal Care, and Other Industries

The first studied features of cyanobacterial EPS envisaging industrial exploitation were the high viscosity of their aqueous solutions, and the capabilities to stabilize emulsions and to form gels with good tensile strength (reviewed by Colica and De Philippis 2014). This interesting rheological behavior is mostly determined by the complex composition, structure, and high molecular mass (MM) of the cyanobacterial EPS. The most promising feature is their ability to stabilize the flow properties of aqueous solutions against changes in temperature, ionic strength, and pH. In addition, the amphiphilic character contributes to their possible use in the stabilization of emulsions, as bioflocculants and/or viscosifiers (Table 3). The pseudoplastic behavior usually observed for aqueous solutions of these polymers is important for the food industry, in order to provide good sensory qualities, flavor release, and suspending properties in the production of, e.g., cake mixtures, salad dressings, sauces, puddings, and dairy products, as well as in the cosmetic industry, for the production of lotions, creams, and gels. These polymers can also be used to disperse hydrocarbons or oils in water or food preparations, in the secondary recovery of petroleum, or as a stain remover. This is the case of emulcyan, an extracellular sulfated heteropolysaccharide containing fatty acids and peptides, produced by the filamentous cyanobacterium *Phormidium* J-1, which is capable of forming emulsions of hydrocarbons and/or oils in water and that can act as a bioflocculant, clarifying the water column in natural turbid-water habitats (Fattom and Shilo 1985).

Due to other natural features, namely moisturizer, photoprotector, antioxidant, and anti-inflammatory properties, the application of cyanobacterial EPS is also being studied in the field of cosmetics, cosmeceuticals (i.e., cosmetic products with active

Table 3 Examples of cyanobacterial EPS and their applications in food and personal care industries

Cyanobacteria	Polymers	Applications	References
Aphanothece sacrum	Sacran (EPS)	Moisture retention	Okajima et al. (2008)
Cyanothece sp. CCY 0110	Cyanoflan (RPS)	Emulsification	Mota et al. (2020)
Lyngbya stagina	EPS	Viscosifier	Jindal et al. (2013)
Nostoc commune	Sheath	Moisture absorption and retention, Antioxidant, Photo-protection (MAAs)	Li et al. (2011), Nazifi et al. (2015)
Nostoc flagelliforme	EPS	Flocculation and emulsification	Han et al. (2014)
Nostoc punctiforme	Sheath	Photo-protection (scytonemin)	Soule et al. (2016)
Phormidium J-1	Emulcyan (RPS)	Flocculation and emulsification	Fattom and Shilo (1985)

ingredients resulting in a pharmaceutical therapeutic benefit), and nutraceuticals (i.e., nutritional supplements) (Pierre et al. 2019). For instance, the EPS produced by the edible *Nostoc commune* have strong moisture absorption and retention capacities (Table 3), greater than urea and chitosan, having a high potential to be used as humectants in moisturizers without the need to be combined with undesirable occlusive agents (Li et al. 2011). In addition, these EPS have antioxidant activities since they are capable of scavenging both superoxide anions and hydroxyl radicals in a dose-dependent manner, and they can increase antioxidant enzymes activity, and decrease the content of lipid peroxidation. Other example is the moisture retention capacity of Sacran, a giant anionic polysaccharide extracted from the cyanobacterium *Aphanothece sacrum*, which is tenfold higher than hyaluronic acid (Okajima et al. 2008). It has been suggested that the moisture retention capacity of cyanobacterial EPS is due to strong interactions between water molecules and the hydrophilic hydroxyl groups of the polysaccharides (Derikvand et al. 2016). The photo-protective compounds that often appear within the EPS (scytonemin and MAAs) have also great potential to be used as ingredients in sunscreens due to their inherent good solubility, photostability, and effective UVA absorption properties (Derikvand et al. 2016). These properties can be beneficial for several personal care products beyond the traditional skin protection and suntan lotions, including daytime facial moisturizers, lipsticks, and makeup.

Despite some cyanobacterial EPS exhibit similar or even better properties than the commercially available microbial EPS, such as xanthan gum and chitosan (Li et al. 2011; Mota et al. 2020), it is still difficult to compete to the well-established products in the market. Therefore, only polymers with novel and unique properties may be capable to overcome this challenge. Nonetheless, the interest in cyanobacterial-based products by the cosmetic industry is evident since several of them can be found at the European Commission Database for information on

cosmetic substances and ingredients (CosIng), such as *Aphanothece sacrum* "exopolysaccharides" with stated functions of absorbent, emulsion stabilizing, film forming, and viscosity controlling.

6.3 Biomedical

A wide range of potential biomedical applications have been described for cyanobacterial EPS, which make them very attractive for the development of innovative bioproducts, such as drugs, nanocarriers, coatings, or scaffolds. The majority of the envisaged applications are based on the biological activity of the cyanobacterial EPS, e.g., antioxidant, immunostimulatory, cytotoxic, pro-inflammatory, antiviral, and antimicrobial (Table 4). These activities mostly result from a complex interaction of several structural features, including the sugar residue composition, molecular mass, sulfation level, distribution of sulfate groups along the polysaccharide backbone, and stereochemistry (Pereira et al. 2019b). The high sulfate content is one of the most promising cyanobacterial EPS features in the biomedical context, having been associated to different bioactivities, such as antiviral (Reichert et al. 2017), antioxidant (Parwani et al. 2014), anticoagulant (Flamm and Blaschek 2014a), and antimicrobial (Challouf et al. 2011). Nonetheless, only in a few cases, the bioactivities exhibited were undoubtedly associated with the EPS features, and usually this association is inferred based on other compositionally similar bioactive polymers from other bacterial sources or eukaryotic organisms (mainly macroalgae).

At the top of the list of the most studied bioactive EPS are those produced by the filamentous edible cyanobacterium *Arthrospira platensis*, well known as *Spirulina*, for which several patents were filled. At least nine biological activities, e.g., antiviral and immunostimulatory, are well described for different types of EPS from *A. platensis* (Table 4). However, at the industrial level, EPS form *A. platensis* that are still considered as a co-product from the extraction of high-value compounds (e.g., phycocyanin pigment used as natural colorant) (Pierre et al. 2019). In contrast, the antitumor activity of cyanobacterial EPS is one of the least studied bioactivities; however, recent works demonstrated that completely distinct cyanobacterial EPS have strong antiproliferative effects and/or efficiently trigger apoptosis in different tumor cell lines (Flores et al. 2019b; Li et al. 2018). For example, the polymer produced by a *Synechocystis* Δ*sigF* EPS-overproducing mutant decreases the viability of melanoma, thyroid, and ovary carcinoma cells by inducing high levels of apoptosis, through p53 and caspase-3 activation (Flores et al. 2019b), and the nostoglycan isolated from *Nostoc sphaeroides* colonies suppresses the proliferation of several types of tumor cell lines due to its antioxidant properties and induces apoptosis in human lung adenocarcinoma A549 cells via caspase-dependent pathway (Li et al. 2018).

However, other cyanobacterial EPS with no apparent biological activity can also be interesting for the pharmaceutical or biomedical field, for example, the RPS produced by the unicellular strain *Cyanothece* sp. CCY 0110 that showed to be a promising vehicle for topical and oral administration of therapeutic macromolecules

Table 4 Examples of cyanobacterial EPS and their biological activities and/or applications in biomedicine

Cyanobacteria	Polymers	Activities/applications	References
Anabaena variabilis; A. anomala; A. oryzae; Tolypothrix tenuis	EPS	Hemostatic blood clotting agent, wound dressing/ healing, antibacterial, antioxidant	Parwani et al. (2014)
Aphanothece sacrum	Sacran (EPS)	Anti-inflammatory, hydrogel-like scaffold	Ngatu et al. (2012), Okajima et al. (2008)
Arthrospira platensis (*Spirulina*)	Immulina (EPS)	Immunostimulatory, pro-inflammatory	Løbner et al. (2008)
	Spirulan (EPS)	Antiviral, tissue remodeling and migration, stimulation of anticoagulant proteoglycan secretion, antithrombin, anti-atherogenic, antitumor	Kaji et al. (2002), Lee et al. (2007), Mader et al. (2016), Mishima et al. (1998)
	EPS	Antimicrobial (bactericidal, bacteriostatic), antioxidant, bacterial anti-adhesive, antiviral	Reichert et al. (2017), Challouf et al. (2011)
Cyanothece sp. CCY 0110	Cyanoflan (RPS)	Controlled drug delivery, anti-adhesive coating	Estevinho et al. (2019), Costa et al. (2020)
Nostoc commune	EPS	Inhibition of human complement, biomaterial for coatings/membranes, capping agent of silver nanoparticles (bactericidal)	Morsy et al. (2014), Brull et al. (2000)
Nostoc sp.	EPS	Antitussive, bronchodilator, immunomodulatory	Uhliariková et al. (2020)
Nostoc sp.; *Synechocystis* sp.	EPS	Antifungal film	Morales-Jiménez et al. (2020)
Nostoc flagelliforme	Nostoflan (EPS)	Antiviral, Antitumor	Yue et al. (2011), Kanekiyo et al. (2007)
Nostoc sphaeroides	Nostoglycan (EPS)	Antitumor, Antioxidant	Li et al. (2018)
Nostoc microscopicum	EPS	Bactericidal, Antibiofilm	Ramachandran et al. (2019)
Synechocystis aquatilis	EPS	Inhibition of human complement, Anticoagulant	Flamm and Blaschek (2014a)
Synechocystis sp. PCC 6803	RPS	Antitumor	Flores et al. (2019b)

(Estevinho et al. 2019). This polymer was also used to produce an anti-adhesive coating capable of efficiently preventing the adhesion of relevant etiological agents, aiming at developing an antibiotic-free alternative to fight catheter-associated urinary tract infections (Costa et al. 2020).

The lack of knowledge about the mode of action and the production mechanisms of cyanobacterial EPS hinders the approval of these polymers as qualified

commodity materials by the policy agencies worldwide. In addition, this approval process is even more restrictive regarding products with biological activity and for biomedical use. So far, only *Arthrospira* (namely *A. platensis* and *A. maxima* biomass/extracts) successfully accomplished the high-quality criteria to be commercialized worldwide as dietary supplement and cosmetic product. Another significant milestone was reached using *A. platensis* carbohydrate-based molecules, such as a peptidoglycan complex that is being tested as anticancer drug in patients with advanced pancreatic cancer (Khan et al. 2019). This molecule reached the phase I in December 2016, but the results of this study are still not currently available.

Overall, although very promising *in vitro* results have been obtained testing cyanobacterial EPS, more comprehensive studies are needed to confirm these results *in vivo*, as well as to fully understand the mechanisms and the EPS features associated with their bioactivity.

7 Conclusions and Future Perspectives

The complexity of the chemical composition and structure, rheological behavior, and diversity of bioactivities of the cyanobacterial EPS results in an almost endless list of biotechnological applications. In addition, with the expected growth of the universal current "green trend," the development of novel natural solutions with better performance and positive cost-benefit is a must. However, despite the recent advances on the knowledge on cyanobacterial EPS biosynthetic pathways, the identification of key components and the elucidation of their regulatory networks is mandatory to control EPS production/EPS characteristics. Enduring efforts to improve the photobioreactors in terms of design, operation, and scaleup, together with modern genetic/protein engineering approaches, can significantly improve the economics and feasibility of cyanobacteria/cyanobacterial EPS industrial production. In addition, cost-effective methodologies covering the entire product development, including biomass production, product processing and purification, and packaging and marketing, still need to be further addressed to make cyanobacteria/cyanobacterial extracellular polymers economically attractive.

Acknowledgments This work was financed by FEDER – Fundo Europeu de Desenvolvimento Regional funds through the COMPETE2020 – Operacional Programme for Competitiveness and Internationalisation (POCI), Portugal 2020, and by Portuguese funds through FCT – Fundação para a Ciência e a Tecnologia/Ministério da Ciência, Tecnologia e Ensino Superior in the framework of the project POCI-01-0145-FEDER-028779 (PTDC/BIA-MIC/28779/2017).

References

Agostoni M, Montgomery BL. Survival strategies in the aquatic and terrestrial world: the impact of second messengers on cyanobacterial processes. Life (Basel). 2014;4(4):745–69.

Brull LP, Huang Z, Thomas-Oates JE, Paulsen BS, Cohen EH, Michaelsen TE. Studies of polysaccharides from three edible species of *Nostoc* (cyanobacteria) with different colony

morphologies: structural characterization and effect on the complement system of polysaccharides from *Nostoc Commune*. J Phycol. 2000;36(5):871–81.
Challouf R, Trabelsi L, Dhieb RB, El Abed O, Yahia A, Ghozzi K, Ammar JB, Omran H, Ouada HB. Evaluation of cytotoxicity and biological activities in extracellular polysaccharides released by cyanobacterium *Arthrospira platensis*. Braz Arch Biol Technol. 2011;54(4):831–8.
Chamizo S, Adessi A, Certini G, De Philippis R. Cyanobacteria inoculation as a potential tool for stabilization of burned soils. Restor Ecol. 2020;28:S106–14.
Chen L, Wang G, Hong S, Liu A, Li C, Liu Y. UV-B-induced oxidative damage and protective role of exopolysaccharides in desert cyanobacterium *Microcoleus vaginatus*. J Integr Plant Biol. 2009;51(2):194–200.
Colica G, De Philippis R. Exopolysaccharides from cyanobacteria and their possible industrial applications. In: Sharma NK, Rai AK, Stal LJ, editors. Cyanobacteria: an economic perspective. Chichester: Wiley; 2014. p. 197–207.
Colica G, Li H, Rossi F, Li DH, Liu YD, De Philippis R. Microbial secreted exopolysaccharides affect the hydrological behavior of induced biological soil crusts in desert sandy soils. Soil Biol Biochem. 2014;68:62–70.
Costa B, Mota R, Tamagnini P, Martins MCL, Costa F. Natural cyanobacterial polymer-based coating as a preventive strategy to avoid catheter-associated urinary tract infections. Mar Drugs. 2020;18(6):279.
De Philippis R, Colica G, Micheletti E. Exopolysaccharide-producing cyanobacteria in heavy metal removal from water: molecular basis and practical applicability of the biosorption process. Appl Microbiol Biotechnol. 2011;92:697–708.
de Porcellinis AJ, Klähn S, Rosgaard L, Kirsch R, Gutekunst K, Georg J, Hess WR, Sakuragi Y. The non-coding RNA Ncr0700/PmgR1 is required for photomixotrophic growth and the regulation of glycogen accumulation in the cyanobacterium *Synechocystis* sp. PCC 6803. Plant Cell Physiol. 2016;57(10):2091–103.
Delattre C, Pierre G, Laroche C, Michaud P. Production, extraction and characterization of microalgal and cyanobacterial exopolysaccharides. Biotechnol Adv. 2016;34:1159–79.
Derikvand P, Llewellyn CA, Purton S. Cyanobacterial metabolites as a source of sunscreens and moisturizers: a comparison with current synthetic compounds. Eur J Phycol. 2016;52(1):43–56.
Di Pippo F, Ellwood NTW, Guzzon A, Siliato L, Micheletti E, De Philippis R, Albertano PB. Effect of light and temperature on biomass, photosynthesis and capsular polysaccharides in cultured phototrophic biofilms. J Appl Phycol. 2012;24(2):211–20.
Estevinho BN, Mota R, Leite JP, Tamagnini P, Gales L, Rocha F. Application of a cyanobacterial extracellular polymeric substance in the microencapsulation of vitamin B12. Powder Technol. 2019;343:644–51.
Fan Q, Lechno-Yossef S, Ehira S, Kaneko T, Ohmori M, Sato N, Tabata S, Wolk CP. Signal transduction genes required for heterocyst maturation in *Anabaena* sp. strain PCC 7120. J Bacteriol. 2006;188:6688–93.
Fattom A, Shilo M. Production of emulcyan by *Phormidium* J-1: its activity and function. FEMS Microbiol Lett. 1985;31:3–9.
Fisher ML, Allen R, Luo Y, Iii RC. Export of extracellular polysaccharides modulates adherence of the cyanobacterium *Synechocystis*. PLoS One. 2013;8(9):e74514.
Flamm D, Blaschek W. A sulfated cyanobacterial polysaccharide proven as a strong inhibitor of human complement activity in an in vitro assay. Planta Med. 2014a;80(12):1009–16.
Flamm D, Blaschek W. Exopolysaccharides of *Synechocystis aquatilis* are sulfated arabinofucans containing N-acetyl-fucosamine. Carbohydr Polym. 2014b;101:301–6.
Flores C, Tamagnini P. Looking outwards: isolation of cyanobacterial released carbohydrate polymers and proteins. JoVE. 2019;147:e59590.
Flores C, Santos M, Pereira SB, Mota R, Rossi F, De Philippis R, Couto N, Karunakaran E, Wright PC, Oliveira P, Tamagnini P. The alternative sigma factor SigF is a key player in the control of secretion mechanisms in *Synechocystis* sp. PCC 6803. Environ Microbiol. 2019a;21:343–59.
Flores C, Lima RT, Adessi A, Sousa A, Pereira SB, Granja PL, De Philippis R, Soares P, Tamagnini P. Characterization and antitumor activity of the extracellular carbohydrate polymer from the cyanobacterium *Synechocystis* Δ*sigF* mutant. Int J Biol Macromol. 2019b;136:1219–27.

Foster JS, Havemann SA, Singh AK, Sherman LA. Role of mrgA in peroxide and light stress in the cyanobacterium *Synechocystis* sp. PCC 6803. FEMS Microbiol Lett. 2009;293:298–304.

Gonzalez A, Riley KW, Harwood TV, Zuniga EG, Risser DD. A tripartite, hierarchical sigma factor cascade promotes hormogonium development in the filamentous cyanobacterium *Nostoc punctiforme*. mSphere. 2019;4:e00231–19.

Han P, Sun Y, Wu X, Yuan Y, Dai Y, Jia S. Emulsifying, flocculating, and physicochemical properties of exopolysaccharide produced by cyanobacterium *Nostoc flagelliforme*. Appl Biochem Biotechnol. 2014;172(1):36–49.

Han P, Shen S, Wang H, Sun Y, Dai Y, Jia S. Comparative metabolomic analysis of the effects of light quality on polysaccharide production of cyanobacterium *Nostoc flagelliforme*. Algal Res. 2015;9:143–50.

Herrero A, Stavans J, Flores E. The multicellular nature of filamentous heterocyst-forming cyanobacteria. FEMS Microbiol Rev. 2016;40(6):831–54.

Huang G, Fan Q, Lechno-Yossef S, Wojciuch E, Wolk CP, Kaneko T, Tabata S. Clustered genes required for the synthesis of heterocyst envelope polysaccharide in *Anabaena* sp. Strain PCC 7120. J Bacteriol. 2005;187(3):1114–23.

Jindal N, Singh DP, Khattar JIS. Optimization, characterization, and flow properties of exo-polysaccharides produced by the cyanobacterium *Lyngbya stagnina*. J Basic Microbiol. 2013;53:902–12.

Jittawuttipoka T, Planchon M, Spalla O, Benzerara K, Guyot F, Cassier-Chauvat C, Chauvat F. Multidisciplinary evidences that *Synechocystis* PCC6803 exopolysaccharides operate in cell sedimentation and protection against salt and metal stresses. PLoS One. 2013;8:e55564.

Kaji T, Shimada S, Yamamoto C, Fujiwara Y, Jung-Bum L, Hayashi T. Inhibition of the association of proteoglycans with cultured vascular endothelial cell layers by calcium and sodium spirulan. J Heal Sci. 2002;48(3):250–5.

Kanekiyo K, Hayashi K, Takenaka H, Lee JB, Hayashi T. Anti-herpes simplex virus target of an acidic polysaccharide, nostoflan, from the edible blue-green alga *Nostoc flagelliforme*. Biol Pharm Bull. 2007;30(8):1573–5.

Kehr JC, Dittmann E. Biosynthesis and function of extracellular glycans in cyanobacteria. Life. 2015;5:164–80.

Khan T, Date A, Chawda H, Patel K. Polysaccharides as potential anticancer agents-a review of their progress. Carbohydr Polym. 2019;210:412–28.

Khayatan B, Meeks JC, Risser DD. Evidence that a modified type IV pilus-like system powers gliding motility and polysaccharide secretion in filamentous cyanobacteria. Mol Microbiol. 2015;98:1021–36.

Lee J, Hou X, Hayashi K, Hayashi T. Effect of partial desulfation and oversulfation of sodium spirulan on the potency of anti-herpetic activities. Carbohydr Polym. 2007;69(4):651–8.

Li H, Xu J, Liu Y, Ai S, Qin F, Li Z, Zhang H, Huang Z. Antioxidant and moisture-retention activities of the polysaccharide from *Nostoc commune*. Carbohydr Polym. 2011;83:1821–7.

Li H, Su L, Chen S, Zhao L, Wang H, Ding F, Chen H, Shi R, Wang Y, Huang Z. Physicochemical characterization and functional analysis of the polysaccharide from the edible microalga *Nostoc sphaeroides*. Molecules. 2018;23(2):508.

Løbner M, Walsted A, Larsen R, Bendtzen K, Nielsen CH. Enhancement of human adaptive immune responses by administration of a high-molecular-weight polysaccharide extract from the cyanobacterium *Arthrospira platensis*. J Medicinal Food. 2008;11(2):313–22.

Lombard V, Golaconda Ramulu H, Drula E, Coutinho PM, Henrissat B. The carbohydrate-active enzymes database (CAZy) in 2013. Nucleic Acids Res. 2014;42(1):490–5.

Low KE, Howell PL. Gram-negative synthase-dependent exopolysaccharide biosynthetic machines. Curr Opin Struct Biol. 2018;53:32–44.

Mader J, Gallo A, Schommartz T, Handke W, Nagel C, Günther P, Brune W, Reich K. Calcium spirulan derived from *Spirulina platensis* inhibits herpes simplex virus 1 attachment to human keratinocytes and protects against herpes labialis. J Allergy Clin Immunol. 2016;137(1):197–203.

Miranda H, Immerzeel P, Gerber L, Hörnaeus K, Lind SB, Pattanaik B, Lindberg P, Mamedov F, Lindblad P. Sll1783, a monooxygenase associated with polysaccharide processing in the unicellular cyanobacterium *Synechocystis* PCC 6803. Physiol Plant. 2017;161(2):182–95.

Mishima T, Murata J, Toyoshima M, Fujii H, Nakajima M, Hayashi T, Kato T, Saiki I. Inhibition of tumor invasion and metastasis by calcium spirulan (Ca-SP), a novel sulfated polysaccharide derived from a blue-green alga, *Spirulina platensis*. Clin Exp Metastasis. 1998;16(6):541–50.

Morales-Jiménez M, Gouveia L, Yáñez-Fernández J, Castro-Muñoz R, Barragán-Huerta BE. Production, preparation and characterization of microalgae-based biopolymer as a potential bioactive film. Coatings. 2020;10(2):120.

Morsy FM, Nafady NA, Abd-Alla MH, Elhady DA. Green synthesis of silver nanoparticles by water soluble fraction of the extracellular polysaccharides/matrix of the cyanobacterium *Nostoc commune* and its application as a potent fungal surface sterilizing agent of seed crops. Univers J Microbiol Res. 2014;2(2):36–43.

Mota R, Guimaraes R, Buttel Z, Rossi F, Colica G, Silva CJ, Santos C, Gales L, Zille A, De Philippis R, Pereira SB, Tamagnini P. Production and characterization of extracellular carbohydrate polymer from *Cyanothece* sp. CCY 0110. Carbohydr Polym. 2013;92:1408–15.

Mota R, Rossi F, Andrenelli L, Pereira SB, De Philippis R, Tamagnini P. Released polysaccharides (RPS) from *Cyanothece* sp CCY 0110 as biosorbent for heavy metals bioremediation: interactions between metals and RPS binding sites. Appl Microbiol Biotechnol. 2016;100:7765–75.

Mota R, Vidal R, Pandeirada C, Flores C, Adessi A, De Philippis R, Nunes C, Coimbra MA, Tamagnini P. Cyanoflan: a cyanobacterial sulfated carbohydrate polymer with emulsifying properties. Carbohydr Polym. 2020;229:115525.

Mugnai G, Rossi F, Felde VJMNL, Colesie C, Büdel B, Peth S, Kaplan A, De Philippis R. The potential of the cyanobacterium *Leptolyngbya ohadii* as inoculum for stabilizing bare sandy substrates. Soil Biol Biochem. 2018;127:318–28.

Nazifi E, Wadab N, Asano T, Nishiuchi T, Iwamuro Y, Chinaka S, Matsugo S, Sakamoto T. Characterization of the chemical diversity of glycosylated mycosporine-like amino acids in the terrestrial cyanobacterium *Nostoc commune*. J Photoch Photobio B. 2015;142:154–68.

Ngatu NR, Okajima MK, Yokogawa M, Hirota R, Eitoku M, Muzembo BA, Dumavibhat N, Takaishi M, Sano S, Kaneko T, Tanaka T, Nakamura H, Suganuma N. Anti-inflammatory effects of sacran, a novel polysaccharide from *Aphanothece sacrum*, on 2, 4, 6-trinitrochlorobenzene–induced allergic dermatitis in vivo. Ann Allergy Asthma Immunol. 2012;108(2):117–22.

O'Toole GA, Wong GCL. Sensational biofilms: surface sensing in bacteria. Curr Opin Microbiol. 2016;30(1999):139–46.

Okajima MK, Bamba T, Kaneso Y, Hirata K, Fukusaki E, Kajiyama S, Kaneko T. Supergiant ampholytic sugar chains with imbalanced charge ratio form saline ultra-absorbent hydrogels. Macromolecules. 2008;41(12):4061–4.

Parwani L, Bhatnagar M, Bhatnagar A, Sharma V. Antioxidant and iron-chelating activities of cyanobacterial exopolymers with potential for wound healing. J Appl Phycol. 2014;26(3):1473–82.

Pereira S, Zille A, Micheletti E, Moradas-Ferreira P, De Philippis R, Tamagnini P. Complexity of cyanobacterial exopolysaccharides: composition, structures, inducing factors and putative genes involved in their biosynthesis and assembly. FEMS Microbiol Rev. 2009;33:917–41.

Pereira SB, Mota R, Vieira CP, Vieira J, Tamagnini P. Phylum-wide analysis of genes/proteins related to the last steps of assembly and export of extracellular polymeric substances (EPS) in cyanobacteria. Sci Rep. 2015;5:14835.

Pereira SB, Santos M, Leite JP, Flores C, Eisfeld C, Büttel Z, Mota R, Rossi F, De Philippis R, Gales L, Morais-Cabral JH, Tamagnini P. The role of the tyrosine kinase Wzc (Sll0923) and the phosphatase Wzb (Slr0328) in the production of extracellular polymeric substances (EPS) by *Synechocystis* PCC 6803. MicrobiologyOpen. 2019a;8:e753.

Pereira SB, Sousa A, Santos M, Araújo M, Serôdio F, Granja P, Tamagnini P. Strategies to obtain designer polymers based on cyanobacterial extracellular polymeric substances (EPS). Int J Mol Sci. 2019b;20:5693.

Pierre G, Delattre C, Dubessay P, Jubeau S, Vialleix C, Cadoret JP, Probert I, Michaud P. What is in store for EPS microalgae in the next decade? Molecules. 2019;24(23):4296.

Ramachandran L, Marappa N, Sethumadhavan K, Nooruddin T. Glycoprotein prompted plausible bactericidal and antibiofilm outturn of extracellular polymers from *Nostoc microscopicum*. Appl Biochem Biotechnol. 2019;191:284–98.

Reichert M, Bergmann SM, Hwang J, Buchholz R, Lindenberger C. Antiviral activity of exopolysaccharides from *Arthrospira platensis* against koi herpesvirus. J Fish Dis. 2017;40(10): 1441–50.

Riley KW, Gonzalez A, Risser DD. A partner-switching regulatory system controls hormogonium development in the filamentous cyanobacterium *Nostoc punctiforme*. Mol Microbiol. 2018;109 (4):555–69.

Risser DD, Meeks JC. Comparative transcriptomics with a motility-deficient mutant leads to identification of a novel polysaccharide secretion system in *Nostoc punctiforme*. Mol Microbiol. 2013;87(4):884–93.

Rossi F, De Philippis R. Exocellular polysaccharides in microalgae and cyanobacteria: chemical features, role and enzymes and genes involved in their biosynthesis. In: Borowitzka MA, et al., editors. The physiology of microalgae, developments in applied phycology, vol. 6. Springer International Publishing Switzerland; 2016. p. 565–90.

Rossi F, Mugnai G, De Philippis R. Complex role of the polymeric matrix in biological soil crusts. Plant Soil. 2018;429:19–34.

Schmid J. Recent insights in microbial exopolysaccharide biosynthesis and engineering strategies. Curr Opin Biotechnol. 2018;53:130–6.

Singh S, Kant C, Yadav RK, Reddy YP, Abraham G. Cyanobacterial exopolysaccharides: composition, biosynthesis, and biotechnological applications. In: Mishra AK, Tiwari DN, Rai AN, editors. Cyanobacteria: from basic science to applications. Academic; 2019. p. 347–58.

Soule T, Shipe D, Lothamer J. Extracellular polysaccharide production in a scytonemin-deficient mutant of *Nostoc punctiforme* under UVA and oxidative stress. Curr Microbiol. 2016;73(4): 455–62.

Stuart RK, Mayali X, Lee JZ, Everroad RC, Hwang M, Bebout BM, Weber PK, Pett-Ridge J, Thelen MP. Cyanobacterial reuse of extracellular organic carbon in microbial mats. ISME J. 2016;10(5):1240–51.

Takenaka H, Yamaguchi Y. Commercial-scale culturing of cyanobacteria: an industrial experience. In: Sharma NK, Rai AK, Stal LJ, editors. Cyanobacteria: an economic perspective. Chichester: Wiley; 2014. p. 293–302.

Tamaru Y, Takani Y, Yoshida T, Sakamoto T. Crucial role of extracellular polysaccharides in desiccation and freezing tolerance in the terrestrial cyanobacterium *Nostoc commune*. Appl Environ Microbiol. 2005;71:7327–33.

Tien CJ. Biosorption of metal ions by freshwater algae with different surface characteristics. Process Biochem. 2002;38:605–13.

Uhliariková I, Šutovská M, Barboríková J, Molitorisová M, Kim HJ, Park YI, Matulová M, Lukavský J, Hromadková Z, Capek P. Structural characteristics and biological effects of exopolysaccharide produced by cyanobacterium *Nostoc* sp. Int J Biol Macromol. 2020;160: 364–71.

Wang Y, Lechno-Yossef S, Gong Y, Fan Q, Wolk CP, Xu X. Predicted glycosyl transferase genes located outside the HEP island are required for formation of heterocyst envelope polysaccharide in *Anabaena* sp. strain PCC 7120. J Bacteriol. 2007;189(14):5372–8.

Xiao R, Zheng Y. Overview of microalgal extracellular polymeric substances (EPS) and their applications. Biotechnol Adv. 2016;34:1225–44.

Yue SJ, Jia SR, Yao J, Dai YJ. Nutritional analysis of the wild and liquid suspension cultured *Nostoc flagelliforme* and antitumor effects of the extracellular polysaccharides. Adv Mater Res. 2011;345:177–82.

Zhang L, Selão TT, Nixon PJ, Norling B. Photosynthetic conversion of CO_2 to hyaluronic acid by engineered strains of the cyanobacterium *Synechococcus* sp. PCC 7002. Algal Res. 2019;44: 101702.

Zhao C, Li Z, Li T, Zhang Y, Bryant DA, Zhao J. High-yield production of extracellular type-I cellulose by the cyanobacterium *Synechococcus* sp. PCC 7002. Cell Discov. 2015;1:15004.

Zuniga EG, Boateng KK, Bui NU, Kurnfuli S, Muthana SM, Risser DD. Identification of a hormogonium-polysaccharide specific gene set conserved in filamentous cyanobacteria. Mol Microbiol. 2020;114(4):597–608.

Glycosaminoglycans

9

Hajer Radhouani, Susana Correia, Cristiana Gonçalves, Rui L. Reis, and Joaquim Miguel Oliveira

Contents

Abstract

Glycosaminoglycans (GAGs), also known as mucopolysaccharides, are linear anionic compounds composed of repeating disaccharide units and classified into four groups: heparin and heparan sulfates, chondroitin sulfate and dermatan

H. Radhouani (✉) · S. Correia · C. Gonçalves · R. L. Reis · J. M. Oliveira
3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Zona Industrial da Gandra, Barco, Guimarães, Portugal

ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães, Portugal
e-mail: hajer.radhouani@i3bs.uminho.pt; susana.correia@i3bs.uminho.pt; cristiana.goncalves@i3bs.uminho.pt; rgreis@i3bs.uminho.pt; miguel.oliveira@i3bs.uminho.pt

J. M. Oliveira et al. (eds.), *Polysaccharides of Microbial Origin*,
https://doi.org/10.1007/978-3-030-42215-8_12

sulfate, keratan sulfate, and hyaluronan/hyaluronic acid. These large complex carbohydrate molecules are present in every mammalian tissue where they are known to bind and regulate a broad range of proteins involved in a myriad of physiological and pathological processes. GAGs are found in (in)vertebrate animals, implying a conserved function in the animal kingdom. Though, today there is an increasing number of examples of GAGs of microbial origin. There are concerns such as environmental impact, presence of undesirable animal products, and contamination risks that have necessitated alternate sources for industrial GAG production. The GAGs produced by microorganisms (microbial GAGs) are renewable resources and meet current market demands. Besides, these microbial GAGs are less complex and lack some modifications usually observed in animal GAGs. In fact, certain bacteria such as *Escherichia coli*, *Pasteurella multocida*, and *Streptococcus* have the necessary enzyme machinery to produce simple, nonsulfated GAGs, such as hyaluronan, heparosan, and chondroitin, among many more. Due to the recent expansion of GAG demand, a summary of the molecular structures, biosynthesis, physiologic functions, and clinical applications of the four primary groups of GAGs, and also a brief description of the microbial production of GAGs, is of particular interest.

Keywords

Bacteria · Glycosaminoglycans · Microbial production · Biological properties · Pharmaceutical applications

1 Introduction

Glycosaminoglycans (GAGs) are long linear anionic polysaccharide compounds composed of repeating disaccharide monomers that are present in animal tissues (Casale and Crane 2019). GAGs are constituted of combined sulfated or nonsulfated uronic acids (glucuronic acid or iduronic acid) and amino sugars (glucosamine or galactosamine). From the combination of different uronic acids and amino sugars, four main groups of GAGs can be distinguished: heparin (HEP) and heparan sulfates (HPS), chondroitin sulfate (CS) and dermatan sulfate (DS), keratan sulfate (KS), and hyaluronan or hyaluronic acid (HA) (Neves et al. 2020). These heteropolysaccharides can occur with different sulfation degrees and patterns that affect their biological function, with exception of HA, the only nonsulfated GAG (Soares da Costa et al. 2017).

Sulfated GAGs are synthesized by specific enzymes in the Golgi apparatus of the cell, whereas hyaluronan (HA) is synthesized by transmembrane proteins called HA synthases. While HA is not linked to a protein and produced from its reducing end, sulfated GAGs are synthesized from the nonreducing end as side chains attached to a protein that forms proteoglycans (PGs) (Fig. 1) (Köwitsch et al. 2018).

Their functions within the body are widespread and determined principally by their molecular structure. These polysaccharides are present in the extracellular matrix (ECM) of all animal cell surfaces and some are known to bind a number of

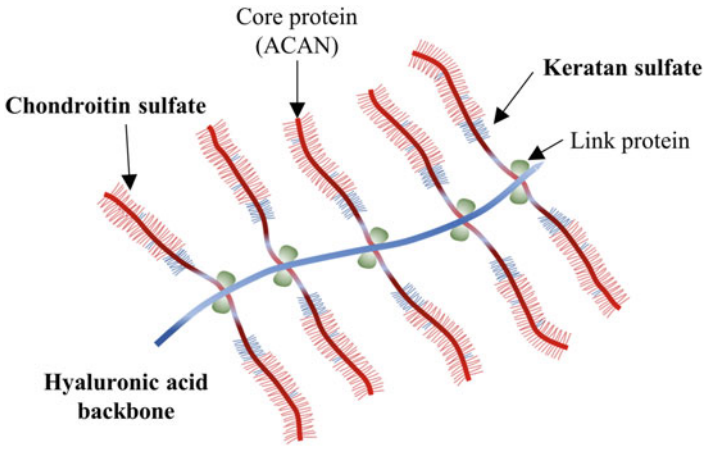


Fig. 1 Structure of an aggrecan-type complex proteoglycan

different proteins, including cytokines, growth factors, chemokines, enzymes, and adhesion molecules (Jackson et al. 1991; Gandhi and Mancera 2008). The function of GAGs was believed to be limited to structural scaffolding and cell hydration; however, evidence now suggests that GAGs play a crucial role in cell signaling, which attends to modulate a vast amount of biochemical processes (Linhardt and Toida 2004). Some of these processes include regulation of cell growth and proliferation, promotion of cell adhesion, anticoagulation, and wound repair, among others (Casale and Crane 2019).

Over the past few decades, GAGs are being used extensively in the pharmaceutical industry because of their multiple biological properties. Likewise, the therapeutic potential of GAGs and their mimetics for the treatment of many diseases, including thrombosis, thrombophlebitis, cancer, inflammation, infection, wound healing, lung diseases, Alzheimer's disease, etc., are being actively investigated (Weitz and Harenberg 2017; Pomin and Mulloy 2018; Morla 2019; Neves et al. 2020). Usually, the production of GAGs is obtained from mammalian tissues mainly generated in slaughterhouses: rooster combs, cartilage (tracheas and nasal from bovine and swine), and umbilical cords. However, as a consequence of concerns due to bovine spongiform encephalopathy (BSE) and undesired contaminations causing possible allergic inflammation responses, the extraction of GAGs from microorganisms has received an increasing attention. The microbial production of GAGs can generate best yields with higher concentrations at lower costs and with more efficiency (Mishra and Pavelko 2006; Vázquez et al. 2013; Delbarre-Ladrat et al. 2014). The main microbial glycosaminoglycans are those produced by Group C *Streptococcus* (GCS), *Escherichia coli* K5, *E. coli* K4, and *Pasteurella multocida*. In fact, these bacteria have the necessary enzyme machinery to produce simple, nonsulfated GAGs, such as hyaluronan, heparosan, and chondroitin. One of the most important commercial sources of hyaluronan polysaccharides for surgical, ophthalmic, and viscoelastic applications is GCS (Nwodo et al. 2012; Cimini et al. 2018).

Herein, the current knowledge of the four primary groups of GAGs regarding their structure, biosynthesis, properties, microbial production, and clinical applications are overviewed.

2 Structural Features

Glycosaminoglycans are structurally complex molecules, with varying lengths, backbone sugars, and modifications. GAGs are linear and highly negatively charged polysaccharides due to the presence of uronic acid residues and sulfate groups. These biopolymers are composed of repeating disaccharide subunits, which are made of an amino sugar and a hexuronic acid, that have molecular weights of roughly 10–100 kDa. These building blocks consist of variously N-substituted glucose- or galactosamine linked to glucuronic or iduronic acid (Almer et al. 2018; Morla 2019).

These disaccharides can suffer modifications at multiple positions by epimerization, sulfation, and/or acetylation, resulting in the different major categories of GAGs (HEP/HPS, CS/DS, KS, and HA) (Gandhi and Mancera 2008). Except for HA, all GAGs are negatively charged due to the presence of varying amounts of sulfate and carboxyl groups within their structure. Thus, HA is the only GAG that is not sulfated and, hence, is the least negatively charged GAG, while HEP is the most negatively charged (Pomin and Mulloy 2018). The molecular structure of each of the major categories appears below.

2.1 Heparin (HEP) and Heparan Sulfate (HPS)

Heparin and heparan sulfate (Fig. 2) contain repeating disaccharide units of *N*-acetylglucosamine and hexuronic acid residues. The hexuronic acid residue glucuronic acid is identified in heparan sulfate, while iduronic acid is present in heparin (Casale and Crane 2019). The major repeating disaccharide sequence of heparin (75–90%) is [→4)-2-O-sulfo-α-L-ido-pyranosyluronic acid (IdoAp) (1→4)-2-deoxy-2-*N*-,6-*O*-sulfo-α-D-glucopyranosylsamine (GlcNp) (1→], while minor sequences contain β-D-glucopyranosyluronic acid (GlcAp) residues, a reduced content of sulfo groups as well as *N*-acetylation. Regarding HPS, this polysaccharide has a similar structure with heparin, primarily containing nonsulfated disaccharides [→4) β -D-GlcAp (1→4)-α-D-GlcNpAc (1→] and monosulfated disaccharides, such as [→4) β -D-GlcAp (1→4)-6-sulfo-α-D-*N*-GlcNpAc]. HEP and HPS are composed of sulfated linear polysaccharide combinations, with a molecular weight (MW) ranging from 5000 to 40,000 and a MW average from 10,000 to 25,000 kDa (Zhang et al. 2010). HPS polysaccharides are abundant components of the cell surface and extracellular matrix of all multicellular animals, whereas HEP is present within most cells and contains higher levels of sulfo groups and more iduronic acid. Heparin is usually extracted from animal organs, predominantly porcine intestinal mucosa (Meneghetti et al. 2015).

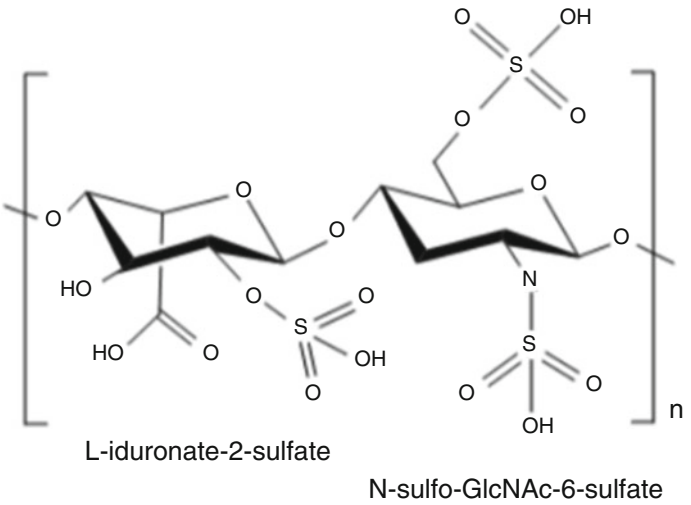


Fig. 2 Heparin and Heparan sulfate structures they are composed of L-iduronate (IdoA) or D-glucuronate (GlcA) and *N*-sulfo-D-glucosamine-6-sulfate

2.2 Chondroitin Sulfate (CS) and Dermatan Sulfate (DS)

Chondroitin sulfate (Fig. 3A) and dermatan sulfate (also known as chondroitin sulfate-B, Fig. 3B) are similar in their structural composition to HPS. Their disaccharide repeat consists of *N*-acetylgalactosamine and hexuronic acids – iduronic acid in CS and glucuronic acid in DS (Raman et al. 2005). The CS/DS family of GAGs is composed by alternating 1→3, 1→4 linked 2-amino, 2-deoxy-α-D-galactopyranose ($GalN_pAc$) and uronic acid (β-D-$GlcA_p$ in CS and α-L-$IdoA_p$ in DS) residue. Moreover, CS-GAGs also have domain structures similar to HPS-GAGs. The molecular weights of CS/DS range from 2 to 50 kDa. Furthermore, CS and DS can be identified on the cell surface and in the extracellular matrix like HPS (Suflita et al. 2015; Casale and Crane 2019).

2.3 Keratan Sulfate (KS)

Keratan sulfate (Fig. 4) contains a disaccharide repeat composed of galactose and *N*-acetylglucosamine; the sulfation patterns may be present on either unit, although with increased frequency on *N*- acetylglucosamine. As stated before, KS is the only sulfated GAG that is not connected by a tetrasaccharide linker compound to the PG protein core (Prydz 2015). KS has two distinct forms, originally designated as KS-I and KS-II based on differences between cornea and cartilage KS. However, a third type of KS linkage (mannose-*O*-Ser) has been identified that has been named KS-III. KS is comprised primarily of 6-*O*-sulfo-$GlcN_pAc$ and galactose (Gal) (which may

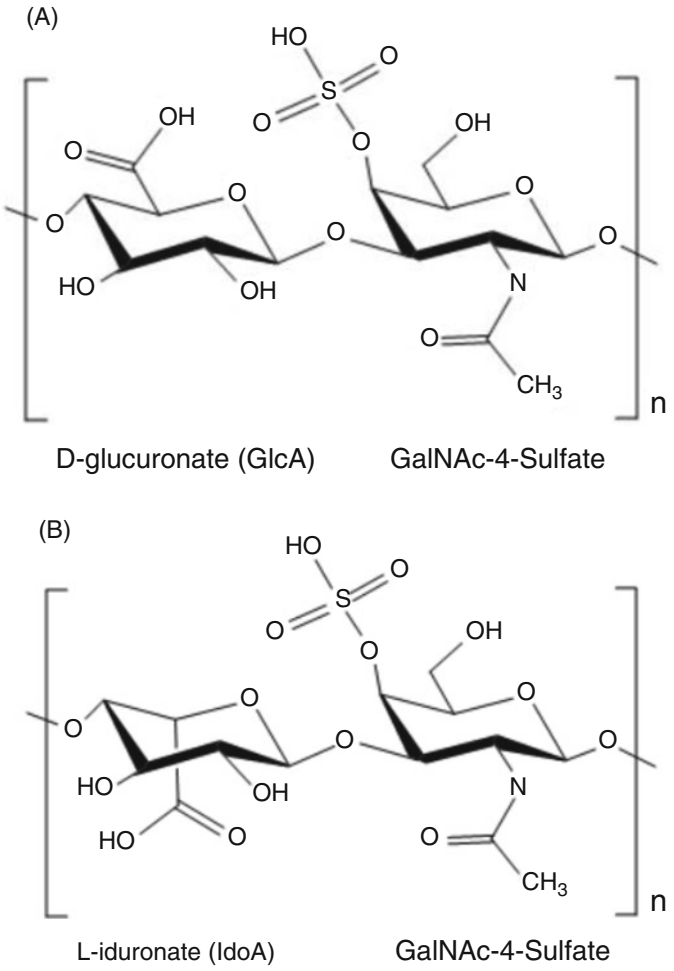


Fig. 3 Chondroitin sulfate (**A**) and Dermatan sulfate (**B**) structures

contain 6-*O*-sulfo groups). KS has been known in an extensive range of tissues, such as animal cornea, cartilage, bone, neural cells, dermis, ovarian zona pellucida, and even mammal uterine lining (Funderburgh 2000).

2.4 Hyaluronan/Hyaluronic Acid (HA)

Hyaluronan (Fig. 5) is an anionic linear polysaccharide composed of disaccharide units consisting of ([→3)-D-GlcNpAc(1→4)-β-D-GlcAp(1→]) (Moscovici 2015). This homocopolymer has the simplest structure of all GAGs, consisting of sequentially bound glucuronic acid and *N*-acetylglucosamine residues, without the need for

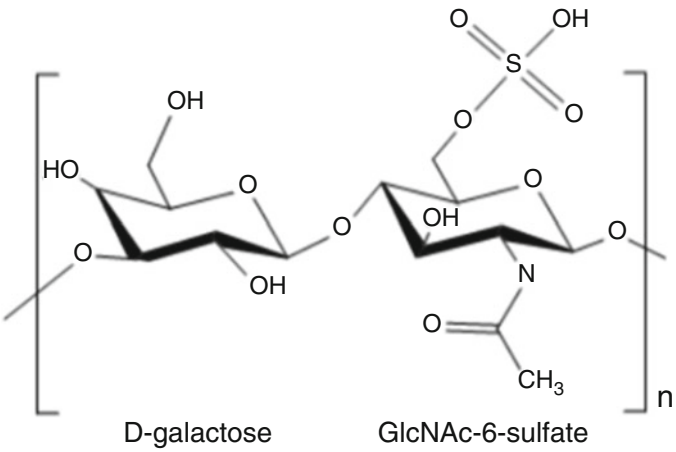


Fig. 4 Keratan sulfate structure

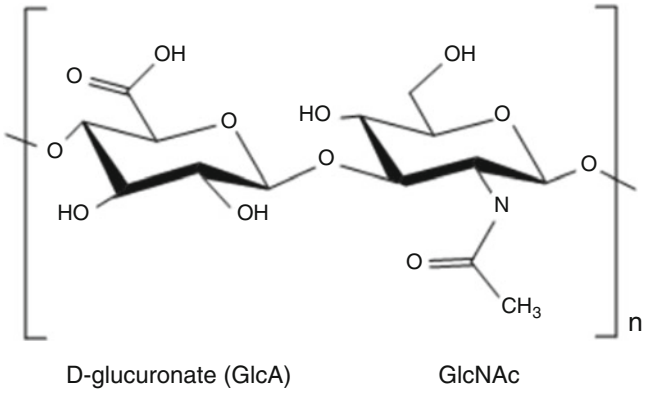


Fig. 5 Hyaluronan structure

additional functional group sulfation in the Golgi apparatus as other GAGs (Ghiselli 2017). This unsulfated GAG is characterized by a very high MW of up to 2000 kDa. Hyaluronan is present in the vitreous humor, cartilage tissue, and synovial fluid (Tamer 2013). The presence of hyaluronan outside of vertebrates has been identified only in a mollusk (Yamada et al. 2011).

3 Biosynthesis

Glycoscience faces many challenges due, mainly, to the nontemplate-driven nature of GAG's biosynthesis and their structural complexity (Lopez Aguilar et al. 2017). The biosynthesis process involves complex reactions that turn the simple glycosaminoglycan backbone into highly heterogeneous structures. The carbon backbone

of the GAG chain may not be modified (e.g., hyaluronan) or may suffer modifications at different regions through sulfation, de-acetylation, and/or epimerization (e.g., heparin, heparan sulfate, chondroitin sulfate, dermatan sulfate, and keratan sulfate) (Datta et al. 2019). One of the critical modifications is the epimerization of D-glucuronic acid to its C5-epimer L-iduronic acid, being this reaction demonstrated to be crucial for animal development (Raedts et al. 2013).

In eukaryotes, the process of GAG biosynthesis takes place in the endoplasmic reticulum and the Golgi apparatus, with the synthesis of five uridine diphosphate (UDP) derived activated sugars (UDP-glucuronic acid, UDP-*N*-acetylglucosamine, UDP-xylose, UDP-galactose, and UDP-*N*-acetylgalactosamine) (Fernández and Warren 1998). These sugars are then secreted through an antiporter transmembrane transporter from the cytoplasm to the Golgi apparatus for further modification (Ghiselli 2017). An exception is HA, which is exclusively synthesized at the plasma membrane by a membrane-bound HA-synthase. Instead of undergoing modification and sulfation in the Golgi apparatus, the HA precursor sugars UDP-glucuronic acid and UDP-*N*-acetylglucosamine are transferred from the cytoplasm to the plasma membrane for further processing without sulfation resulting in HA production (Raedts et al. 2013; Casale and Crane 2019).

To apply their biological function, GAGs are further processed by a series of modification steps such as sulfation of functional groups by the action of the sulfate donor compound 3'-phosphoadenosine-5'-phosphosulfate (PAPS). It is important to highlight that the accessibility of PAPS for GAG's sulfation will disturb the biosynthetic rate of production of the sulfated GAGs (Ghiselli 2017). Moreover, these sulfated GAG chains which were synthesized in the Golgi apparatus are added to proteoglycan. Regarding the GAGs heparin/heparan sulfate, chondroitin sulfate, and dermatan sulfate, the tethering process happens through a serine amino acid residue present on the protein core that relates to a common tetrasaccharide linker between the GAG and PG. Moreover, the only sulfated GAG that is not related to a PG protein core by this process is keratan sulfate and is instead linked by several other compounds depending on the subtype of keratan sulfate (I, II, and III) (Prydz 2015).

4 Industrial Production of GAGs

Nowadays, the commercial production of GAGs is commonly based on three origin categories (1) industrial production from animals, (2) industrial production using microbial cells, and (3) industrial production using eukaryotes (Datta et al. 2019).

Most of the commercially available GAGs have so far been extracted and purified from mammalian tissues, such as heparin from bovine and porcine mast cells that are found in intestine and lung tissues, chondroitin from animal (bovine, chicken and porcine) cartilage, dermatan sulfate from mucosal tissue and bovine and porcine intestine or porcine skin, and hyaluronan from rooster combs, umbilical cords, synovial fluids, skin or vitreous humor (Datta et al. 2019).

This often highlights health concern issues on viruses and prions, as well as some lower vertebrates that are potentially endangered. The tissue extraction method has

many disadvantages such as insufficient raw materials, nonuniform products, waste liquid pollution, and potential pathogenic factors (Jin et al. 2019). Recently, in order to avoid health issues and wider ecosystem damages derived from the extensive uses of mammalian and fishery wastes as substrate, diverse investigations on the industrial production of GAGs from microorganisms have been described (Vázquez et al. 2013; Datta et al. 2019). It is important to point out that prokaryotic GAGs are usually less complex than the eukaryotic ones due to an absence of chemical processes such as sulfation modification (Raedts et al. 2011).

5 Bacterial Production of GAGs

Certain bacteria have the capacity to synthetize simple, nonsulfated GAGs, such as chondroitin, heparosan, and hyaluronan. These bacterial GAGs, known as capsular polysaccharides, are commonly produced by *Streptococcus* sp., *Escherichia coli* K5, *E. coli* K4, and *Pasteurella multocida* (Fallacara et al. 2018; Datta et al. 2019). These polysaccharides represent amazing substitutes for GAGs of animal origin (Nwodo et al. 2012).

Some examples of the most common bacterial GAGs from serotypes of *Streptococcus*, *E. coli* and *P. multocida*, among others, are described in the following sections.

5.1 Bacterial Hyaluronan

Hyaluronan production through bacterial fermentation started in the 1960s, but its production on industrial scale was firstly achieved by Shiseido in the 1980s (Liu et al. 2011). It is important to highlight that bacterial fermentation produces hyaluronan with high molecular weight and purity, but the risk of some contamination still exists such as bacterial endotoxins, proteins, nucleic acids, and heavy metals (Hussain et al. 2017).

On the other hand, bacterial hyaluronan production has a great advantage since bacteria can be metabolically and/or physiologically improved to produce with high molecular weight and purity. Nowadays, there is a rising interest on using microorganisms such as pathogenic streptococci or safe recombinant hosts for bacterial hyaluronan production since they have the necessary hyaluronan synthase (Boeriu et al. 2013). Recently, the most significant alternative has been the development of bacterial hyaluronan production by the gram-positive *Streptococcus* sp. and gram-negative bacteria *P. multocida*, which can naturally produce hyaluronan (Schiraldi et al. 2010; Cimini et al. 2018).

Group C streptococci (*S. equi* subsp. *equi* and *S. equi* subsp. *zooepidemicus*) have been shown to produce hyaluronan with a higher quality and quantity than Group A streptococci or pathogenic bacterium *P. multocida*. Microbial fermentation of *S. zooepidemicus* is considered as one the most proficient and widespread processes of hyaluronan production. More recently, a recombinant *S. thermophilus* was

developed with the ability to produce 1.2 $g.L^{-1}$ hyaluronan with a molecular weight similar to the wild type strain (Izawa et al. 2011; Zakeri et al. 2017).

Research on hyaluronan production optimization through bacterial fermentation attended yield improvement, molecular weight increase, and also polymer polydispersity reduction, parameters which are fundamental for hyaluronan applications. The main characteristic was to regulate the molecular weight through several parameters such as optimized process conditions, improved bacteria, or even using metabolic engineering (Fallacara et al. 2018).

Recent research in alternative systems for sustainable production of hyaluronan proposed new microbial strains such as *E. coli*, *Bacillus* sp., *Agrobacterium* sp., and *Lactobacillus lactis*. Hence, nowadays researchers have an interest to develop new strains like *Pichia pastoris* (*Komagataella pastoris*), Lactic acid bacteria (*Lactococcus lactis*), *Bacillus subtilis* and *Corynebacterium glutamicum*, and other safe strains to recreate the production pathway for HA synthesis (Vázquez et al. 2013; Datta et al. 2019).

5.2 Bacterial Chondroitin

Another example of a GAG-like polysaccharide produced by bacterial strains is chondroitin; the microbial synthesis of this biomolecule is, now, one of the most potential alternatives to animal sources of chondroitin/CS. Pathogenic bacteria such as *E. coli* K4 and *P. multocida* type F are known to produce a capsular polysaccharide (CPS) that possessed a disaccharide repeat like unsulfated chondroitin and thus can be used as a starting biopolymer for further chemo/enzymatic modifications to CS with the desired sulfation degree and pattern (Deangelis et al. 2013; Cimini et al. 2018).

The capsules of *P. multocida* type A, D, and F could be extracted upon treatment with different glycosaminoglycan hydrolases. These CPS are constituted of hyaluronan, *N*-acetylheparosan (heparosan or unsulfated, unepimerized heparin), and unsulfated chondroitin, respectively (DeAngelis and White 2004). In the beginning, *P. multocida*, an important gram-negative pathogenic bacterium, was one of the preferred bacteria to produce chondroitin; however, its cholera pathogenicity has impeded and reduced its interest (Naccari et al. 2009; Vázquez et al. 2013).

Another member of the Pasteurellaceae family, *Avibacterium paragallinarum* genotype A, was also described to produce a chondroitin capsule. It is important to point out that a fragment of its genome draft sequence revealed high homology to the capsule gene clusters of *P. multocida* type F and *E. coli* K4 (Wang et al. 2018).

5.3 Bacterial Heparosan

Heparosan is biosynthesized as a polysaccharide capsule in bacteria including *E. coli* K5 and *P. multocida* type D, being the common precursor molecule of both heparin and heparan sulfate which have similar backbone structures to the commercial heparin.

These bacterial polysaccharides have been synthesized by *E. coli* K5 in the non-sulfated forms and in the sulfated forms by *E. coli* K4. Moreover, heparan sulfates, produced by *P. multocida*, are characterized by higher molecular weights (200 and 300 kDa) than those produced by *E. coli* strains (DeAngelis and White 2004).

Contrary to animal sources, no postpolymerization modifications occur on the K5 heparosan molecule, which makes this K5 polysaccharide a valuable substrate to investigate the enzymes in heparin biosynthesis and a potential precursor for heparin chemo-enzymatic synthesis (Vann et al. 1981; Raedts et al. 2011). Recent research demonstrated that the extracted heparosan from microbial sources could be modified chemoenzymatically in order to produce the anticoagulant heparin (Bhaskar et al. 2015). Besides, other study demonstrated that mammalian cells that naturally produce heparan sulfate can be metabolically modified for heparin production. The recovery and modification of these polysaccharides into biologically active GAG-like polysaccharides, such as heparin or heparan sulfate, will serve as an important substitute for animal derived GAGs (Baik et al. 2012; Datta et al. 2019).

6 Properties and Clinical Applications

Glycosaminoglycans, as one main part of the glycocalyx, are involved in a myriad of biological and therapeutic functions, as well as in several diseases. Certain GAGs are known to bind and regulate a number of different proteins, such as growth factors, cytokines, morphogens, chemokines, enzymes, and cell adhesion molecules (Gandhi and Mancera 2008; Mende et al. 2016). Additionally, GAGs play a key role in cell signaling and development, axonal growth, anticoagulation, angiogenesis, tumor progression, and metastasis. Uncontrolled progenitor cell proliferation leads to malignant tissue transformation and cancer (Morla 2019). GAG bioactivity is conferred by intrinsic structural features such as sugar compositions, glycosidic bonds, and sulfation patterns and degrees. Since GAGs are engaged in a variety of biological processes, their use in drug development has been for long interesting in the pharmaceutical industry (Table 1), and more recently in tissue engineering, as shown in Fig. 6 (Lane et al. 2017; Köwitsch et al. 2018; Morla 2019).

6.1 Hyaluronan

The most abundant nonsulfated GAG, hyaluronan, has numerous important functional roles, such as signaling activity during embryonic morphogenesis, pulmonary and vascular diseases, and wound healing. HA also acts in the lubrication of synovial joints and joint movement; it has been described to function as space filler, wetting agent, flow barrier within the synovium, protector of cartilage surfaces and has undergone clinical testing as a means of relieving arthritis (Ondresik et al. 2017).

Moreover, the best-known physicochemical property of HA is its aptitude to form hydrogels. This property allows HA to be used as a vehicle to make specific hydrogel formulations for regenerative medicine. HA-based products used to be applied in

Table 1 Summary of the most common GAGs (DeAngelis 2002; Gandhi and Mancera 2008; Raedts et al. 2011; Köwitsch et al. 2018)

GAGs	Main disaccharide	Sulfation pattern	Molecular weight	(In)vertebrate sources	Potential therapeutic application
HPS	-4)GlcAβ(1-4) GlcNAc-α(1-	GlcA(IdoA) at C2, GlcNAc (NS) at C6, C3	10–70 kDa	Component of cell surface membranes, ECM	Anti-inflammatory, antitumor
HEP	-4)IdoAα(1-4) GlcNS-α(1-	Like heparan sulfate but heavier	5–40 kDa	Component of intracellular granules of mast cells lining the arteries of the lungs, liver, skin	Anticoagulant/ antithrombotic, anti-inflammatory, wound healing (OA), antitumor, promoter of cell growth and differentiation in tissue engineering, hydration therapies, and protective function on cosmetic industry
CS	-4)GlcAβ(1-3) GalNAc-β(1-	GalNAc at C4/C6 and GlcA at C2	5–50 kDa	Cartilage, tendons, ligaments, bone, heart valves	Anti-inflammatory, wound healing (OA), antitumor, promoter of cell growth, and differentiation in tissue engineering
DS	-4)IdoAα(1-3) GalNAc-β(1-	GalNAc at C4/C6 and IdoA at C2	15–40 kDa	Skin, blood vessels, heart valves, tendons, lungs	Anticoagulant/ antithrombotic
KS	-3)Galβ(1-4) GlcNAc-β(1-	Gal and GlcNAc at C6	4–19 kDa	Cornea, bone, cartilage	Antiadhesive, active ingredient in eye drops
HA	-4)GlcAβ(1-3) GlcNAc-β(1-	Unsulfated polysaccharide	4–8000 kDa	Synovial fluid, vitreous humor, ECM of loose connective tissue	Anti-inflammatory, antitumor, wound healing, promoter of cell growth and differentiation in tissue engineering, lubricating agent of synovial joint fluid, hydration therapies, and wrinkle prevention on cosmetic industry

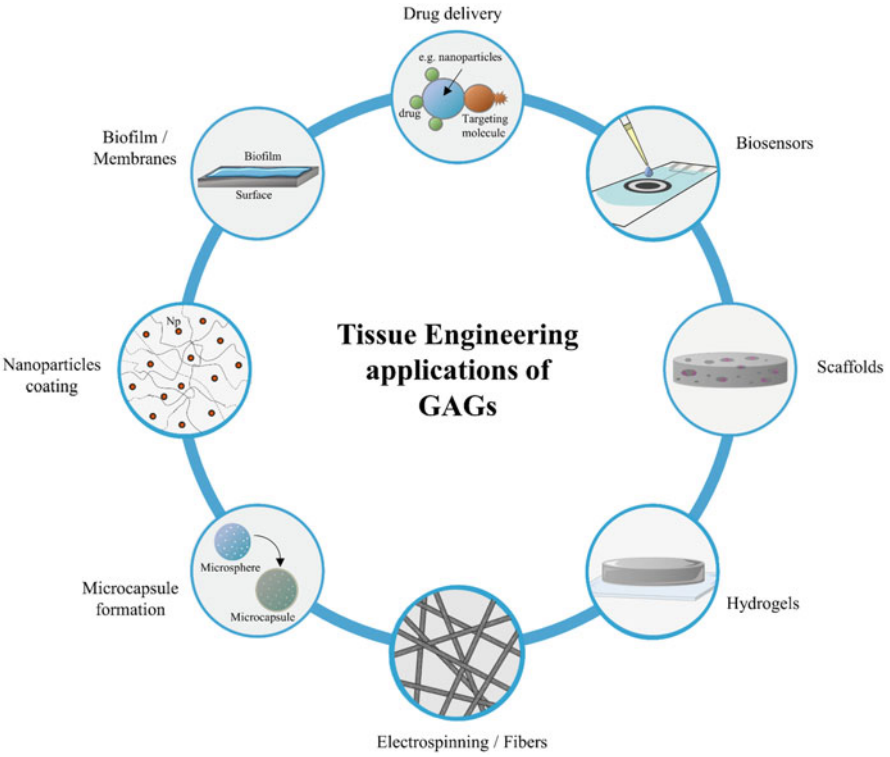


Fig. 6 GAGs and derivatives in some tissue engineering applications

cosmetics to, firm, soften and smooth skin by dint of its hydrating properties and inherent regenerative; HA is an important functional component of the extracellular matrix (ECM) of skin and has shown clinical utility in preventing formation of postsurgical adhesions (Zhu et al. 2017). Due to its relative ease to be extracted and modified and its capability to form 3D structures, HA has become the most used GAG in medical product development (Fallacara et al. 2018; Pomin and Mulloy 2018).

6.2 Chondroitin Sulfate and Dermatan Sulfate

The dermatan molecule has residues of L-iduronic acid, which is commonly considered as the principal difference between chondroitin sulfate and dermatan sulfate. CS is essential in cell–cell interaction and communication, and DS reveals a potential antithrombotic effect. These two GAGs also have remarkable functions in growth factor signaling, neurite outgrowth, cell division, morphogenesis, inflammation, and infection (Hayes and Melrose 2018).

Chondroitin sulfate has an anticoagulant activity and so can be used also as a natural alternative for heparin in several pharmaceutical industries and also as a dietary supplement to treat arthritis pain and inflammation, and as hydrogels for cartilage tissue engineering (De Jesus Raposo et al. 2015). In fact, this polysaccharide can be used as an alternative treatment in cases of osteoarthritis, and occasionally even osteoporosis, because of its essential roles in cartilage and other connective tissues. The use of CS for arthritis treatment is usually related to the use of glucosamine, another key constituent of cartilage tissues (Schiraldi et al. 2010).

On the other hand, dermatan sulfate is used as a stabilizer for growth factors and cytokines. This biomolecule demonstrated a significant anticoagulant property. The anticoagulant activity of dermatan sulfate inhibits thrombin, having no effect on the clotting cascade and in platelet function. With its anticoagulant and antithrombotic activity, DS could be considered a good alternative for heparin, one of the most popular and widely used anticoagulants (Cardoso et al. 2016). Moreover, DS is thought to be involved in wound repair, in cell differentiation, morphogenesis, and migration, which is why this substance is used for cosmetic and clinical applications (Sobczak et al. 2018).

6.3 Heparin and Heparan Sulfate

Heparin is an invaluable drug for the treatment of coagulation and thrombotic disorders and is listed as one of the World Health Organizations essential drugs. It has been widely used as an anticoagulant for more than 80 years and is characterized by several biological activities with potential clinical applications, such as anti-inflammatory, angiogenic and antiangiogenic, anticancer and antiviral activities. It has effects on lipoprotein lipase, on smooth muscle proliferation, and on inhibition of complement activation, among others (Zhang et al. 2010).

Furthermore, due to its high charge density, HEP is also important in cell–cell interactions involving adhesion proteins, cell–cell communication involving chemokines, and cell signaling involving growth factors (Suflita et al. 2015). When crosslinked (physically and chemically), heparin hydrogels have been developed for a variety of clinical applications including the encapsulation of fibroblasts, differentiation of stem cells, and liver tissue engineering applications (Roberts and Martens 2016).

Because of the presence of sulfated groups in the HPS molecule, the polysaccharide is capable to bind to numerous proteins and thus regulates biological processes such as coagulation. Moreover, HPS can bind to several polypeptides like growth factors as well as cellular receptor complexes (Cheng et al. 2017). Recently, it has been used in the development of some new antineoplastic drugs along with new drug delivery systems and also in diagnostics (Weissmann et al. 2016).

6.4 Keratan Sulfate

Keratan sulfate is one of the principal functional components in cornea, particularly in the maintenance of corneal transparency, and it has antiadhesive properties. This

polysaccharide can be used as an active ingredient in eye drops for the treatment of certain visual dysfunctions. This biomolecule has been detected in several neural and epithelial tissues, in which KS expression responds to wound healing, physiological variations, and embryonic development. It has been revealed that KS has functional roles in cellular recognition of protein ligands, axonal guidance, cell motility, in embryo implantation, and in decreasing the immune response in diseases like osteoarthritis in cartilage (Caterson and Melrose 2018).

7 Conclusions and Future Trends

Glycosaminoglycans, with their exceptional properties and diverse applications, have proved to be remarkable materials for medical, pharmaceutical, and cosmetic applications. Most of GAGs are extracted from animal sources to produce active biopolymers to use in several applications. However, GAG-producing microorganisms are renewable resources and could meet current market demands, but remain largely unexplored. Microbial GAG production has the additional and important advantage that bacteria can be physiologically and/or metabolically improved for better biopolymer production. Thus, there is an urgent need to develop industrial-scale microbial production processes of GAGs, with new microorganisms that could be further engineered. Genome sequencing and bioinformatics analysis could be used to discover novel GAG-producing bacteria and/or optimize microbial GAG production. The availability of new genome sequences, together with advances in metabolic engineering and analytical tools, could be applied to evaluate the correlation between cell metabolism and new biologically active GAG compounds. Further investigations on designing new metabolic engineering approaches will also be needed to construct microbial cells that will produce biopolymers with desired structures for a certain application.

Acknowledgments H. Radhouani and C. Gonçalves were supported by the Foundation for Science and Technology (FCT) from Portugal, with references CEECIND/00111/2017 and SFRH/BPD/94277/2013, respectively. J. M. Oliveira thanks the FCT for the fund provided under the Investigador FCT 2015 (IF/01285/2015) program. S. Correia and this work were funded by the R&D Project KOAT – Kefiran exopolysaccharide: Promising biopolymer for use in regenerative medicine and tissue engineering, with reference PTDC/BTMMAT/29760/2017 (POCI-01-0145-FEDER-029760), financed by FCT and co-financed by FEDER and POCI.

References

Almer J, Gesslbauer B, Kungl AJ. Therapeutic strategies to target microbial protein–glycosaminoglycan interactions. Biochem Soc Trans. 2018;46(6):1505–15. https://doi.org/10.1042/BST20170485.

Baik JY, Gasimli L, Yang B, et al. Metabolic engineering of Chinese hamster ovary cells: towards a bioengineered heparin. Metab Eng. 2012;14(2):81–90. https://doi.org/10.1016/j.ymben.2012.01.008.

Bhaskar U, Li G, Fu L, et al. Combinatorial one-pot chemoenzymatic synthesis of heparin. Carbohydr Polym. 2015;122:399–407. https://doi.org/10.1016/j.carbpol.2014.10.054.

Boeriu CG, Springer J, Kooy FK, et al. Production methods for Hyaluronan. Int J Carbohydr Chem. 2013;624967(1–14). https://doi.org/10.1155/2013/624967.

Cardoso MJ, Costa RR, Mano JF. Marine origin polysaccharides in drug delivery systems. Mar Drugs. 2016;14(2):34. https://doi.org/10.3390/md14020034.

Casale J, Crane JS. Biochemistry, glycosaminoglycans. 2019. In: StatPearls. https://pubmed.ncbi.nlm.nih.gov/31335015/. Treasure Island (FL): StatPearls Publishing; 2021. PMID: 31335015.

Caterson B, Melrose J. Keratan sulfate, a complex glycosaminoglycan with unique functional capability. Glycobiology. 2018;28(4):182–206. https://doi.org/10.1093/glycob/cwy003.

Cheng F, Belting M, Fransson LÅ, Mani K. Nucleolin is a nuclear target of heparan sulfate derived from glypican-1. Exp Cell Res. 2017;354(1):31–9. https://doi.org/10.1016/j.yexcr.2017.03.021.

Cimini D, Restaino OF, Schiraldi C. Microbial production and metabolic engineering of chondroitin and chondroitin sulfate. Emerg Top Life Sci. 2018;2(3):349–61. https://doi.org/10.1042/etls20180006.

Datta P, Linhardt RJ, Sharfstein ST. Industrial production of glycosaminoglycans. In: Encyclopedia of microbiology. 2019;681–90. https://doi.org/10.1016/B978-0-12-809633-8.12224-1.

De Jesus Raposo MF, De Morais AMB, De Morais RMSC. Marine polysaccharides from algae with potential biomedical applications. Mar Drugs. 2015;13(5):2967–3028. https://doi.org/10.3390/md13052967.

DeAngelis PL. Microbial glycosaminoglycan glycosyltransferases. Glycobiology. 2002;12(1):9R–16R. https://doi.org/10.1093/glycob/12.1.9r.

DeAngelis PL, White CL. Identification of a distinct, cryptic heparosan synthase from *Pasteurella multocida* Types A, D, and F. J Bacteriol. 2004;186(24):8529–32. https://doi.org/10.1128/JB.186.24.8529-8532.2004.

Deangelis PL, Liu J, Linhardt RJ. Chemoenzymatic synthesis of glycosaminoglycans: re-creating, re-modeling and re-designing nature's longest or most complex carbohydrate chains. Glycobiology. 2013;23(7):764–77. https://doi.org/10.1093/glycob/cwt016.

Delbarre-Ladrat C, Sinquin C, Lebellenger L, et al. Exopolysaccharides produced by marine bacteria and their applications as glycosaminoglycan-like molecules. Front Chem. 2014;2:85. https://doi.org/10.3389/fchem.2014.00085. eCollection 2014.

Fallacara A, Baldini E, Manfredini S, Vertuani S. Hyaluronic acid in the third millennium. Polymers (Basel). 2018;10(7):701. https://doi.org/10.3390/polym10070701.

Fernández CJ, Warren G. In vitro synthesis of sulfated glycosaminoglycans coupled to inter-compartmental golgi transport. J Biol Chem. 1998;273(30):19030–9. https://doi.org/10.1074/jbc.273.30.19030.

Funderburgh JL. Keratan sulfate: structure, biosynthesis, and function. Glycobiology. 2000;10(10):951–8. https://doi.org/10.1093/glycob/10.10.951.

Gandhi NS, Mancera RL. The structure of glycosaminoglycans and their interactions with proteins. Chem Biol Drug Des. 2008;72(6):455–82. https://doi.org/10.1111/j.1747-0285.2008.00741.x.

Ghiselli G. Drug-mediated regulation of glycosaminoglycan biosynthesis. Med Res Rev. 2017;37(5):1051–94. https://doi.org/10.1002/med.21429.

Hayes AJ, Melrose J. Glycans and glycosaminoglycans in neurobiology: key regulators of neuronal cell function and fate. Biochem J. 2018;475(15):2511–45. https://doi.org/10.1042/BCJ20180283.

Hussain Z, Thu HE, Katas H, Bukhari SNA. Hyaluronic acid-based biomaterials: a versatile and smart approach to tissue regeneration and treating traumatic, surgical, and chronic wounds. Polym Rev. 2017;57(4):594–630. https://doi.org/10.1080/15583724.2017.1315433.

Izawa N, Serata M, Sone T, et al. Hyaluronic acid production by recombinant *Streptococcus thermophilus*. J Biosci Bioeng. 2011;111(6):665–70. https://doi.org/10.1016/j.jbiosc.2011.02.005.

Jackson RL, Busch SJ, Cardin AD. Glycosaminoglycans: molecular properties, protein interactions, and role in physiological processes. Physiol Rev. 1991;71(2):481–539. https://doi.org/10.1152/physrev.1991.71.2.481.

Jin X, Zhou Z, Wang Y, et al. Microbial synthesis of glycosaminoglycans with synthetic biology strategies. Sci Sin Vitae. 2019;49(5):553–62. https://doi.org/10.1360/n052018-00249.
Köwitsch A, Zhou G, Groth T. Medical application of glycosaminoglycans: a review. J Tissue Eng Regen Med. 2018;12(1):e23–e41. https://doi.org/10.1002/term.2398.
Lane RS, Ange KS, Zolghadr B, et al. Expanding glycosaminoglycan chemical space: towards the creation of sulfated analogs, novel polymers and chimeric constructs. Glycobiology. 2017;27(7):646–56. https://doi.org/10.1093/glycob/cwx021.
Linhardt RJ, Toida T. Role of glycosaminoglycans in cellular communication. Acc Chem Res. 2004;37(7):431–38. https://doi.org/10.1021/ar030138x.
Liu L, Liu Y, Li J, et al. Microbial production of hyaluronic acid: current state, challenges, and perspectives. Microb Cell Factories. 2011;10:99. https://doi.org/10.1186/1475-2859-10-99.
Lopez Aguilar A, Briard JG, Yang L, et al. Tools for studying glycans: recent advances in chemoenzymatic glycan labeling. ACS Chem Biol. 2017;12(3):611–21. https://doi.org/10.1021/acschembio.6b01089.
Mende M, Bednarek C, Wawryszyn M, et al. Chemical synthesis of glycosaminoglycans. Chem Rev. 2016;116(14):8193–255. https://doi.org/10.1021/acs.chemrev.6b00010.
Meneghetti MCZ, Hughes AJ, Rudd TR, et al. Heparan sulfate and heparin interactions with proteins. J R Soc Interface. 2015;12(110):0589. https://doi.org/10.1098/rsif.2015.0589.
Mishra A, Pavelko T. Treatment of chronic elbow tendinosis with buffered platelet-rich plasma. Am J Sports Med. 2006;34:1774–8. https://doi.org/10.1177/0363546506288850.
Morla S. Glycosaminoglycans and glycosaminoglycan mimetics in cancer and inflammation. Int J Mol Sci. 2019;20(8):1963. https://doi.org/10.3390/ijms20081963.
Moscovici M. Present and future medical applications of microbial exopolysaccharides. Front Microbiol. 2015;6:1012. https://doi.org/10.3389/fmicb.2015.01012.
Naccari C, Cristani M, Cimino F, et al. Common buzzards (Buteo buteo) bio-indicators of heavy metals pollution in Sicily (Italy). Environ Int. 2009;35:594–8. https://doi.org/10.1016/j.envint.2008.11.002.
Neves MI, Araújo M, Moroni L, et al. Glycosaminoglycan-inspired biomaterials for the development of bioactive hydrogel networks. Molecules. 2020;25(4):978. https://doi.org/10.3390/molecules25040978.
Nwodo UU, Green E, Okoh AI. Bacterial exopolysaccharides: functionality and prospects. Int J Mol Sci. 2012;13(11):14002–15. https://doi.org/10.3390/ijms131114002.
Ondresik M, Maia FRA, Morais AD, et al. Management of knee osteoarthritis. Current status and future trends. Biotechnol Bioeng. 2017;114:717–39. https://doi.org/10.1002/bit.26182.
Pomin VH, Mulloy B. Glycosaminoglycans and proteoglycans. Pharmaceuticals. 2018;11(1):27. https://doi.org/10.3390/ph11010027.
Prydz K. Determinants of glycosaminoglycan (GAG) structure. Biomol Ther. 2015;5(3):2003–22. https://doi.org/10.3390/biom5032003.
Raedts J, Kengen SWM, Van Der Oost J. Occurrence of L-iduronic acid and putative D-glucuronyl C5-epimerases in prokaryotes. Glycoconj J. 2011;28(2):57–66. https://doi.org/10.1007/s10719-011-9324-7.
Raedts J, Lundgren M, Kengen SWM, et al. A novel bacterial enzyme with d-glucuronyl C5-epimerase activity. J Biol Chem. 2013;288(34):24332–9. https://doi.org/10.1074/jbc.M113.476440.
Raman R, Sasisekharan V, Sasisekharan R. Structural insights into biological roles of protein-glycosaminoglycan interactions. Chem Biol. 2005;12(3):267–77. https://doi.org/10.1016/j.chembiol.2004.11.020.
Roberts JJ, Martens PJ. Engineering biosynthetic cell encapsulation systems. In Poole-Warren L, Martens P, Green R (Ed.). Biosynthetic polymers for medical applications. Woodhead Publishing. 2015;205–39. https://doi.org/10.1016/B978-1-78242-105-4.00009-2 2016.
Schiraldi C, Cimini D, De Rosa M. Production of chondroitin sulfate and chondroitin. Appl Microbiol Biotechnol. 2010;87(4):1209–20. https://doi.org/10.1007/s00253-010-2677-1.

Soares da Costa D, Reis RL, Pashkuleva I. Sulfation of glycosaminoglycans and its implications in human health and disorders. Annu Rev Biomed Eng. 2017;19:1–26. https://doi.org/10.1146/annurev-bioeng-071516-044610.

Sobczak AIS, Pitt SJ, Stewart AJ. Glycosaminoglycan neutralization in coagulation control. Arterioscler Thromb Vasc Biol. 2018;38(6):1258–70. https://doi.org/10.1161/ATVBAHA.118.311102.

Suflita M, Fu L, He W, et al. Heparin and related polysaccharides: synthesis using recombinant enzymes and metabolic engineering. Appl Microbiol Biotechnol. 2015;99(18):7465–79. https://doi.org/10.1007/s00253-015-6821-9.

Tamer TM. Hyaluronan and synovial joint: function, distribution and healing. Interdiscip Toxicol. 2013;6(3):111–25. https://doi.org/10.2478/intox-2013-0019.

Vann WF, Schmidt MA, Jann B, Jann K. The structure of the capsular polysaccharide (K5 Antigenn) of urinary-tract-infective *Escherichia coli* 010:K5:H4: a polymer similar to Desulfo-Heparin. Eur J Biochem. 1981;6(3):111–25. https://doi.org/10.1111/j.1432-1033.1981.tb05343.x.

Vázquez JA, Rodríguez-Amado I, Montemayor MI, et al. Chondroitin sulfate, hyaluronic acid and chitin/chitosan production using marine waste sources: characteristics, applications and eco-friendly processes: a review. Mar Drugs. 2013;11(3):747–74. https://doi.org/10.3390/md11030747.

Wang TT, Zhu CY, Zheng S, et al. Identification and characterization of a chondroitin synthase from *Avibacterium paragallinarum*. Appl Microbiol Biotechnol. 2018;02(11):4785–97. https://doi.org/10.1007/s00253-018-8926-4.

Weissmann M, Arvatz G, Horowitz N, et al. Heparanase-neutralizing antibodies attenuate lymphoma tumor growth and metastasis. Proc Natl Acad Sci USA. 2016;113(3):704–9. https://doi.org/10.1073/pnas.1519453113.

Weitz JI, Harenberg J. New developments in anticoagulants: past, present and future. Thromb Haemost. 2017;117(7):1283–8. https://doi.org/10.1160/TH16-10-0807.

Yamada S, Sugahara K, Özbek S. Evolution of glycosaminoglycans. Commun Integr Biol. 2011;4(2):150–8. https://doi.org/10.4161/cib.4.2.14547.

Zakeri A, Rasaee MJ, Pourzardosht N. Enhanced hyluronic acid production in *Streptococcus zooepidemicus* by over expressing HasA and molecular weight control with Niscin and glucose. Biotechnol Reports. 2017;16:65–70. https://doi.org/10.1016/j.btre.2017.02.007.

Zhang F, Zhang Z, Linhardt RJ. Glycosaminoglycans. In: Handbook of Glycomics. Cummings RD, Pierce JM (Eds). 2010;59–80. San Diego: Academic Press.

Zhu Z, Wang Y-M, Yang J, Luo X-S. Hyaluronic acid: a versatile biomaterial in tissue engineering. Plast Aesthetic Res. 2017;4:219–27. https://doi.org/10.20517/2347-9264.2017.71.

Part II

Fungal and Microalgal Polysaccharides

Botryosphaeran

10

An Unusual Exocellular Fungal (1→3)(1→6)-β-D-Glucan with Notable Biomedical Applications

Robert F. H. Dekker and Aneli M. Barbosa-Dekker

Contents

Abstract

Botryosphaeran [(1→3)(1→6)-β-D-glucan] is an exopolysaccharide (EPS) produced by the ascomycete *Botryosphaeria rhodina* MAMB-05 when cultivated on glucose medium. Botryosphaeran has an unusual chemical structure in the sense that one of its two appendage residues (gentiobiose) attached to the (1→3)-linked backbone chain is rare, the other, glucose, is common to most β-glucans of this type. A *family* of botryosphaerans is produced on different carbohydrate substrates. Botryosphaeran is neither mutagenic nor genotoxic, and presents antioxidant, anticlastogenic, hypoglycemic, hypocholesterolemic, and hypotriglyceridemic

R. F. H. Dekker (✉)
Universidade Tecnológica Federal do Paraná (UTFPR), Programa de Pós-Graduação em Engenharia Ambiental, Câmpus Londrina, Londrina, Paraná, Brazil

A. M. Barbosa-Dekker
Beta-Glucan Produtos Farmoquímicos - EIRELI, Lote 24A, Bloco Zircônia, Universidade Tecnológica Federal do Paraná, Avenida João Miguel Caram, Londrina, Paraná, Brazil

J. M. Oliveira et al. (eds.), *Polysaccharides of Microbial Origin*,
https://doi.org/10.1007/978-3-030-42215-8_63

activities and reduced the development of Walker-256 bearing tumors and cachexia syndrome in rats. Botryosphaeran exhibited antiproliferation of cancer cells that is associated with cell cycle arrest, apoptosis, necrosis, and oxidative stress in breast carcinoma MCF-7 cells, and cell cycle arrest in human T-lymphocyte tumorigenic cells. Botryosphaeran is commercially used in formulated cosmetic products to promote skin health and treat skin conditions.

Keywords

Family of botryosphaerans · Sulfonated botryosphaeran · Antimutagenicity · Anti-obesogenicity · Hypoglycemia · Dyslipidemia · Breast cancer MCF-7 · Walker 256 tumor

1 Introduction

The discovery of botryosphaeran (an exopolysaccharide) evolved from a fungal isolate collected from a colony of fungi growing on a stem canker of a eucalyptus tree. The isolate was purified to an axenic culture, designated MAMB-05, morphologically characterized as a species of the genus *Botryosphaeria* (taxa *Botryosphaeriaceae*) (Barbosa et al. 1996), and molecularly identified to the species level as *Botryosphaeria rhodina* (Saldanha et al. 2007).

Cultivation of isolate MAMB-05 on glucose media by submerged fermentation resulted in increased viscosity of the culture fluid suggesting the presence of an exocellular biopolymer. On treating the cell-free fermentation broth with ethanol, a white precipitate resulted, which when isolated and subjected to total acid hydrolysis, yielded glucose as the only hydrolysis product, indicating that the biopolymer was an exopolysaccharide constituted of D-glucose residues; i.e., a D-glucan (Dekker and Barbosa 2001). After isolation, purification, and chemical structural characterization, the exopolysaccharide was identified as a (1→3)(1→6)-β-D-glucan, and named botryosphaeran (Barbosa et al. 2003).

The polysaccharide group of β-D-glucans consist chiefly of a backbone chain comprising β-(1→3)-linked D-glucose residues containing appendages comprising D-glucose units through β-(1→6)-linkages (Stone 2009). The chemistry and biology of the (1→3)-β-D-glucans have been widely studied and have attracted much interest because of their unique structure-biological function relationships (Stone and Clarke 1992; Bacic et al. 2009).

Besides being produced exocellularly, the (1→3)(1→6)-linked β-D-glucans also participate as structural elements in the cell wall of fungi including yeasts, where they are found in a matrix along with other components including proteins and other polysaccharides: chitin, α- and β-glucans of various glycosidic linkages, and mannans (Gow et al. 2017).

The β-glucans vary in fine physical structure, molecular size, degree of branching, and conformation (random coil, single and triple helices), properties that confer a crucial role on their biological properties (Leung et al. 2006; Wang et al. 2017). The

β-glucans possess strong antioxidant activity through scavenging of free radicals thereby avoiding cellular damage by reactive oxygen species (ROS) that trigger oxidative stress (Wang et al. 2013, 2016).

The (1→3)-β-D-glucans possess biological response modifying activities such as immunostimulation and immunomodulation (Bohn and BeMiller 1995), and are well recognized in effectively treating human disease conditions that includes obesity, dyslipidemia, diabetes, inflammation, cardiovascular diseases, and cancers (Chen and Raymond 2008; Vetvicka and Vetvickova 2012; Wouk et al. 2021).

The biological activities of the β-glucans are manifested through internal cell-signaling events on binding to the carbohydrate-binding domain on surface receptors (Dectin-1, Toll-like Receptors (TLR), Complement Receptor CR-3) of immune cells, where they are recognized as pathogen-associated molecular patterns, and initiate the *innate* and *adaptive* immune response of the host resulting in the systemic activation of the whole immune system. This triggers the secretion of cytokines (signaling proteins) and chemokines (small chemo-attractant cytokines) that influence cellular functions.

2 Chemical Structure of Botryosphaeran

The chemical structure of the botryosphaeran (designated **EPS_{GLC}**) from *Botryosphaeria rhodina* MAMB-05 cultivated on glucose was determined through methylation analysis, Smith degradation, Fourier-Transform Infra-Red (FT-IR), and ^{13}C Nuclear Magnetic Resonance (NMR) spectroscopy and provided precise details on the sugar moiety, nature and configuration of the glycosidic linkages, and the polysaccharide's appendages (Barbosa et al. 2003). Methylation and acid hydrolysis of the permethylated polysaccharide revealed the presence of 2,3,4,6-tetra-*O*-methyl-glucose (**I**, *nonreducing terminal unit*), 2,4,6-tri-*O*-methyl-glucose (**II**, *3-O-substituted glucosyl residue*), 2,3,4-tri-*O*-methyl-glucose (**III**, *6-O-substituted glucosyl residue*), and 2,4-di-*O*-methyl-glucose (**IV**, *3,6-di-O-substituted unit*) as the main methylated sugar derivatives (molar proportions 26:36:16:22, respectively). Methylation analysis demonstrated that botryosphaeran is a glucan consisting of a (1→3)-linked glucose backbone substituted with approximately 22% branch points on C-6 of the glucose moiety. Periodate oxidation and Smith degradation revealed side chains consisting of both single glucose residues, and short-chained (1→6)-β-linked glucosyl disaccharides.

FT-IR spectroscopy confirmed β-anomeric carbons in botryosphaeran and (1→3)-di-*O*-substituted glucose residues. ^{13}C NMR spectral analysis on purified botryosphaeran and the periodate-oxidized polysaccharide revealed that all the carbon lines were well resolved, and chemical shifts were congruent with literature values. ^{13}C NMR analysis revealed no peaks resonating around δ 100.0 ppm, typical of α-configuration of anomeric carbons. Peaks resonating between δ 103.3 and 102.9 ppm provided clear evidence that only β-anomeric carbons were present.

The structural investigation concluded that botryosphaeran was a (1→3)-β-D-glucan with (1→6)-β-linked single glucose and gentiobiose appendages attached at random along the backbone chain at a frequency of one branch point (glucose or

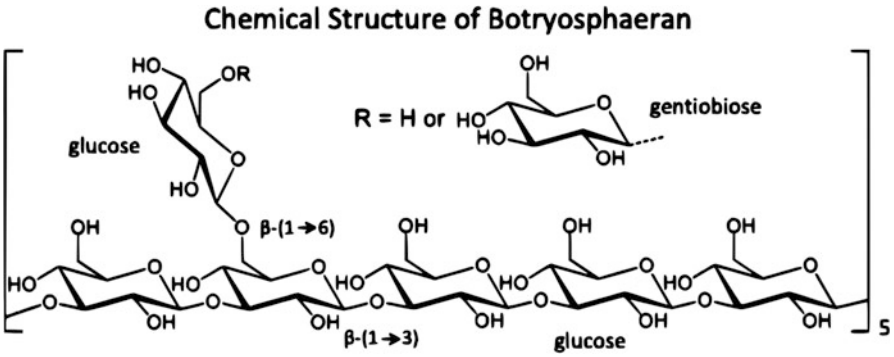


Fig. 1 The chemical structure of botryosphaeran – (1→3)(1→6)-β-D-glucan – an exopolysaccharide produced by the ascomycete *Botryosphaeria rhodina* MAMB-05 when cultivated on glucose medium

gentiobiose) to every five glucose residues (Barbosa et al. 2003). The proportion of glucose-to-gentiobiose along the backbone chain was 6:16 (Fonseca et al. 2011). The structure of botryosphaeran (EPS_{GLC}) is shown in Fig. 1.

2.1 A Family of Botryosphaerans

When *Botryosphaeria rhodina* was cultivated on other carbohydrate substrates (galactose, mannose, fructose; lactose, sucrose), the fungus produced exopolysaccharides, which after isolation and acid hydrolysis produced only glucose; i.e., they were glucans. FT-IR spectroscopy of the exopolysaccharides presented confirmation they all possessed a (1→3)-β-glucan backbone, thus constituting a *family* of botryosphaerans (Steluti et al. 2004).

Although the carbohydrate sources did not change the chemical structure of the backbone chain of botryosphaeran, they strongly influenced the degree of branching by glucose and gentiobiose on the polysaccharide chain. When *Botryosphaeria rhodina* was grown on either fructose or sucrose, exopolysaccharides designated $\mathbf{EPS_{FRU}}$ and $\mathbf{EPS_{SUC}}$, respectively, resulted (Corradi da Silva et al. 2005). Methylation analysis of EPS_{FRU} and EPS_{SUC} revealed they both constituted a typical botryosphaeran structure comprising a backbone chain of β-(1→3)-linked glucose units with side chains of a single glucose residue, and also a short-chained (1→6)-β-linked glucosyl disaccharide (gentiobiose), in keeping with the chemical structure of botryosphaeran (EPS_{GLC}).

EPS_{FRU} was more branched (31%) than botryosphaerans, EPS_{GLC} and EPS_{SUC}, having a branch point to every three glucose units along the backbone chain. Additionally, the relatively low amount of the tri-*O*-methyl derivative (**II**, *3-O-substituted glucosyl residue*) of EPS_{FRU} by comparison to the tetra-*O*-methyl (**I**) and the di-*O*-methyl (**IV**) derivatives presented evidence of a highly branched structure. Furthermore, the low value of the tri-*O*-methyl (**III**) derivative suggested

that the backbone chain of EPS_{FRU} consisted essentially of consecutive (1→3)-linked D-glucose residues with side branching on C-6.

FT-IR and ^{13}C NMR spectra of EPS_{FRU} and EPS_{SUC} revealed very similar data to that obtained for EPS_{GLC}, the only difference being in the intensity of the ^{13}C NMR signals of EPS_{FRU}. The most important difference was the higher amount of the 6-*O*-substituted derivative in EPS_{FRU} compared to EPS_{SUC}. This evidence was confirmed by the intensity of the signal at δ 70.1 and correlated to glucose residues with substitution on C-6 with glucose or gentiobiose residues, in accord with the methylation results.

The degree of branching for EPS_{SUC} (21%) (Corradi da Silva et al. 2005) was similar to that of EPS_{GLC} (22%) (Barbosa et al. 2003). The proportion of glucose to gentiobiose along the backbone chain for EPS_{FRU} was 16:15, and for EPS_{SUC}, 12:9 (Fonseca et al. 2011).

Our studies on botryosphaeran indicated that the extent of substitution of repetitive branching residues could be modified through the source of carbohydrate during fungal growth. Manipulation of the nutrient media, however, did not change the structure or molecular constitution of the backbone chain of the botryosphaerans.

All three botryosphaerans exist in a triple helix conformation (Giese et al. 2008), which strongly influences biological activity (Yoshitomi et al. 2005), and all exhibited free-radical scavenging properties and antioxidant activities (Giese et al. 2015).

2.2 Sulfonylation of Botryosphaeran: Anticoagulant and Antiviral Activities

Certain physical structural features of (1→3)-β-glucans (e.g., molecular weight, degree of polymerization, chemical nature of the side-branch residues, and degree of ramification as well as conformation of the constituent chains in solution) can lead to solubility problems in aqueous solutions (Wang et al. 2017). The problem of water solubility can be resolved by adding appropriate functional groups (e.g., sulfonyl) to hydroxyl positions along the polysaccharide chain (Kagimura et al. 2015).

Sulfonylation of polysaccharides introduces a charged group (anionic sulfonate) that improves water-solubility, but also changes the biological functions of the modified polysaccharide, e.g., mimicking heparin activity, i.e., anticoagulation (Jiao et al. 2011). Sulfonated polysaccharides, naturally derived from various plant sources including algae and microorganisms, and those chemically derived by sulfonylation reactions, are well known to present anticoagulant activity.

A blood clot is initiated with the activation of platelets that is followed by a cascade of coagulation events catalyzed by enzymes (serine proteases) leading to the formation of thrombin, which acts in the conversion of fibrinogen to fibrin to form a stable cross-linked clot. Heparin has the property of an anticoagulant, and interferes in this process preventing blood clotting, and acts by inhibiting antithrombin of the intrinsic pathway of the coagulation cascade. The biological mechanism of sulfonated polysaccharides occurs by the potentiation of plasma cofactors that are natural inhibitors of the coagulation cascade of events (Melo et al. 2004).

Anticoagulation activity of a sulfonated polysaccharide can be determined by specific assays monitoring clot formation of blood plasma, and measures the clotting times taken for the formation of activated partial thromboplastin (APTT; intrinsic phase of coagulation), thrombin (TT; conversion of plasmatic fibrinogen to fibrin), and prothrombin (PT; extrinsic phase of coagulation). Heparin is used as the reference standard. The coagulation time in the last stage of the cascade is the conversion of fibrinogen to fibrin by thrombin.

Botryosphaerans EPS_{GLC} and EPS_{FRU} sulfonylated using chlorosulfonic acid in formamide solvent readily became water-soluble. The sulfonated botryosphaerans showed an absorption peak at 259 nm in the ultraviolet (UV) spectrum, which was due to the introduced sulfonate group, while FT-IR spectral analysis supported evidence of the introduced sulfonate groups in both botryosphaerans (Mendes et al. 2009; Brandi et al. 2011). ^{13}C NMR spectral analysis of the sulfonated polysaccharides showed that the C-6 signals shifted to around δ 68.0 compared to the parent botryosphaeran (δ 60.7–61.1) indicating that most of the hydroxyl-free C-6 on glucose was substituted by the introduced sulfonate groups. The primary hydroxyl groups on position C-6 is more accessible for sulfonation than the secondary hydroxyl groups, which may be sterically hindered when introducing sulfonate groups. The presence of the β-(1→6)-glucosidic linkages in the side chains may also sterically reduce the number of primary hydroxyl groups available for sulfonation. Brandi et al. (2011) presented evidence that sulfonate substitution on botryosphaeran was absent from hydroxyls at C-2 and C-4, presumably due to steric hindrance.

When the sulfonated botryosphaerans were assayed in blood clotting tests, they exhibited anticoagulation activity compared to the parent, unmodified polysaccharide (Mendes et al. 2009; Brandi et al. 2011). The APTT and TT tests of highly branched sulfonated botryosphaeran (S-EPS_{FRU}) indicated significant in vitro anticoagulant activity that was both dose- and degree of sulfonation-dependent. The prolongation of APTT in the presence of S-EPS_{FRU} indicated inhibition of the intrinsic pathway of coagulation, while the extension of the TT time indicated inhibition of the conversion of fibrinogen into fibrin (Brandi et al. 2011). The anticoagulant action of S-EPS_{FRU} is most likely related to the activation of antithrombin that leads to the inhibition of thrombin formation, as the times of clotting were severely prolonged. Un-sulfonated botryosphaeran did not inhibit any of the three coagulation tests, hence has no anticoagulant activity (Mendes et al. 2009; Brandi et al. 2011).

Besides anticoagulation activity, sulfonated polysaccharides are also well known for antiviral activity by inhibiting infection of viruses (Chen and Huang 2018), and do this by interacting with the positively charged motifs on glycoproteins of the virus responsible for binding to cell surface receptors on the human host cell. Sulfonylation of botryosphaeran (EPS_{SUC}) exhibited strong antiviral activity against the enveloped viruses *Dengue* and *Herpes simplex* (Sacchelli et al. 2019).

The parent unmodified botryosphaeran moderately inhibited acyclovir-sensitive (HSV-KOS) and acyclovir-resistant (HS-VAR) strains of *Herpes simplex* infection in Vero cells (monkey kidney epithelial cells), and demonstrated that native

botryosphaeran exhibited antiherpetic activity. Sulfonated botryosphaerans of low (0.4) and high (1.1) degrees of substitution (DS) potently inhibited infection against HSV-KOS and HSV-AR. Likewise, dengue viral (DENV-3) infection of Vero cells was weakly inhibited by the parent botryosphaeran, but inhibition was remarkably stronger for the two sulfonated botryosphaerans. Apparently, the presence of sulfonate groups on the polysaccharide chain, and the DS, were important characteristics of antiviral activity of botryosphaeran (Sacchelli et al. 2019).

3 Antimutagenicity and Chemopreventive Effect of Botryosphaeran

New compounds with potential nutraceutical and biomedical applications need *first* to be screened for mutagenic and genotoxic activities, as this property assesses cellular damage on the host. There are a number of tests considered biomarkers for assessing mutagenic and genotoxic damage. These can be conveniently performed on animal models and mammalian cell-lines, and include: cell viability and cell proliferation – MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay (Mosmann 1983), micronucleus test (Hayashi 2016), comet assay (Tice et al. 2000), and the Ames test – uses bacteria (*Salmonella typhimurium* strains) instead of animals (Maron and Ames 1983). Genotoxicity can be assessed by the cytokinesis-block micronucleus assay (Fenech 1993).

Another important characteristic in assessing a new compound is whether it can exert a chemoprotective effect to reduce mutations induced by common chemotherapeutic drugs to treat cancers (e.g., cyclophosphamide, doxorubicin, methyl methanesulfonate, bleomycin), or mutagens (benzo[a]pyrene). These drugs can be used experimentally as cytostatic agents to induce cellular damage in micronucleus formation in bone marrow and peripheral blood cells of model lab animals.

Botryosphaeran was demonstrated to be non-mutagenic, nor cytotoxic or genotoxic, and exhibited strong antimutagenic activity against the *in vivo* DNA-damaging effect of cyclophosphamide, reducing the clastogenic (aneugenic) effect of cyclophosphamide-induced micronucleus formation in bone marrow and peripheral blood cells in Swiss mice (Miranda et al. 2008). In a further study using a higher dose of botryosphaeran (30 mg/kg body weight (b.w.)/day/15 days), Silva-Sena et al. (2018) supported the findings of Miranda et al. (2008), and confirmed that botryosphaeran was not mutagenic nor genotoxic in mice (young and adult of both genders). Botryosphaeran, furthermore, exhibited strong antimutagenic activity being able to reduce the number of micronucleated cells formed by clastogenic cyclophosphamide.

Similar studies with mammalian cell-lines: Chinese hamster lung fibroblasts (V79), rat hepatocarcinoma cells (HTC), and cultured normal and tumor (leukemic) human T lymphocytes also confirmed that botryosphaeran was neither mutagenic nor genotoxic, and exhibited chemoprotective effects against the clastogenic effects of doxorubicin and methyl methanesulfonate (Malini et al. 2016), and the mutagen benzo[a]pyrene (Kerche-Silva et al. 2017).

The findings that botryosphaeran did not present clastogenic or aneugenic damage in mice, nor in several mammalian cell-lines, indicated that it was safe for human use, and thus possessed GRAS status (Generally Recommended As Safe). An interesting feature of botryosphaeran was its strong chemoprotective effect against DNA damage and cell-death induced by chemotherapeutic drugs. This property, and its immunopotentiation function, could find applications of botryosphaeran as a coadjuvant in vaccines, and in treating cancers to lessen the side effects of therapeutic drugs.

4 Anti-Obesogenic Activity of Botryosphaeran

Obesity, an overweight condition prevalent worldwide, is based upon a body mass index (BMI) exceeding 30 kg/m^2, and is a strong risk factor for metabolic dysfunction, diabetes, dyslipidemia, and hepatic steatosis that can lead to cardiovascular diseases and cancer. Obesity occurs when there is an energy imbalance with caloric intake far exceeding caloric expenditure. Obesity is characterized by an excess of white adipose tissue stored in the body as an energy source. The incidence of obesity along with diabetes, dyslipidemia, hepatic steatosis, and their associated comorbidities is a worldwide health problem increasing at an alarming rate.

Fungal (1→3)-β-glucans are known to reduce the risk of obesity and diabetes, and are effective in treating human hyperglycemia, hyperlipidemia, and hypercholesterolemia that pose cardiovascular risks (Chen and Raymond 2008); Wouk et al. 2021). Studies have revealed that β-glucans can reduce body weight through weight control and metabolism (Rahmani et al. 2019).

Silva et al. (2018) induced obesity in rats by feeding a high-fat and high-sugar diet over an 8-week period, and observed symptoms of glucose intolerance, insulin resistance, dyslipidemia, and hepatic steatosis. Treatment with botryosphaeran (12 mg/kg. body weight (b.w.)/day over 15 days) attenuated obesity (reduced weight gains and adipose tissue mass), hepatic steatosis (reduced total lipids, triglycerides, and cholesterol), dyslipidemia (reduced the levels of triglycerides and very-low density lipoprotein (VLDL), and increased high density lipoprotein (HDL) levels), and improved glucose intolerance and insulin sensitivity, as well as increasing AMP-activated protein-kinase (AMPK) and Forkhead transcription factor, FOXO3a, activities in adipose tissue (Silva et al. 2018).

AMPK plays a key role in metabolism, especially in cellular energy homeostasis. AMPK regulates several metabolic enzymes and transcription factors including the FOXO forkhead transcription factors, and signaling pathways in liver, adipose tissue, skeletal muscle, and heart that contribute to cellular metabolism of glucose and lipids (Kahn et al. 2005). FOXO is involved in regulating energy metabolism (Calnan and Brunet 2008).

Obese rats bearing Walker-256 tumors presented accumulation of adipose tissue mass, reduced muscle mass, glucose intolerance, insulin resistance, hyperglycemia, anemia, leukocytosis, and thrombocytopenia. Botryosphaeran corrected insulin resistance and hyperglycemia, modulated cholesterol levels, and increased total

leukocyte and lymphocyte counts. Obesity increased tumor development and cachexia syndrome (prognostic in cancer patients, which is characterized by weight loss, anorexia, asthenia, and anemia), and was significantly higher for the obese group than the nonobese group of rats. Treatment with botryosphaeran (12 mg/kg. b. w./day/15 days) attenuated these parameters. The immune response is thought to contribute to lowering the development of tumor, and botryosphaeran effectively attenuated tumor growth (Comiran et al. 2021).

In non-obese rats bearing Walker-256 tumor, treatment with a higher dose of botryosphaeran (30 mg/kg. b.w./day/15 days) maintained glycemia within the normal limits, and ameliorated the hypertriglyceridemic condition. Botryosphaeran modulated the levels of glucose, triglycerides and HDL-cholesterol, corrected macrocytic anemia (condition of enlarged red blood cells with low hemoglobin content), and significantly reduced tumor development and cancer cachexia syndrome by increasing apoptosis (ordered cascade of enzymatic events leading to cell death) of the tumor cells through increasing bax expression in the rats (Geraldelli et al. 2020a). Bax, known as the bcl-2-like protein 4, is a member of the *Bcl-2* gene family that acts as a regulator of cell death by either inhibiting or inducing apoptosis.

In obese rats with Walker-256 bearing tumor, botryosphaeran treatment at the same dose (30 mg/kg b.w./day for 15 days) significantly decreased tumor growth and the intensity of the neoplastic cachexia syndrome, and corrected the macrocytic anemia condition. The mechanism is thought to be associated with the reduction of visceral adipose tissue, the improvement of insulin sensitivity, and increased activity of FOXO3a observed in the tumor tissue (Geraldelli et al. 2020b).

From the aforementioned studies on botryosphaeran, we conclude that this unusual fungal (1→3)(1→6)-β-D-glucan presented anti-obesogenic activity beneficial in the obese condition that may be associated with other comorbidities, such as dyslipidemia, insulin resistance, and cancer.

5 Hypoglycemia and Hypocholesterolemia Exhibited by Botryosphaeran

5.1 Improving the Diabetic Condition

Diabetes, a debilitating chronic metabolic disorder, is characterized by hyperglycemia (high glucose levels) due to defects in insulin secretion and insulin action. Epidemiological studies have presented evidence that diabetes is linked to an increased risk of cancer and cardiovascular diseases with the prevailing factor being the high glucose levels. The diabetic condition is manifested by insulin resistance and insufficient insulin secreted by the endocrine system (β cells) of the pancreas. Hyperglycemia also results in the generation of reactive oxygen/nitrogen species that severely impact on the diabetic condition. Management of the diabetic condition focuses primarily on controlling hyperglycemia through administering insulin or drugs like metformin, depending upon the type of diabetes mellitus; type 1 (genetic, severe deficiencies of insulin), and type 2 (late-onset and prediabetic

condition manifested by glucose intolerance). Managing diabetes is essential in order to decrease, or avoid, other conditions that include cardiovascular-related diseases and cancer, among others.

Cereals and mushrooms contain β-glucans that effectively lower blood glucose levels through dietary changes that are helpful for diabetics. Such products display antiglycemic properties and reduce the condition of diabetes, and the risk of developing diabetes, without experiencing undesirable side effects common to drugs used to treat the diabetic condition.

Diabetes can be induced experimentally in lab animal models through injection of diabetogenic agents such as alloxan (class of pyrimidones) and streptozotocin (alkylating antineoplastic agent). They selectively cause injury to the insulin-secreting β cells of the pancreas that leads to type-1 diabetes.

Treatment of streptozotocin-induced diabetic male rats with botryosphaeran (12 mg/kg. b.w./day) lowered blood glucose levels by 52% over a 15-day period, and was accompanied by a reduced intake of ration and an increase in the median body weight gain, as well as in the efficiency of food conversion in the diabetic animals. Botryosphaeran was furthermore observed to exert a protective effect by reducing the symptoms of cachexia in the type-1 diabetic animals (Miranda-Nantes et al. 2011). In related studies, obese rats presented glucose intolerance because of the obesogenic condition, and treatment with botryosphaeran (12 mg/kg. b.w./day) over 15 days was effective in decreasing obesity (reduced feed intake, weight gain, and periepididymal and mesenteric adipose tissue weights), and improved glucose intolerance (reduced plasma glucose levels, 26%) and insulin sensitivity in the obese animals (Silva et a. 2018). In another related study using 18-month old male knockout LDLr$^{-/-}$ mice genetically predispositioned to hyperlipidemia, treatment with botryosphaeran (30 mg/kg b.w./day) over 15 days improved glucose intolerance reducing the plasma glucose levels (36%) (Silva-Sena et al. (2018).

5.2 Improving the Dyslipidemic Condition

Dyslipidemia is often related to obesity, and is the condition of abnormally elevated levels of cholesterol and lipids in the circulating blood system, and is the primary cause of cardiovascular diseases such as atherosclerosis, cardiac infarction, and stroke (ischemic and hemorrhagic) (Wouk et al. 2021). Prevention and management of this condition is crucial to decrease the alarming number of morbidities and mortalities related to heart diseases. Dyslipidemia is associated with hypertriglyceridemia and hypercholesterolemia, and its prevention decreases fat accumulation and manages obesity.

The control of cholesterol can be successfully targeted by statins (lipid-lowering drugs) that block metabolic pathways leading to the synthesis of cholesterol. Statins, although effective in reducing cardiovascular diseases, also possess adverse side effects (Goulomb and Evans 2008). A class of natural products with GRAS status that lower cholesterol without experiencing side effects are the (1→3)-β-D-glucans

(Sima et al. 2018). They have been demonstrated to be efficacious in treating hypercholesterolemic activity (Chen and Raymond 2008).

The β-glucans are categorized in the polysaccharide group that constitutes "dietary fibers" and remain undigested in their passage through the human gastrointestinal tract. Fiber is characterized by its physical properties that feature their soluble or insoluble nature. The β-glucans as fibers present health benefits that have been reported to include reduction of bowel transit time thus preventing constipation and reducing the risk of colorectal cancer. Besides lowering cholesterol and regulating glucose blood levels in the respective management of dyslipidemia and diabetes, they also promote the growth of beneficial gut microflora (gut microbiota) producing short-chain fatty acids that influence metabolism in the host (Brennan and Cleary 2005).

Botryosphaeran treatment (12 mg/kg. b.w./day) of rats preconditioned to hyperlipidemia and fed a high-fat diet over 15 days reduced the total plasma cholesterol and LDL-cholesterol (*bad* cholesterol) levels by 18.6% and 27%, respectively (Miranda-Nantes et al. 2011). In a related but independent study, male knockout $LDLr^{-/-}$ mice developed elevated plasma cholesterol levels and atherosclerosis on feeding a high-fat diet. Treatment with botryosphaeran (30 mg/kg. b.w./day) over 15 days attenuated the lipidic profiles reducing them by 84%, and decreased LDL-cholesterol by 86%. Additionally, the deposition of lipids in the aorta decreased by ~33% in the mice group tested compared with a control, thus lowering the cardiovascular risk of atherosclerosis (Silva-Sena et al. 2018). Botryosphaeran can therefore serve as a promising agent to treat cardiovascular-related diseases with potential for use as a nutraceutical to treat these metabolic conditions.

In a further related study from our research group, Silva et al. (2018) demonstrated that obese rats induced on a high-fat and high-sugar diet showed significant increases in weight gain and adipose tissue mass, and presented hepatic steatosis and dyslipidemia. Treatment of the obese rats with botryosphaeran (12 mg/kg b.w.) over 15 days attenuated obesity significantly reducing feed intake, weight gain, deposition of periepididymal and mesenteric fat, reduced VLDL-cholesterol, and raised HDL-cholesterol (*good* cholesterol) levels. Furthermore, botryosphaeran corrected dyslipidemia by reducing the content of total lipids, triglycerides, and cholesterol in the obese animals. Botryosphaeran modulated the expression of AMPK and FOXO3a proteins in adipose tissue of the obese rats.

6 Anticancer Activity of Botryosphaeran

Cancer, a multifactorial disease, is one of the principal causes of deaths in the world and is increasing globally. Cancer treated by chemotherapy and radiotherapy bears the hallmark of debilitating side effects that can contribute to cancer mortality. In targeting cancers, it's important to develop appropriate treatments with minimal side effects. In this respect, β-glucans of the β-(1→3)-linked kind are recognized as possessing anticancer activity, and bear no side effects (Vetvicka and Vetvickova 2012). Their anticancer effects are mediated directly on cancer cells via cytotoxicity,

and indirectly through immuno-modulation and metabolism (Chan et al. 2009; Batbayar et al. 2012).

Botryosphaeran mediated cell-signaling pathways in human breast carcinoma MCF-7 cells leading to suppression of tumorigenesis, decreased proliferation of cancer cells, and promoted apoptosis, necrosis, and oxidative stress. The anti-proliferative effect of botryosphaeran was mediated by the actions of AMPK and FOXO3a (Queiroz et al. 2015).

AMPK activates the regulation of cancer cell proliferation and is important in inhibiting cellular growth and promoting apoptosis in cancer cells (Shackelford and Shaw 2009). AMPK can activate FOXO3a and consequently promotes cell cycle arrest, apoptosis, and tumor suppression (Brunet et al. 1999; Dijkers et al. 2000).

Activated FOXO3a induces the transcription of certain target genes involved in cell-cycle arrest, cell-death, and tumor suppression, and this effect was induced by botryosphaeran in MCF-7 cells (Queiroz et al. 2015), and in Walker-256 tumor-bearing obese rats (Geraldelli et al. 2020b). In MCF-7 cells, botryosphaeran increased the intracellular expression of mRNA's of p53 and p27 (involved in cell-cycle arrest) and bax (apoptosis regulator), and cleaved caspase-3 proteins involved in apoptotic pathways, confirming this occurred via FOXO3a activity (Queiroz et al. 2015). Malini et al. (2015) reported cell-cycle arrest by botryosphaeran in human tumorigenic (Jurkat) T-lymphocytes.

Botryosphaeran increased oxidative stress in breast cancer MCF-7 cells, and this effect was associated with increased generation of ROS with consequent apoptosis and necrosis. Oxidative stress is the imbalance between the production of reactive oxygen species (superoxide anion radical, hydroxyl radical, hydrogen peroxide, and organic hydroperoxides), and the endogenous protective actions of enzymes (superoxide dismutase, catalase, among others) and non-enzymatic antioxidants (natural products that includes plant polyphenols, vitamins C and E, and also β-glucans, such as botryosphaeran; Giese et al. 2015).

In the presence of the pro-oxidant hydrogen peroxide, botryosphaeran increased ROS levels in the MCF-7 cells above that generated by hydrogen peroxide alone, indicating that oxidative stress induced by botryosphaeran was responsible for cancer cell death (Queiroz et al. 2015). Oxidative stress generated by ROS can promote cell apoptosis, and consequently reduce cell viability and stimulate AMPK activity (Queiroz et al. 2014).

In Walker-256 tumor-bearing rats (non-obese), botryosphaeran (30 mg/kg b.w. over 15 days) significantly reduced tumor development and cancer cachexia syndrome through increasing apoptosis of tumor cells via bax protein expression (Geraldelli et al. 2020a). In related work with obese rats bearing the Walker-256 tumor, botryosphaeran decreased tumor growth and the intensity of the neoplastic cachexia syndrome, and increased FOXO3a activity (Geraldelli et al. 2020b). Botryosphaeran acted in a concentration-dependent manner decreasing tumor growth and cachexia syndrome in both obese and non-obese rats bearing Walker-256 tumors (Comiran et al. 2021).

The relative differences in tumor development between non-obese and obese male rats bearing Walker-256 tumors, and following treatment with botryosphaeran

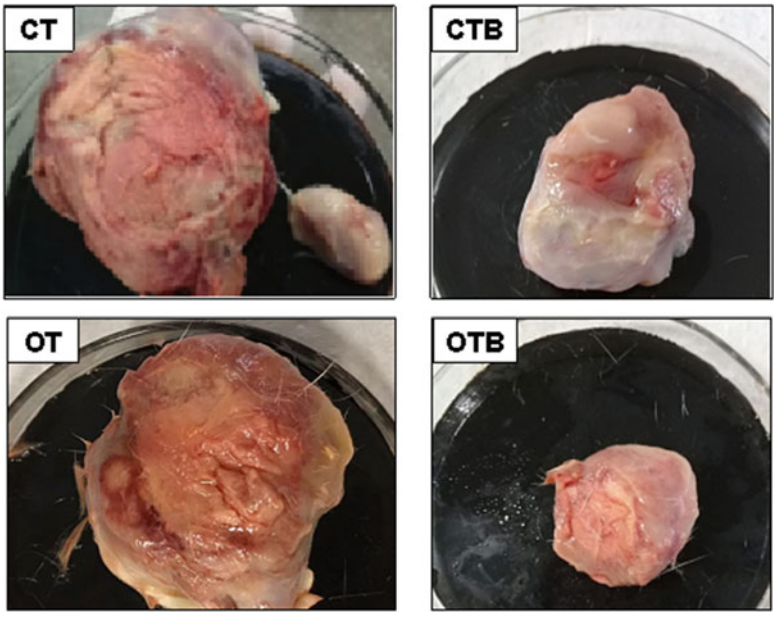


Fig. 2 The relative sizes of Walker-256 bearing tumors in non-obese (CT) and obese (OT) male rats, and after 15 days of treatment with botryosphaeran (CTB and OTB)

(30 mg/kg. b.w./day) for 15 days, is shown in Fig. 2, which indicates a notable reduction in the size and weight of the tumors before and after treatment with botryosphaeran.

7 Conclusion and Future Perspectives

As aforementioned, botryosphaeran manifests many biological activities that find applications as alternative and complementary medicines. The uniqueness of this fungal β-glucan activity we believe is influenced by the chemistry of the side-chain residues, especially the di-glucoside unit, gentiobiose, in synergy with glucose, as well as the degree of branching of substituents along the backbone chain.

Our research continues to unravel new knowledge on botryosphaeran that leads to further applications. Three commercial cosmetic products have evolved from ~20 years of studies on botryosphaeran. They include a formulated cosmetic facial crème, body lotion, and a serum all containing β-glucan that promote skin health and treat skin conditions. Unpublished data has revealed that botryosphaeran promotes healing of skin wounds that leads to re-epithelialization of the wound area, and the synthesis of collagen fibers. Additionally, botryosphaeran possesses antinociceptive activity (removal of pain sensation), an important factor in treating wound injuries.

In wider applications, botryosphaeran and its chemical derivative, carboxymethyl-botryosphaeran, have been used to construct novel electrochemical

sensors and biosensors as platform materials assembled on simple electrodes to rapidly and quantitatively measure various drug components, e.g., dopamine and spironolactone (Coelho et al. 2019), hydroquinone (Mattos et al. 2019), desloratadine (Salamanca-Neto et al. 2020), dopamine and paracetamol (Eisele et al. 2019), and quercetin (Gomes et al. 2020).

Acknowledgments The authors are grateful to Danielle Geraldelli and Dr. Eveline Queiroz (Núcleo de Pesquisa e Apoio Didático em Saúde, Instituto de Ciências da Saúde, Universidade Federal de Mato Grosso, Sinop-MT, Brazil) for the provision of photographs of rats bearing the Walker-256 tumors.

References

Bacic A, Fincher G, Stone BA, editors. Chemistry, biochemistry and biology of (1→3)-β-glucans and related polysaccharides. Cambridge: Academic; 2009.

Barbosa AM, Dekker RFH, St. Hardy GE. Veratryl alcohol as an inducer of laccase by an ascomycete, *Botryosphaeria* sp., when screened on the polymeric dye poly R-478. Lett Appl Microbiol. 1996;23:93–6.

Barbosa AM, Steluti RM, Dekker RFH, Cardoso MS, Corradi da Silva ML. Structural characterization of botryosphaeran: a (1→3;1→6)-β-D-glucan produced by the ascomyceteous fungus, *Botryosphaeria* sp. Carbohydr Res. 2003;338:1691–8.

Batbayar S, Lee DH, Kim HW. Immunomodulation of fungal β-glucan in host defense signaling by dectin-1. Biomol Ther. 2012;20:433–45.

Bohn JA, BeMiller JN. (1→3)-β-D-glucans as biological response modifiers: a review of structure-functional activity relationships. Carbohydr Polym. 1995;28:3–14.

Brandi J, Oliveira EC, Monteiro NK, Vasconcelos AFD, Dekker RFH, Barbosa AM, Silveira JLM, Mourao PAS, Corradi da Silva ML. Chemical modification of botryosphaeran: structural characterization and anticoagulant activity of a water-soluble sulfonated (1→3)(1→6)-β-D-glucan. J Microbiol Biotechnol. 2011;21:1036–42.

Brennan CS, Cleary LJ. The potential use of cereal (1→3,1→4)-β-D-glucans as functional food ingredients. J Cereal Sci. 2005;42:1–13.

Brunet A, Bonni A, Zigmond MJ, Lin MZ, Juo P, Hu LS, Anderson MJ, Arden KC, Blenis J, Greenberg ME. Akt promotes cell survival by phosphorylating and inhibiting a forkhead transcription factor. Cell. 1999;96:857–68.

Calnan DR, Brunet A. The FoxO code. Oncogene. 2008;27:2276–88.

Chan G, Chan W, Sze D. The effects of β-glucan on human immune and cancer cells. J Hematol Oncol. 2009;2:25.

Chen L, Huang G The antiviral activity of polysaccharides and their derivatives, Int J Biol Macromol. 2018;115:77–82.

Chen J, Raymond K. Beta-glucans in the treatment of diabetes and associated cardiovascular risks. Vasc Health Risk Manag. 2008;4:1265–72.

Coelho JH, Eisele APP, Valezi CF, Mattos GJ, Schirmann JG, Dekker RFH, Barbosa-Dekker AM, Sartori ER. Exploring the exocellular fungal biopolymer botryosphaeran for laccase-biosensor architecture and application to determine dopamine and spironolactone. Talanta. 2019;204:475–83.

Comiran PK, Ribeiro MC, Silva JHG, Martins KO, Santos IA, Chiaradia AEF, Silva AZ, Dekker RFH, Barbosa-Dekker AM, Alegranci P, Queiroz AIF. Botryosphaeran attenuates tumor development and the cancer cachexia syndrome in Walker-256 tumor-bearing obese rats and improves the metabolic and hematological profiles of these rats. Nutr Cancer 2021;73:1175–1192.

Corradi da Silva MDL, Izeli NL, Martinez PF, Silva IR, Constantino CJL, Cardoso MS, Barbosa AM, Dekker RFH, Silva GVJ. Purification and structural characterisation of (1→3;1→6)-β-D-

glucans (botryosphaerans) from *Botryosphaeria rhodina* grown on sucrose and fructose as carbon sources: a comparative study. Carbohydr Polym. 2005;61:10–7.

Dekker RFH, Barbosa AM. Effect of aeration and veratryl alcohol on the production of two laccases by the ascomycete *Botryosphaeria* sp. Enzym Microb Technol. 2001;28:81–8.

Dijkers PF, Medema RH, Lammers JWJ, Koenderman L, Coffer PJ. Expression of the pro-apoptotic Bcl-2 family member Bim is regulated by the forkhead transcription factor FKHR-L1. Curr Biol. 2000;10:1201–4.

Eisele APP, Valezi CF, Mazziero T, Dekker RFH, Barbosa-Dekker AM, Sartori ER. Layering of a film of carboxymethyl-botryosphaeran onto carbon black as a novel sensitive electrochemical platform on glassy carbon electrodes for the improvement in the simultaneous determination of phenolic compounds. Sensors Actuators B Chem. 2019;287:18–26.

Fenech M. The cytokinesis-block micronucleus technique and its application to genotoxicity studies in human populations. Environ Health Perspect. 1993;101(Suppl 3):101–7.

Fonseca PRMS, Dekker RFH, Barbosa AM, Silveira JLM, Vasconcelos AFD, Monteiro NK, Aranda-Selverio G, Corradi da Silva ML. Thermal and rheological properties of a family of botryosphaerans produced by *Botryosphaeria rhodina* MAMB-05. Molecules. 2011;16:7488–501.

Geraldelli D, Ribeiro MC, Medeiros TC, Comiran PK, Martins KO, Oliveira MF, Oliveira GA, Dekker RFH, Barbosa-Dekker AM, Alegranci P, Queiroz EAIF. Tumor development in rats and cancer cachexia are reduced by treatment with botryosphaeran by increasing apoptosis and improving the metabolic profile. Life Sci. 2020a;252:117608.

Geraldelli D, Ribeiro MC, Medeiros TC, Comiran PK, Martins KO, Oliveira MF, Oliveira GA, Dekker RFH, Barbosa-Dekker AM, Alegranci P, Queiroz EAIF. Botryosphaeran, a (1→3)(1→6)-β-D-glucan, reduces tumor development and cachexia syndrome in obese male rats by increasing insulin sensitivity and FOXO3a activity. Int J Biol Macromol. 2020b;165:985–994.

Giese EC, Dekker RFH, Barbosa AM, da Silva R. Triple helix conformation of botryosphaeran, a (1→3;1→6)-β-D-glucan produced by *Botryosphaeria rhodina* MAMB-05. Carbohydr Polym. 2008;74:953–6.

Giese EC, Gascon J, Anzelmo G, Barbosa AM, Alves da Cunha MA, Dekker RFH. Free- radical scavenging properties and antioxidant activities of the β-glucans: botryosphaeran, laminarin, curdlan and lasiodiplodan. Int J Biol Macromol. 2015;72:125–30.

Gomes A, Mattos GJ, Coldibeli B, Dekker RFH, Barbosa Dekker AM, Sartori ER. Covalent attachment of laccase to carboxymethyl-botryosphaeran in aqueous solution for the construction of a voltammetric biosensor to quantify quercetin. Bioelectrochemistry. 2020;135:107543.

Gow NAR, Latge J, Munro CA. The fungal cell wall: structure, biosynthesis and function. Microbiol Spectr. 2017;5:1–25.

Goulomb, BA, Evans MA. Statin adverse effects: A review of the literature and evidence for a mitochondrial mechanism, Am. J. Cardiovasc Drugs 2008;8:373–418.

Hayashi M. The micronucleus test-most widely used in vivo genotoxicity test. Genes Environ. 2016;38:18. (pages 1–6)

Jiao G, Yu G, Zhang J, Ewart HS. Chemical structures and bioactivities of sulfated polysaccharides from marine algae. Mar Drugs. 2011;9:196–233.

Kagimura FY, Cunha MAA, Barbosa AM, Dekker RFH, Malfatti CRM. Biological activities of derivatized D-glucans: a review. Int J Biol Macromol. 2015;72:588–98.

Kahn BB, Alquier T, Carling D, Hardie DG. AMP-activated protein kinase: ancient energy gauge provides clues to modern understanding of metabolism. Cell Metab. 2005;1:15–25.

Leung MYK, Liu C, Koon JCM, Fung KP. Polysaccharide biological response modifiers. Immunol Lett. 2006;105:101–14.

Malini M, Souza MF, Oliveira MT, Antunes LMG, Figueiredo SG, Barbosa AM, Dekker RFH, Cólus IM. Modulation of gene expression and cell cycle by botryosphaeran, a (1→3)(1→6)-β-d-glucan in human lymphocytes. Int J Biol Macromol. 2015;77:214–21.

Malini M, Camargo MS, Hernandes LC, Vargas-Rechia CG, Varanda EA, Barbosa AM, Dekker RFH, Matsumoto ST, Antunes LMG, Cólus IMS. Chemopreventive effect and lack of genotoxicity and mutagenicity of the exopolysaccharide botryosphaeran on human lymphocytes. Toxicol In Vitr. 2016;36:18–25.

Maron DM, Ames BN. Revised methods for the *Salmonella mutagenicity* test. Mutat Res, Environ Mutagen Relat Subj. 1983;113:173–215.

Mattos GJ, Moraes JT, Barbosa EM, PC PCC, RFH D, Barbosa-Dekker AM, Sartori ER. Laccase stabilized on β-D-glucan films on the surface of carbon black/gold nanoparticles: a new platform for electrochemical biosensing. Bioelectrochemistry. 2019;129:116–23.

Melo FR, Pereira MS, Foguel D, Mourão PAS. Antithrombin-mediated anticoagulant activity of sulfated polysaccharides: different mechanisms for heparin and sulfated galactans. J Biol Chem. 2004;279:20824–35.

Mendes SF, Santos O, Barbosa AM, Vasconcelos AFD, Aranda-Selverio G, Monteiro NK, Dekker RFH, Pereira MS, Tovar AMF, Mourão PAS, Corradi da Silva ML. Sulfonation and anticoagulant activity of botryosphaeran from *Botryosphaeria rhodina* MAMB-05 grown on fructose. Int J Biol Macromol. 2009;45:305–9.

Miranda CCBO, Dekker RFH, Serpeloni JM, Fonseca EAI, Cólus IMS, Barbosa AM. Anticlastogenic activity exhibited by botryosphaeran, a new exopolysaccharide produced by *Botryosphaeria rhodina* MAMB-05. Int J Biol Macromol. 2008;42:172–7.

Miranda-Nantes CCBO, Fonseca EAI, Zaia CTBV, Dekker RFH, Khaper N, Castro IA, Barbosa AM. Hypoglycemic and hypocholesterolemic effects of botryosphaeran from *Botryosphaeria rhodina* MAMB-05 in diabetes-induced and hyperlipidemia conditions in rats. Mycobiology. 2011;39:187–93.

Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods. 1983;65:55–63.

Queiroz EAIF, Puukila S, Eichler R, Sampaio SC, Forsyth HL, Lees SJ, Barbosa AM, Dekker RFH, Fortes ZB, Khaper N. Metformin induces apoptosis and cell cycle arrest mediated by oxidative stress, AMPK and FOXO3a in MCF-7 breast cancer cells. PLoS One. 2014;9:e98207.

Queiroz EAIF, Fortes ZB, da Cunha MAA, Barbosa AM, Khaper N, Dekker RFH. Antiproliferative and pro-apoptotic effects of three fungal exocellular β-glucans in MCF-7 breast cancer cells is mediated by oxidative stress, AMP-activated protein kinase (AMPK) and the Forkhead transcription factor, FOXO3a. Int J Biochem Cell Biol. 2015;67:14–24.

Rahmani J, Miri A, Černevičiūtė R, Thompson J, de Souza NN, Sultana R, Kord Varkaneh H, Mousavi SM, Hekmatdoost A. Effects of cereal beta-glucan consumption on body weight, body mass index, waist circumference and total energy intake: a meta-analysis of randomized controlled trials. Complement Ther Med. 2019;43:131–9.

Sacchelli BAL, Galhardi LCF, Linhares REC, Lopes JL, Dekker RFH, Barbosa-Dekker AM, Orsato A. Botryosphaeran and sulfonated derivatives as novel antiviral agents for Herpes simplex and dengue fever. Int J Biol Macromol. 2019;138:334–9.

Salamanca-Neto CAR, Olean-Oliveira A, Scremin J, Ceravolo GS, Dekker RFH, Barbosa-Dekker AM, Teixeira MFS, Sartori ER. Carboxymethyl-botryosphaeran stabilized carbon nanotubes aqueous dispersion: a new platform design for electrochemical sensing of desloratadine. Talanta. 2020;210:120642.

Saldanha RL, Garcia JE, Dekker RFH, Vilas-Bôas LA, Barbosa AM. Genetic diversity among *Botryosphaeria* species and their correlation with cell wall lytic enzyme production. Braz J Microbiol. 2007;38:259–64.

Shackelford DB, Shaw RJ. The LKB1-AMPK pathway: metabolism and growth control in tumor suppression. Nat Rev Cancer. 2009;9:563–75.

Silva AZ, Costa FP, Souza IL, Ribeiro MC, Giordani M, Queiroz DA, Luvizotto RAM, Nascimento AF, Bomfim GF, Sugizaki MM, Dekker RFH, Barbosa-Dekker AM, Queiroz EAIF. Botryosphaeran reduces obesity, hepatic steatosis, dyslipidaemia, insulin resistance and glucose intolerance in diet-induced obese rats. Life Sci. 2018;211:147–56.

Silva-Sena GG, Malini M, Delarmelina JM, Dutra JCV, Gervásio SV, Leal MAS, Costa Pereira TM, Barbosa-Dekker AM, Dekker RFH, de Paula F, Batitucci MCP. *In vivo* antimutagenic and antiatherogenic effects of the (1→3)(1→6)-β-D-glucan botryosphaeran. Mutat Res, Genet Toxicol Environ Mutagen. 2018;826:6–14.

Sima P, Vannucci L, Vetvicka V. β-Glucans and cholesterol. Int J Mol Med. 2018;41:1799–808.

Steluti RM, Giese EC, Piggato MM, Sumiya AFG, Covizzi LG, Job AE, Cardoso MS, Corradi da Silva ML, Dekker RFH, Barbosa AM. Comparison of botryosphaeran production by the ascomyceteous fungus *Botryosphaeria* sp., grown on different carbohydrate carbon sources, and their partial structural features. J Basic Microbiol. 2004;44:480–6.

Stone BA. Chemistry of β-glucans. In: Stone BA, Clark AE, Fincher GB, Basic A, editors. Chemistry and biology of (1→3)-β-glucans and related polysaccharides. Burlington: Elsevier Press; 2009. p. 5–46.

Stone BA, Clarke AE, editors. Chemistry and biology of (1→3)-β-glucans. Bundoora: La Trobe University Press; 1992.

Tice RR, Agurell E, Anderson D, Burlinson B, Hartmann A, Kobayashi H, Miyamae Y, Rojas E, Ryu JC, Sasaki YF. Single cell gel/comet assay: guidelines for *in vitro* and *in vivo* genetic toxicology testing. Environ Mol Mutagen. 2000;35:206–21.

Vetvicka V, Vetvickova J. β(1→3)-glucan in cancer treatment. Am J Immunol. 2012;8:38–43.

Wang H, Liu Y, Qi Z, Wang S, Liu S, Li X, Wang H, Xia X. An overview on natural polysaccharides with antioxidant properties. Curr Med Chem. 2013;20:2899–913.

Wang J, Hu S, Nie S, Yu Q, Xie M. Reviews on mechanisms of *in vitro* antioxidant activity of polysaccharides. Oxidative Med Cell Longev. 2016;2016:5692852.

Wang Q, Sheng X, Shi A, Hu H, Yang Y, Liu L, Fei L, Liu H. β-Glucans: relationships between modification, conformation and functional activities. Molecules. 2017;22:257.

Wouk J, Dekker RFH, Queiroz, EAIF, Barbosa-Dekker AM. β-Glucans as a panacea for a healthy heart? Their roles in preventing and treating cardiovascular diseases. Int J Biol Macromol 2021; 177:176–203.

Yoshitomi H, Sakaguchi N, Kobayashi K, Brown GD, Tagami T, Sakihama T, Hirota K, Tanaka S, Nomura T, Miki I, Gordon S, Akira S, Nakamura T, Sakaguchi S. A role for fungal β-glucans and their receptor dectin-1 in the induction of autoimmune arthritis in genetically susceptible mice. J Exp Med. 2005;201:949–60.

Chitin and Its Derivatives

11

Applications in Biomedical Devices

Simone S. Silva, J. M. Gomes, L. C. Rodrigues, and Rui L. Reis

Contents

Abstract

Chitin and chitosan are well-known natural polymers that have been considered a sustainable feedstock for the production of high-value biomaterials. In particular, the wide availability of the main sources of chitin, e.g., squid pens, shrimp, crab shells, insects, and fungal cell walls, is associated to different methodologies for its extraction, namely, classical methods based on strong acids, alkali, and enzymes, or alternative ones like the use of ionic liquids and/or natural deep eutectic solvents. The latter method offers opportunities to extract

S. S. Silva (✉) · J. M. Gomes · L. C. Rodrigues · R. L. Reis
3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Zona Industrial da Gandra, Barco, Guimarães, Portugal

ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães, Portugal
e-mail: simonesilva@i3bs.uminho.pt

J. M. Oliveira et al. (eds.), *Polysaccharides of Microbial Origin*,
https://doi.org/10.1007/978-3-030-42215-8_13

and process chitin directly from the raw materials, preserving the chitin qualities. The global market of chitin is continuously increasing across different fields, namely, the textile industry, packaging, technology, and biomedical field. In the biomedical field, many chitin and chitosan-based matrices, namely, membranes, hydrogels, nano−/microfibers, and scaffolds have been produced using various methodologies such as blending chitin and chitosan with other natural/synthetic polymers (such as alginate, gelatin, polycaprolactone), metals and/or ceramics (such as HAp, SiO_2, TiO_2, ZrO_2). Besides, the functional groups of chitin and chitosan can be modified for the development of a broad range of derivatives with a wide range of applications. The achieved architectures have the suitable physicochemical and biological performance for applications in wound repair, bone regeneration, cartilage repair, and infectious diseases. In this chapter is performed an overview of the recent research on the extraction, production, and applications of chitin and chitosan-based matrices envisaging their biomedical potential.

Keywords

Chitin · Chitosan · Marine residues · Biomaterials · Biomedical applications

Abbreviations

(H-HTCC)-NS/MS	N-(2-hydroxypropyl)-3-trimethyl chitosan
[Amim]Br	1-allyl-3-methylimidazolium bromide
[Amim]Cl	1-allyl-3-methylimidazolium chloride
[Bmim]Cl	1-butyl-3-methylimidazolium chloride
[Bmim]OAc	1-butyl-3-methylimidazolium acetate
[Emim]OAc	1-ethyl-3-methylimidazolium acetate
AIBN	2,2′-azobisisobutyronitrile
Bio-IL	Biocompatible ionic liquids
BMSC	Bone marrow-derived stem cells
C-CBM	Collagen—chitin biomimetic membrane
CDI	1,1-carbonyldiimidazole
CMCS	Carboxymethyl chitosan sulfate
COP	Collagen peptide
DA	Degree of acetylation
DD	Degree of deacetylation
DES	Deep eutectic solvent
ECM	Extracellular matrix
EGCG	Epigallocatechin gallate
ESCs	Epidermal stem cells
GAG	Glycosaminoglycans
Hap	Hydroxyapatite
ILs	Ionic liquids

MSCs	Mesenchymal stem cells
MTGase	Microbial transglutaminase
MW	Molecular weight
NACOSs	N-acetyl chitooligosaccharides
NS/MS	Nano/microspheres
PCL	Polycrapolactone
PEI	Polyethylenimine
SF	Silk fibroin
TEOS	Tetraethylorthosilicate
TGF-β	Transforming growth factor- β

1 Introduction

Chitin is a naturally occurring polymer, and it has even been reported to be one of the most abundant biomolecules on earth, with an estimated annual production of 10^{11}–10^{14} tons. Chitin is present in a variety of biomass, such as squid pens, shrimp, crab shells, insects, and fungal cell walls (Bastiaens et al. 2019). The wide availability of the raw material used to obtain chitin and its derivatives associated with various facets of the global market of chitin that appears across different fields, namely, textile industry, packaging, biotechnology, and biomedical field, suggests that the market potential for chitin is rising. In fact, according to different key players, it is expected to boost the growth of the global market for chitin during the forecast period. The global Chitin market size is predicted to gain a CAGR of 3.8% in the forecast period of 2020–2025. It will be expected to reach USD 59 million by 2025, from the actuals USD 50 million (Chitin Market Size 2020).

Despite the above trends, the lack of solubility of chitin in water and organic solvents is challenging, and different strategies involving ionic liquids (ILs) (Silva et al. 2017; Silva et al. 2013a; Silva et al. 2013b; Silva et al. 2011; Silva et al. 2020) and deep eutectic solvents (DES) (Vicente et al. 2020; Sharma et al. 2013) have been explored to help to unlock this limitation. Special emphasis is placed on methods conducted with the use of ILs, defined as salts in the liquid state at ambient temperature (Anthony et al. 2002), which allows not only the extraction in higher product yields as compared to traditional reactions but also its transformation in high-value medical devices (Silva et al. 2013a; Silva et al. 2013b; Silva et al. 2011; Shamshina 2019). Then, the solvating power of ILs on the extraction of chitin from shellfish waste represents a total byproduct use concept with practical implications in crustacean processing industries (Patrick et al. 2013).

Another strategy to explore chitin is mainly focused on the use of chitosan (CHT), a partially deacetylated derivative of chitin that is soluble in aqueous acetic acid and, therefore, easier to process. Both chitin and chitosan have been fabricated as

2D-/3D-based matrices, namely, membranes, scaffolds, micro/nanofibers, and hydrogels (Tao et al. 2020; Muzzarelli 2009; Kumar et al. 2010). These architectures have appropriate features such as large surface area, mechanical properties, and biocompatibility needed for tissue regeneration and sustained release of bioactive compounds (Muzzarelli 2009). The feasibility of the use of those matrices as wound dressings for skin repair, drug delivery systems, bone repair, cartilage regeneration, and chronic disease treatment have been investigated intensively (Silva et al. 2013b; Tao et al. 2020; Muzzarelli 2009; Kumar et al. 2010; Saravanan et al. 2016). However, it sometimes implies its physical or chemical modification and/or blending with other polymers (either natural or synthetic) to reach adequate features for its biomedical application.

This chapter addresses an overview of the fundamental features of the extraction from different sources of chitin, its matrices, and potential tissue engineering approaches (Fig. 1).

2 Chitin Extraction and Structure

Chitin is one of the most abundant polysaccharides on earth, existing widely as one of the main components of the cell walls of fungi and algae, exoskeletons of arthropods such as crustaceans (e.g., shrimps and crabs ranging from 15–30%), and insects (up to 60% in special parts such as the flexible portions), radulas of mollusks, and the beaks of cephalopods, providing structural integrity and protection to those animals (Muzzarelli 2009; Muzzarelli 2011). Chitin may be extracted according to chemical and biological extraction methodologies, through deproteinization and demineralization steps (Fig. 2), possessing both strategies advantages and drawbacks (El Knidri et al. 2018; Arnold et al. 2020). The chemical approach is the most commonly used for industrial and commercial proposes due to the reduced extraction time, productivity, and practicality. However, it requires the use of strong acids and bases with the intent to, respectively, dissolve calcium carbonates and proteins, which is associated with many disadvantages, as high costs, reduced purity, and particularly in the environmental field through effluent generation (El Knidri et al. 2018). Decolorization and purification as additional steps are often required to remove pigments and obtain pure and colorless chitin, which is carried out using acetone, sodium hypochlorite, hydrogen peroxide, or potassium permanganate (Pighinelli 2019). To overcome the disadvantageous hazardousness and to achieve a higher quality final product, a more time-consuming biological approach is being employed using proteases from bacteria and lactic acid-producing bacteria to ensure the demineralization and deproteinization steps (El Knidri et al. 2018; Arnold et al. 2020; Tan et al. 2020). The proteolytic enzymes ensure protein hydrolysis through activation of the low pH of the medium, with the advantage of allowing the recovery of enzymes, proteins, and pigments for further application or reuse. Moreover, into fermentative and enzymatic processes, digestive and microbial enzymes able to consume organic material are produced, potentiating as well the

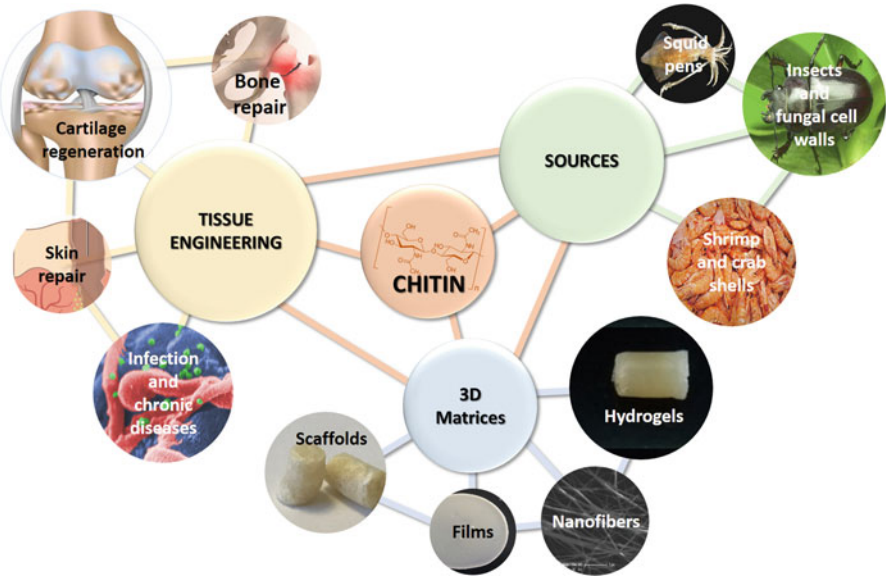


Fig. 1 Overview of the sources, matrices, and tissue engineering applications of chitin

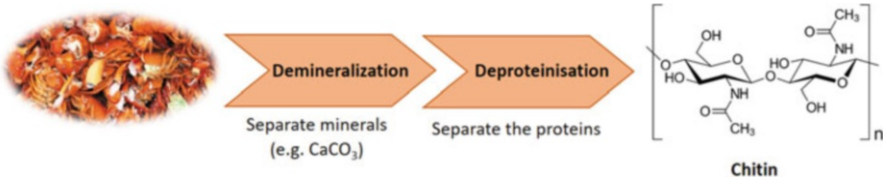


Fig. 2 Steps sequence of chitin extraction from marine wastes

production of hydrolyzed proteins. To increase the effectiveness of the extraction process, a methodology resulting from the combination of the chemical process with biological methods, with the application of microorganisms, is also being explored (Pighinelli 2019). This biotechnological approach presents a new sustainable vision with new quality parameters, offering a more suitable biomaterial, mainly to be applied to health solutions (Pighinelli 2019).

Chitin is a long and regular chain structure made up of β (1 → 4)-linked primary units of N-acetyl D-glucosamine (monomer units of 2-acetamido- 2-deoxy-b-d-glucose) (Silva et al. 2020; Younes and Rinaudo 2015; Pillai et al. 2009) (Fig. 2). Chitin, depending on the nature of the H-bond, can assume three crystalline polymorphic forms designated as α, β, and γ chitin; however, the existence of γ chitin is a controversy (Silva et al. 2020). α- and β-chitins present stable antiparallel and metastable parallel chain alignments being mostly present in different animal species, respectively, α-chitin in arthropods and crustaceans (major sources) and β-chitin in

marine diatoms and squid pens (minor sources) (Younes and Rinaudo 2015; Pillai et al. 2009). The arrangement of the γ-chitin is more complex being composed of three chitin chains; two are parallel, and the third one is arranged in an antiparallel fashion (Silva et al. 2020). The existence of different arrangements leads to differences in the packing and polarities, which consequently promote the existence of distinct physicochemical properties. The strength of the intermolecular forces in β-chitin is much weaker than in α-chitin, β-chitin being observed to be more soluble and reactive (Silva et al. 2020; Hirayama et al. 2018), and α-chitin the most stable form of chitin. Chitin is found to occur as ordered crystalline microfibrous material embedded in a six-stranded protein helix (Qiu and Wang 2017), with reduced reactivity due to the strong intra- and inter-molecular interactions, particularly the H-bonds established between the C = O and NH groups of the adjacent polymeric chains, which make its processability difficult and are the major obstacle to its dissolution, being insoluble in common solvents. However, it can be dissolved in concentrated acids, as hydrochloric, sulfuric, phosphoric, dichloro- and trichloroacetic or formic acids and in hexafluoroisopropanol or hexafluoroacetone, N, N-dimethylacetamide mixture of DMA and LiCl, lithium thiocyanate, $CaBr_2 \cdot H_2O$ saturated methanol, hexafluoroisopropanol alcohol, hexafluoracetone, and N-methyl-2-pyrrolidone and some hot and concentrated neutral salts (El Knidri et al. 2018). Although enabling chitin dissolution, many of those solvents are toxic, corrosive, harmful, or scarcely degradable. Moreover, the most environmentally friendly approach is the possibility to dissolve chitin using ILs (Silva et al. 2011, 2013a, b, 2017). Recent studies described that ILs not only dissolve raw chitin but also isolate it from crustaceans' biomass, making possible the production of chitin fibers or nanofibrous mats via one-pot protocol (Shamshina 2019; Barber et al. 2013). Many of those works suggest that chitin dissolution requires an anion with an increased number of hydrogen bond donors and acceptors (more basic anion), as acetate, able to interact with the chitin H-bonds, destroying them, and leading to chitin crystal dissolution (Uto et al. 2018).

Chitosan, the most important derivative of chitin, is obtained by partial deacetylation of chitin (Daraghmeh et al. 2011); however, during the process also depolymerization reaction occurs, promoting changes into chitosan molecular weight (Younes and Rinaudo 2015). In chitin, the ratio of 2-acetamido-2-deoxy-d-glucopyranose to 2-amino-2-deoxy-d-glucopyranose structural units, commonly named as degree of acetylation (DA), is typically 0.90 indicating the presence of about 5–15% of amino groups due to deacylation that might occur during chitin extraction (Pillai et al. 2009). On its glucose ring, chitin has acetamido groups that can undergo incomplete hydrolysis into primary amine groups, N-deacetylation of chitin, which leads to the formation of chitosan that can be easily dissolved in aqueous acidic solutions, which makes it suitable for various applications.

Similarly to chitin extraction, its deacetylation may also occur according to chemical or enzymatic processes, the chemical one being mostly used for commercial purposes due to its reduced cost and higher efficiency (Younes and Rinaudo 2015). Chemical deacetylation of chitin may likewise involve the use of acids or alkali's solutions; however, due to the increased susceptibility of glycosidic bonds to acids, the resource to alkali deacetylation is most frequently employed (Hajji et al. 2014) producing an insoluble residue of chitosan deacetylated up to ∼85–99%. The

deacetylation reaction may occur under homogeneous or heterogeneous conditions, which may influence the distribution of N-acetyl-D-glucosamine and D-glucosamine residues with some blockwise acetyl group distribution along polymeric chains, consequently promoting a variation of the solubility and degree of aggregation of chitosan leading to changes in their average characteristics (Younes and Rinaudo 2015). According to Younes et al. and its reported fractional factorial design and mathematical model, seven factors may influence deacetylation reaction extension; however, in different extension (Younes et al. 2014). The study revealed a significant influence of the temperature, of the nature of the alkali reagent, of the number of successive baths, of the reaction time, and of the alkali concentration. In contrast, a reduced influence was attributed to the atmospheric conditions (nitrogen or air) and the use of reducing agents (e.g., $NaBH_4$) (Younes and Rinaudo 2015; Younes et al. 2014).

Despite its efficacy, chemical deacetylation also has disadvantages: energy consumption, and waste of concentrated alkaline solution, contributing to an increase in environmental pollution (Younes and Rinaudo 2015).

The resource to chitin deacetylases for the conversion of chitin to chitosan offers the opportunity of producing well-defined chitosan-based on a controlled, non-degradable products (Younes and Rinaudo 2015). However, the deacetylation based on enzymes is not very effective (<10%) (Younes and Rinaudo 2015), pretreatment of chitin substrates are required to improve the accessibility of the acetyl groups to the enzyme, consequently enhancing the deacetylation yield (Tan et al. 2020; Younes and Rinaudo 2015).

3 Chitin and Chitosan Properties

Chitin and chitosan have a unique structure, high charge density, reactive hydroxyl and amino groups, and extensive hydrogen bonding ability. These features can be correlated to properties as, e.g., biocompatibility, biodegradability, nontoxicity, antimicrobial, antioxidant, anti-inflammation action, anticancer effects, among others, which open up a wide range of applications in various fields, especially in the biomedical area. In this context, the physicochemical characteristics, namely, molecular weight (MW), degree of deacetylation (DD), and moisture content of chitin and chitosan have been related to their biological properties. According to the literature (Kim 2018), the antimicrobial effect of chitosan is much higher in comparison to chitin, mainly due to the amino groups that are responsible for the cationic nature of chitosan. In fact, the mechanism of action of chitosan has been associated with its cationic nature, but its antimicrobial activity could also depend on its deacetylation degree. It is also supposed that chitosan presents antimicrobial activities by interacting with the outer membrane or cytoderm of the bacteria (Li et al. 2019). A correlation between the antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*, chitosan MW, and DD at different pH values, revealed that chitosan with low MW and high DD exhibited higher antibacterial activity (Chang et al. 2015).

It has also been observed that the antioxidant activity of chitosan is associated with the presence of the protonated amino groups and its ability to chelate metal ions that induce free radicals formation in cells (Varlamov et al. 2020). Then, many

studies evaluated the antioxidant activity of chitosan with varying MW and DD (Chang et al. 2018). They observed that a decrease in MW and higher DD exhibited the highest scavenging activity against 2,2 diphenyl-1-picrylhydrazyl (DPPH) radicals. Also, investigation on chitosan derivatives obtained after chemical modification or covalent cross-linking can be used to enhance the antioxidant properties of the polymer due to the increased accessibility of functional groups in the reductive reaction and metal ion chelation (Varlamov et al. 2020). Previous studies (Park and Kim 2010) indicated that the hydrolyzed products of chitin, namely, N-acetyl chitooligosaccharides (NACOSs) have a direct radical scavenging effect of intracellular hydrogen peroxide and intracellular glutathione level, suggesting that NACOS act as potent antioxidants in live cells.

Besides antimicrobial and antioxidant action, the DD and MW of chitin, chitosan, and its derivatives affect its antitumor activity. Some studies (Salah et al. 2013) suggested that the antitumor effect of chitin and chitosan may be explained by electrostatic interaction between the charges of the anticancer products and charged functional residues present on internal components and the tumor cell surface. To enhance the antitumor effects of chitin, distinct strategies have been explored involving hydrolysis of chitin to create low molecular weight or even chemical modification on chitin to generate chitin derivatives with better features. Salah et al. (Salah et al. 2013) demonstrated that chitin with low molecular weight, obtained by hydrolysis, has the potential to increase the tumor suppression of THP-1 tumor cells, being an attractive target for selective anticancer drug development.

Moreover, other studies evidenced that lower MW and higher DD of chitosan oligomers were promising parameters for the development of antitumor agents derived from chitosan in in vitro tests with human PC3 (prostate cancer cell), A549 (carcinoma human alveolar basal epithelial cell), and HepG2 (hepatocellular carcinoma cell) (Park et al. 2011). According to Adhikari et al. (Adhikari and Yadav 2018), chitosan and its derivatives could selectively permeate through the cancer cell membranes and demonstrated to have anticancer activity through the cellular enzymatic, antiangiogenic, immune-enhancing, antioxidant defense mechanism, and apoptotic pathways. Moreover, the activity of chitosan and its derivatives enables its use as a carrier of medical drugs and the development of 3D-based systems. Kievit et al. (Kievit et al. 2010) demonstrated that chitosan/alginate scaffolds provided a 3D microenvironment for glioma cells in vitro, which could play a pivotal role in the understanding of these tumors. In other approaches, chitosan involved anticarcinogenic tools on different cancer types such as breast, prostate, and liver (Kim 2018).

4 Chitin and Chitosan-Based Matrices: Processability and Chemical Modification

Both chitin and chitosan present reactive functional groups that allow them to be responsive to chemical modifications (El Knidri et al. 2018). Alkylation may occur between the amino groups and aldehydes and ketones, while other reactions such as o-acetylation, H-bonding with polar atoms, cross-linking, and grafting are a

consequence of the hydroxyl functionality. Generally, chitin and chitosan have been modified by acylation, quaternization, alkylation, hydroxylation, phosphorylation, thiolation, and graft copolymerization (El Knidri et al. 2018; Alves and Mano 2008). Moreover, chitosan may be modified using methylation, acylation, nitration, sulfonation, xanthation, and N-succinylation.

Graft copolymerization is one of the most promising and studied approaches used for chitosan modification (Li et al. 2020). This strategy is important for the development of practically all of the used polymer derivatives through the covalent introduction of new types of side chains and making various molecular designs possible (Li et al. 2020). The properties of the resulting graft copolymers are controlled by the molecular structure, length, and the number of side chains attached. Chitosan contains free amino groups on the deacetylated units and the hydroxyl C3 and C6 carbons on acetylated or deacetylated units that can be grafted (El Knidri et al. 2018). Grafting polymerization initiated by many initiator systems such as ammonium persulfate, potassium persulfate, ceric ammonium nitrate, thiocarbonation potassium bromate, potassium diperiodatocuprate (III), 2,2′-azobisisobutyronitrile (AIBN), and ferrous ammonium sulfate. Moreover, γ-irradiation and enzymes can also be used (Alves and Mano 2008). Moreno-Vásquez et al. successfully prepared epigallocatechin gallate (EGCG)–grafted chitosan by a free radical-induced grafting procedure using a water-soluble redox system of ascorbic acid/hydrogen peroxide as the radical initiator (Moreno-Vásquez et al. 2017). The grafting methodology was efficient, increasing the concentration of linked EGCG. Moreover, the resulting EGCG-grafted chitosan presented higher antioxidant activity, as well as antibacterial activity against *S. aureus* and *Pseudomonas sp*., when compared to the pure counterparts. Enzymes offer many advantages when used in polymer synthesis and modification, including health and safety issues, by the elimination of the hazards associated with the use of other reagents. From an environmental perspective, enzymes selectivity may be exploited to eliminate the need for full waste protection, as well as deprotection steps. Moreover, enzymes specificity allows to precisely modify the macromolecular structure and control the grafted polymer function (El Knidri et al. 2018). Hu et al. used microbial transglutaminase (MTGase) as a catalyst to graft the collagen peptide (COP) molecules on the amino group of carboxymethyl chitosan sulfate (CMCS), following the strategy presented in Fig. 3, improving its antioxidant and anticoagulant activities (Hu et al. 2019).

Chitin and chitosan allow a wide variety of acylation reactions. Chitosan with a higher deacetylation degree is more prone to acylation owing to a decrease in hydrogen bonding ability. The acylation reaction on chitosan may happen with a variety of organic acids and derivatives of organic acids, mainly anhydride and acyl chloride, to introduce aliphatic or aromatic acyl groups to the molecular chain (Wang et al. 2020). The studies also showed that N-acylated chitosan derivatives had enhanced blood compatibility, biocompatibility, and anti-coagulability. ILs have been exploited as potential green processing platforms for chitin and chitosan, being able to dissolve them through disruption of hydrogen bonding (Silva et al. 2017, 2020). Moreover, ILs allow to reduce the dissolution time, while promoting a homogeneous media, which improves the reactions' efficiency. Several ILs have

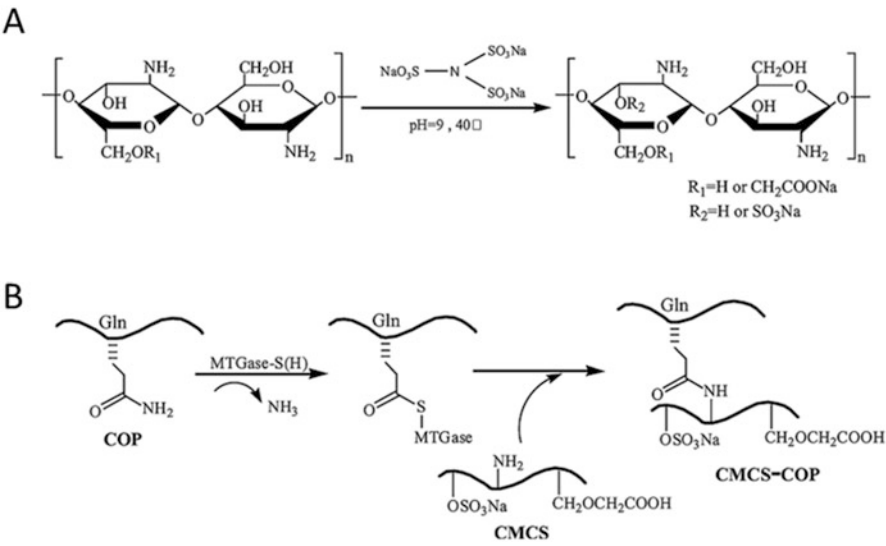


Fig. 3 (**a**) Synthesis of Carboxymethyl chitosan sulfate (CMCS) and (**b**) synthesis of the CMC-Collagen peptide (COP) complex. (Reprinted from Carbohydrate Polymers, 206, Wanqing Hu,Meng Liu,Xiaoshuang Yang,Chang Zhang,Heyi Zhou,Weiguo Xie,Lihong Fan,Min Nie, Modification of chitosan grafted with collagen peptide by enzyme crosslinking, 468–475. Copyright (2019) with permission from Elsevier)

been reported as efficient solutions for the dissolution and processability of chitin and chitosan, being the ones with the acetate and chloride anions the most used (Silva et al. 2011, 2013b, 2017; Wu et al. 2008). In addition, ILs have proved to be an outstanding catalytic medium when used in the above-mentioned reactions, including hydrolysis, acetylation, and graft copolymerization (Silva et al. 2017; Hirayama et al. 2018; Zhang et al. 2009). Imidazolium-based ILs, 1-butyl-3-methylimidazolium chloride ([Bmim]Cl), have been promoting good yields concerning chitosan's depolymerization in the presence of mineral acids by providing the formation of a homogeneous system (Zhang et al. 2009). 1-allyl-3-methylimidazolium bromide ([Amim]Br) and 1-allyl-3-methylimidazolium chloride ([Amim]Cl) were successfully used in the homogeneous acetylation of chitin and chitosan, respectively (Mine et al. 2009; Liu et al. 2013). Once again, imidazolium-based ILs proved to be an excellent reaction medium, resulting in materials with high acetylation degrees. Moreover, the use of ILs in the grafting process proved to enhance the nucleophilicity of amines and increase the stability of the reagent activated complexes, accelerating a nucleophilic substitution reaction, which reduces the reaction time and benefits the reaction control (Silva et al. 2017). The grafting of chitosan was successfully attained using 1-ethyl-3-methylimidazolium acetate ([Emim]OAc) and 1-butyl-3-methylimidazolium acetate ([Bmim]OAc), using ring-opening polymerization of polycaprolactone (PCL) with stannous octoate $Sn(Oct)_2$ as the catalyst, and with polyethylenimine (PEI) and 1,1-carbonyldiimidazole (CDI) as a linking agent (Wang et al. 2013; Chen et al. 2015). The good solubility of chitosan in those

solvents enhanced the high grafting content. Moreover, chitin can be functionalized through its dissolution in [Bmim]OAc, followed by the use of sol-gel method (Silva et al. 2013b). From this approach, by using the extrusion dipping method with a batch containing tetraethylorthosilicate (TEOS)–ethanol–H_2O–HCl, hybrid chitin beads were produced. After, the chitin beads together were loaded into an immersed gellan gum solution, molded to promote agglomeration, and dried by a supercritical assisted agglomeration method. The commonly used ILs, such as the imidazolium-based ones, have some associated toxicological issues, which makes their removal a mandatory step before the evaluation of the biological performance of the final biomaterial. In a recent work, Gomes et al. proposed a biocompatible ionic liquid (Bio-IL), Choline acetate, as a platform for the processing of α-chitin into 3D sponges (Gomes et al. 2020), and evaluated the biological performance of the biomaterial with and without the Bio-IL presence into the final matrix. The developed porous sponges proved to be nontoxic while promoting good cell adhesion, proliferation, and viability when seeded with human adipose stem cells up to 7 days of culture.

Overall, the methods of chemical modification allow to control of the physicochemical properties of the polymers and consequently improve their functionality, leading to a broad range of derivatives with a wide range of applications (El Knidri et al. 2018; Wang et al. 2020).

5 Biomedical Applications of Chitin and its Derivatives

5.1 Skin Regeneration

The treatment of skin lesions requires dressing that ensures physical wound protection, enhances healing, reduces scar formation, and provides antimicrobial protection (Abdelrahman and Newton 2011). Therapeutical tools based on natural resources as chitin, chitosan, and their derivatives offer rational means to design architectures in different shapes, namely, hydrogels, membranes, and sponges. These architectures bring healing perspectives based on their biocompatibility, nontoxicity, and biodegradability cues. On the wound healing context, chitin accelerates macrophage migration and fibroblast proliferation and promotes granulation and vascularization (Muzzarelli 2009). Therefore, many studies are devoted to the description of the beneficial effects of chitin and chitosan for wound healing using in vitro, animal, and clinical studies (Dai et al. 2011; Dong et al. 2019).

Chitin-based matrices such as hydrogels, membranes, scaffolds, sponges, and fibers have been produced as biomaterials for wound dressing applications (Singh et al. 2017; Shamshina et al. 2014). These matrices have high durability, low toxicity, antibacterial activity, and good biocompatibility. For instance, hydrogels of carboxymethyl-chitin (CM-chitin) can be used as carriers for subcutaneous injections, as they protect wound tissues against pathogens infections, enhance cells' growth, and activate macrophages, important characteristics for wound healing (Singh et al. 2017; Synowiecki and Al-Khateeb 2003). Besides that, many chitin-

based wound dressings are commercially available in the form of non-wovens, nanofibrils, composites, films, and sponges (Muzzarelli 2009). Commercially, the HemCon® hemostatic dressing was studied as a topical antimicrobial dressing having favorable effects on healing of excisional wounds that were or were not infected with *Staphylococcus aureus* in mice. This acetate bandage was strongly bactericidal, reduced the number of inflammatory cells in the wounds, and had an overall beneficial effect on wound healing.

Making composites combining chitin with other polymers, e.g., hyaluronic acid, alginate, or even silver or gold particles, known elements of antimicrobial ability, is a good strategy to improve wound healing properties of the final material. Kumar et al. (Kumar et al. 2010) presented a study where β-chitin/nano-silver composite scaffolds were bactericidal against *Escherichia coli,* and *Staphylococcus aureus,* showed good blood clotting ability and cell attachment, being then suggested as suitable candidates for wound dressing applications. Recent studies on chitin/chitosan-derived matrices, as hydrogels, fibers developed using IL platforms demonstrated that these architectures have a positive influence on cell adhesion and proliferation; and superior mechanical strength (Silva et al. 2012, 2020; Shamshina et al. 2014). When applied in a full-dermal-thickness wound model, chitin-based fibers achieved 95–99% closure by day 10 with complete wound closure by day 14 (Shamshina et al. 2014). Shen et al. (Shen et al. 2014) reported the development of the collagen-chitin biomimetic membrane (C-CBM) to act as support to expand epidermal stem cells (ESCs) and develop functional substitutes. C-CBM has great biocompatibility and has degraded when it was subcutaneously transplanted into rat skin. ESCs were cultured, and the resulting construct (ESCs-C-CBM) was used to cover full-thickness skin defects in nude mice for up to 10 weeks post-injury. Nude mice wounds dressed with ESCs-C-CBM produced new skin that was relatively thicker, redder, and more elastic (Fig. 4a). Furthermore, in vivo experiments showed more obvious hair follicle cell proliferation in the full-thickness skin defect nude mice dressed with ESCs-C-CBM compared to those dressed with C-CBM and type I collagen alone (Fig. 4b). The obtained findings indicate that the collagen-modified chitin membrane carried with ESCs has successfully regenerated the whole skin with all the skin appendages and function.

5.2 Bone Repair

Bone is one of the most transplanted tissues being located only after blood (Roseti et al. 2017). It is a tissue, which during the lifetime, undergoes continuous remodeling being able to regenerate itself into a healthy bone after multiple insults (Schroeder and Mosheiff 2011). Conventionally, bone grafting techniques are being used to bone replacement; however, with some drawbacks which require an adjusted bone tissue engineering approach. The design of architectures able to mimic bone in structure, properties, and function has been a challenge for bone tissue engineering that is focused on the creation of 3D-scaffold based on biomaterials and cells that are able to support the formation of new tissue/bone, and, meanwhile, degrade as new

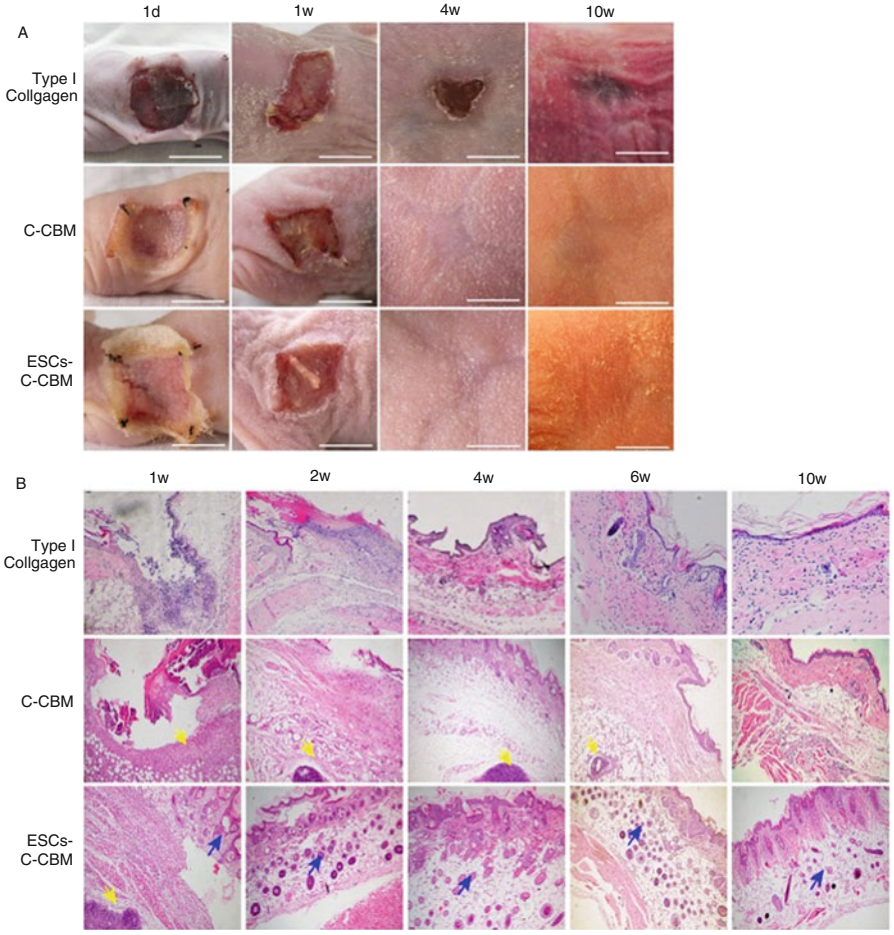


Fig. 4 Observation of nude mice full-thickness defect model. (**a**) Observation of nude mice full-thickness defect model dressed with type I collagen only, C-CBM, or ESCs-C-CBM at 1 d, 1 w, 4 w, and 10 w. Type I collagen group shows that wounds are much slower to heal. Group C-CMB shows the repaired wound skin in the control group was relatively thin and heliotrope with a tendency to bleed; Group ESCs-C-CMB shows the repaired wound in the experimental group was relatively thick and red with re-epithelialization. Scale bar = 1 cm. (**b**) Formation of epidermal nests on the wound surface repaired by epidermal stem cells-collagen-chitin biomimetic (ESCs-C-CBM) membrane compared with C-CBM. Paraffin and stained with hematoxylin and eosin (H&E) stain. Yellow arrows point to chitin, and blue arrows point to epidermal nests increased in the ESC-C-CBM group at 2–10 w (100X). (Reprinted from Shen et al. (2014). Copyright permission from Creative Commons Corporation)

bone is produced (Preethi Soundarya et al. 2018). Different approaches are being employed to develop porous scaffolds capable of regenerating tissues/organs and also to ensure a controlled and targeted release of bioactive agents (Roseti et al. 2017; Preethi Soundarya et al. 2018).

Despite the wide use of chitin into different industrial fields, its usage in bone tissue engineering is limited due to its weak solubility (Preethi Soundarya et al. 2018). In contrast, chitosan is a widely used resource in tissue engineering and, most notably, in bone tissue engineering applications (Muzzarelli 2009; Roseti et al. 2017; Schroeder and Mosheiff 2011; Preethi Soundarya et al. 2018; Mututuvari et al. 2013). Chitosan-based biomaterials envisioning bone tissue applications require the enhancement of their mechanical strength and structural integrity through the addition of other natural/synthetic polymers (such as Alginate, Gelatin, Polycaprolactone), metals, and/or ceramics (such as HAp, SiO_2, TiO_2, ZrO_2, etc.) (Muzzarelli 2009; Saravanan et al. 2016; Preethi Soundarya et al. 2018; Mututuvari et al. 2013; LogithKumar et al. 2016).

Chitin and chitosan-based biomaterials have been reported as bone repair approaches according to different architectures, for example, scaffolds or pastes, being the nanofibrous scaffolds the most commonly applied to bone tissue engineering approaches (Fig. 5).

Chitin/chitosan nanofibrous scaffolds have unique features as large surface area and high porosity, which potentiate cell adhesion and migration, nutrients transportation, and waste discharge (Tao et al. 2020; Rasouli et al. 2019), eventually facilitating tissue regeneration when coordinated with other components (Sofi et al. 2018). Chen et al., have reported a scaffold able to mimic the extracellular matrix and recruit stem cells composed of collagen/chitosan nanofibers (Chen et al. 2011). This architecture allowed cell adhesion, proliferation, and osteogenic differentiation after 7 days of in vitro culture through an upregulation of osteogenic genes signaling, faster calcium deposition, and tissue mineralization, consequently promoting bone regeneration (Chen et al. 2011). Electrospinned films of the same polymeric combination promoted rabbit mesenchymal stem cells (MSCs) proliferation and anabolism, as well as an induced expression of osteogenic genes like osteocalcin, Col-α1, and RUNX2 (Lotfi et al. 2015). It has also contributed to an accelerated bone repair in animal models of skull defects with the production of residual biomaterial particles and no inflammatory response around the transplanted nanofibrous scaffolds (Lotfi et al. 2015). Similar results were obtained implanting chitosan/hydroxyapatite composite nanofibrous scaffold with seeded bone marrow-derived stem cells (BMSCs) into an animal model with skull defects. Those composite nanofibers improved the osseointegration capacity revealing an increase of the newly mineralized tissue at the bone defect site. Coordinated with stem cells, it was also reported that this system promote bone regeneration and in vivo defects repair. When combined to produce hydroxyapatite/collagen/chitosan composite nanofibrous, it was reported an enhancement of the scaffold ability to promote osteogenic differentiation of iPSC-MSCs in vitro and accelerated bone regeneration in vivo (Xie et al. 2016), demonstrating broad applicability in bone tissue engineering approaches.

The nanofibrous scaffolds of chitosan/PCL proved to have beneficial features for cell viability and calcium deposition when cultured in vitro up to 14 days (Tao et al. 2020). The surface organization of the structure designated as shish-kebab-like facilitated mineralization, assuming prospects in osteoinduction applications (Jing et al. 2015) (Fig. 6). The structure was able to present osteoblasts adhesion,

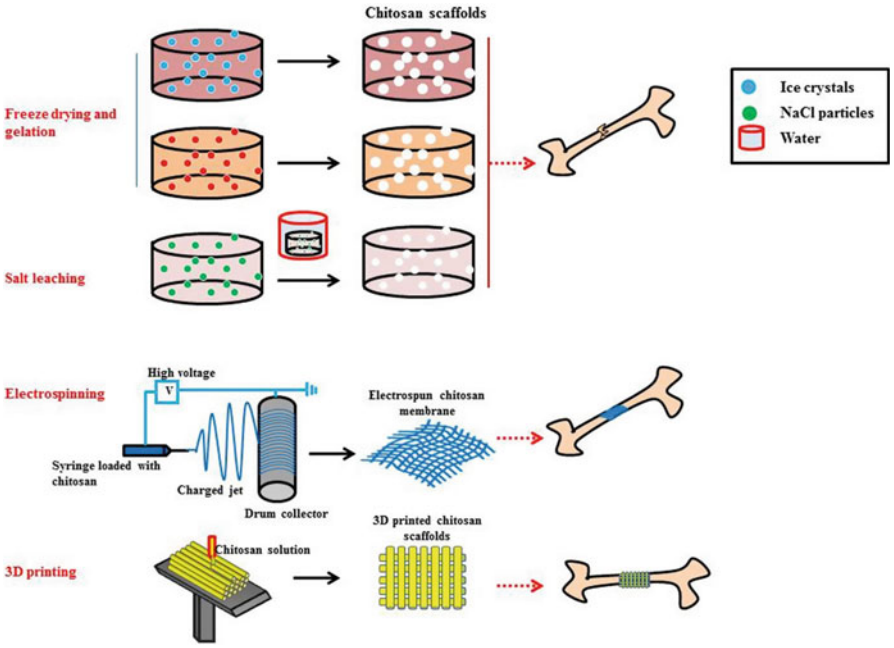


Fig. 5 Schematic representation of the commonly used fabrication methods for producing chitosan-based scaffolds — freeze drying, freeze gelation, salt leaching, electrospinning, and 3D printing (Saravanan et al. 2016). (Reprinted from International Journal of Biological Macromolecules, 93, S. Saravanan,R.S. Leena,N. Selvamurugan, Chitosan based biocomposite scaffolds for bone tissue engineering, 1354–1365. Copyright (2016), with permission from Elsevier)

diffusion, and proliferation combined with chitosan antibacterial properties inhibiting *Staphylococcus epidermidis* colonization in the scaffolds by anionic microgels and cationic oligopeptides deposition hierarchically (Tao et al. 2020).

Furthermore, an alternative procedure was proposed by Nitta et al., through the preparation of scaffolds by mixing chitosan nanofibers in suspension with poly (ethylene glycol) (Nitta et al. 2017). The structures presented an enhanced Young's modulus, have proven to have good hardness and improve mineralization ability proportionally to the increase in chitosan nanofibers content.

Moreover, composite materials based on chitin and hydroxyapatite were prepared through [Bmim]OAc ionic liquids processing (Silva et al. 2013a, b). Silva et al. have reported an enhanced dispersion of the hydroxyapatite across the porous microstructure, with potential to be applied for bone tissue engineering purposes as the presented pore sizes can positively influence osteoblasts viability and proliferation (Silva et al. 2013a, b). Also, hydroxyapatite-chitin-chitosan composite materials, as a self-hardening paste, have been used as bone filling material for guided tissue regeneration (treatment of periodontal bone defects) with promising results. Mututuvari et al. have proposed the processing of chitosan/cellulose/hydroxyapatite multifunctional composites using ([Bmim)]([Cl)] as a solvent (Mututuvari et al.

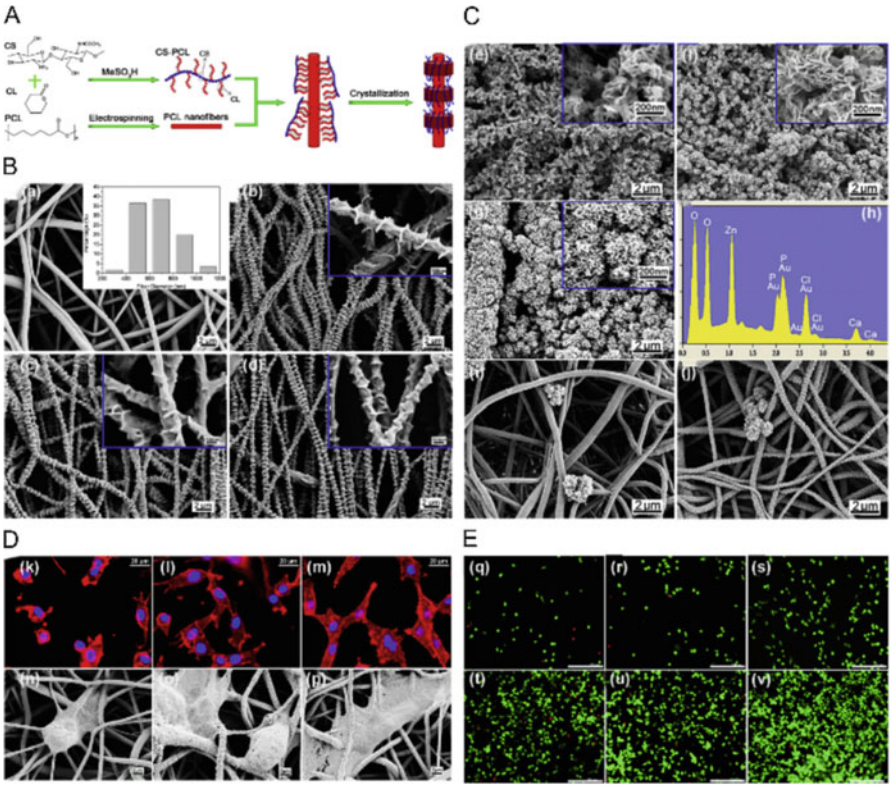


Fig. 6 (**a**) Schematic of fabricating shish-kebab (SK)-like structured scaffolds by CS-PCL. (**b**) SEM images of (a) PCL, (b) PCL–SK(PCL), (c) PCL–SK(CS–PCL6.5), and (d) PCL–SK(CS–PCL8.8). (**c**) SEM images of morphologically mineralized CS-PCL SK-like structured nanofibers (e) 1, (f) 3, and (g) 7 days after mineralization. (h) Elements detected by EDS. The mineralized control groups (i) PCL and (j) PCL-SK nanofibers for 7 days. (**d**) Cytoskeleton staining and SEM images of cells on (k, n) PCL, (l, o) PCL–SK(PCL), and (m, p) PCL–SK(CS–PCL8.8) nanofibers. (E) Live/dead staining showed cells viability on (q, t) PCL, (r, u) PCL–SK (PCL), and (s, v) PCL–SK (CS–PCL8.8) nanofibers for 3 and 7 days. (Copyright permission © 2019 Elsevier Ltd. Tao et al. 2020; Reprinted with permission from Jing et al. (2015). Copyright (2015) American Chemical Society)

2013). From the synergistic combination of the cellulose and chitosan intrinsic features, it was possible to achieve strength, antimicrobial activity, and deliverability, which are adequate features for bone tissue engineering approaches.

Poly (propylene carbonate)/chitosan scaffold presented a porous structure (91.9% porosity) with interconnected spherical macropores. The structure after 14 days of incubation in SBF had deposited abundant apatite crystals and as well attached BMSCs, exhibiting faster osteogenesis. Its implantation in animal model defect revealed almost complete healing after 16 weeks (Zhao et al. 2012).

In summary, biocomposite scaffolds based on chitin or chitosan tested in vitro and in vivo revealed to be nontoxic, biocompatible, osteoconductive, and osteoinductive, promoting good cell adhesion and proliferation, causing an increase in bone

regeneration (Aguilar et al. 2019). In alveolar bone and jawbone regeneration preclinical applications, chitosan has shown promising results accelerating osseointegration and reconstructing critical size defects (Aguilar et al. 2019).

5.3 Cartilage Regeneration

Cartilage has minimal ability to regenerate itself due to its avascular and non-innervated nature and, consequently, limited nutrient supply, as well as chondrocytes limited proliferation (Saravanan et al. 2016). Tissue engineering has been emerging as a promising strategy for cartilage regeneration, and efforts have been made toward the development of adequate biomaterials, which should support, guide, and stimulate tissue growth. Owing to the difficulties related to chitin processing, only a few workers report its use in the pure form for cartilage regeneration. Scaffolds produced using pure β-chitin, or pure chitosan, or different ratios of both polymers showed the same efficiency in supporting chondrocytes growth, using the same chondroitin sulfate concentrations (Kuo and Ku 2008). The outcomes of this work showed that only in the pure β-chitin sponge type II collagen was closer to normal rabbit cartilage. More recently, a microporous hybrid chitin/PCL porous scaffold loaded chondroitin sulfate nanoparticles incorporating the transforming growth factor- β (TGF-β) was developed following the procedure depicted in Fig. 7a, aiming to cue the direct chondrogenesis (Deepthi and Jayakumar 2016). The prolonged-release of TGF-β promoted an increase in the attachment and proliferation of rabbit adipose-derived mesenchymal stem cells, as well as an increase in the proteoglycan deposition, as shown in Fig. 7b. Safranin O was used as an extracellular matrix (ECM) stain to stain proteoglycans in red. The chondrogenic differentiation can be assessed based on the extent of proteoglycan deposited by the cells.

Chitosan-based materials have been widely used for cartilage regeneration mainly due to their desirable features, including biodegradability, a consequence of the physiological depolymerization in the presence of lysozyme, antimicrobial, antitumor, and antioxidant activities, biocompatibility, and minimal body reaction, with an absence of chronic inflammatory response (Chen et al. 2018). Chitosan scaffolds have been widely used for cartilage regeneration, showing satisfactory in vitro outcomes by supporting the cell attachment and proliferation, while maintaining their round morphology as well as good in vivo results (Beck et al. 2017).

Facing the challenges associated with cartilage repair, new methodologies, and biomaterials combinations have been explored. Several studies have stated the use of blends of chitosan/silk fibroin (SF) for the production of porous scaffolds for cartilage regeneration since it has been reported as an attractive biomaterial for the development of 3D porous structures with controllable pore sizes and mechanical properties (Vishwanath et al. 2016; Silva et al. 2008; Panjapheree et al. 2018). In one study, the authors developed chitosan/SF blended porous scaffolds by the freeze-drying method and evaluated the effect of the blend ratio on the properties of the scaffold (Vishwanath et al. 2016). The scaffolds presenting blend ratios of SF/chitosan (80:20) revealed superior results regarding the mechanical properties

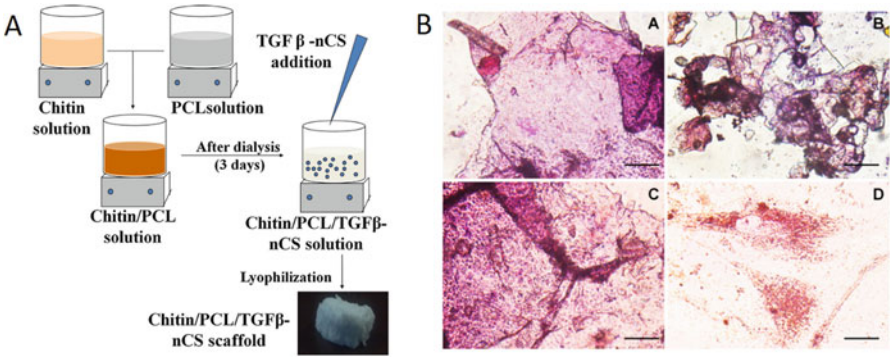


Fig. 7 (**a**) Schematic representation of the procedure used for the production of chitin-PCL-TGF- β -nCS scaffolds; (**b**) Safranin O staining showing proteoglycan deposition after 28 days of chondrogenic differentiation of cells cultured on (**a**) chitin-PCL, (**b**) chitin-PCL-nCS, (**c**) chitin-PCL-TGF- β -nCS, and (**d**) tissue culture plate. Nucleus is stained blue by hematoxylin. Scale bar denotes 100 μm. (Reprinted from International Journal of Biological Macromolecules, 93, S. Deepthi,R. Jayakumar, Prolonged release of TGF-β from polyelectrolyte nanoparticle loaded macroporous chitin-poly(caprolactone) scaffold for chondrogenesis, 1402–1409. Copyright (2016) with permission from Elsevier)

while providing a good cell attachment, viability, and proliferation when cultured with mesenchymal stem cells derived from umbilical cord blood. In addition, the assessment of glycosaminoglycans (GAG) production up to 21 days of culture under chondrogenic differentiation indicates the ability of the scaffolds for cartilage tissue regeneration. Moreover, stable genipin cross-linked chitosan/*bombyx mori* SF sponges, capable of supporting the growth and maintaining the ATDC5 chondrocyte-like cell functions when cultured into the sponge, were produced by Silva et al. (Silva et al. 2008). The sponges were able to promote the adhesion and proliferation of a chondrocyte-like cell line while supporting the deposition of cartilage extracellular matrix (ECM). Porous hybrid chitosan/ECM scaffolds were produced through decellularized bovine cartilage chemically cross-linked with chitosan, by the freeze-drying method (Nasiri and Mashayekhan 2017). The presence of 1% and 2% ECM in the chitosan scaffolds decreased the pore sizes, while at the same time improving its mechanical properties, swelling ability, and biodegradation. Moreover, the scaffolds with 2% ECM presented the best results regarding chondrocytes viability and attachment, demonstrating the potential to be used as a solution for tissue engineering cartilage regeneration.

5.4 Infectious Diseases

Infectious diseases are caused by pathogenic microbes like bacteria, viruses, and fungus (Antabe and Ziegler 2020). Infectious disease will develop when host defense mechanisms get compromised, and the severity of the disease depends on the ability of the pathogen to cause damage to the host and the ability of the host to

resist. In this context, nanotechnology and nanomedicine have shown excellent potential in dealing with a range of different health problems, including viruses, which are considered to be a severe challenge in the medical field. In fact, theranostic nanoparticles were described as promising tools for efficiently and selectively delivering therapeutic moieties, as, e.g., drugs, vaccines, SiRNA, peptide to target sites of infection. As mentioned earlier in this chapter, the chitosan functional groups permit the conjugation of specific ligands and, at the same time, help to target loaded drugs to the site of infection and/or inflammation. Based on that, chitosan nanoparticles offer physical properties that have associated benefits as antimicrobial activity or as drug carriers that can improve antiviral therapy. Chitosan nanoparticles conjugated with the antiviral activity of curcumin, a bioactive compound obtained from *Curcuma longa*, as a composite form, demonstrated to be a powerful multitarget antiviral agent against entry and replication of HCV-4 in hepatoblastoma cells with inhibition of HCV-4a entry by 94.5% (Loutfy et al. 2020).

Actually, the coronavirus (COVID-19) is a highly contagious infectious disease with the potential to evolve and adapt to environmental changes, and it creates significant challenges to treat (Zhang et al. 2020). The COVID-19 is caused by virus SARS-COV-2 (severe acute respiratory syndrome coronavirus 2) and transmitted to humans via respiratory droplets.

Modified chitosan-based nano−/microspheres (NS/MS) were produced with good potential for adsorbing coronaviruses to tackle human coronavirus NL63 (Ciejka et al. 2017). The research also showed that after the addition of N-(2-hydroxypropyl)-3-trimethyl chitosan (H-HTCC)-NS/MS to viral suspensions, there was a decrease in the copy number of viral RNA; this showed good correlation with the amount of added H-HTCC-NS/MS. Based on these studies, HTCC can be fine-tuned to target any coronavirus. Following these directions, recent studies (Milewska et al. 2020) showed that the virus replication of SARS-CoV-2 and MERS-CoV was successfully inhibited by the substituted chitosan HTCC in vitro and ex vivo, suggesting that it can be a promising candidate for the treatment of SARS-CoV-2 patients.

Commercially, Biovanta-Bosti, a leader in chitosan nanoparticle research, developed a 48-h Novochizol TM that can be used to encapsulate any active pharmaceutical ingredient for localized delivery and sustained release in any tissue. Therefore, Novochizol TM could generate intra-pulmonary drug delivery formulations suitable for treating COVID-19 patients.

6 Conclusions

The use of chitin, chitosan, and their derivatives in the biomedical field is continuously growing with the development of alternative strategies not only to produce matrices based on these polymers combined or not with other macromolecules or bioactive compounds; but also to explore them to promote the regeneration of different tissues. The broad chemical modification ability of chitin and chitosan, due to their reactive groups, allows to the extent their use. Furthermore, the

exploitation of ionic liquid platforms opens up opportunities either for efficient chitin extraction, for chitosan and chitin modification, and used as solvents allow to produce high-value biomaterials based on these polymers. Despite the promising findings concerning the use of chitin, due to the difficulties associated with its processing, most of the biomedical studies are mainly focused on the use of chitosan. Chitosan's inherent biodegradability, associated with the additional antimicrobial, antitumor, and antioxidant activities, biocompatibility, and minimal body reaction, make it an attractive polymer for biomedical purposes.

Active research is underway in improving chitin, chitosan-based systems for wound healing since they could introduce valuable properties such as antimicrobial activity, biodegradability, and biocompatibility. In particular, advances in the use of chitin in wound healing have been achieved by combining other polymers, proteins, or even silver or gold particles to enhance the physicochemical and mechanical properties of the resulting matrices.

Chitin/chitosan nanofibers are optimal biomaterials to be applied in bone regenerative engineering because of their excellent biocompatibility and diverse biological functions. Bone engineered chitin/chitosan nanofibrous scaffolds have demonstrated excellent biocompatibility, osteoinduction, and osseointegration. Notably, chitosan nanofibers exhibited an excellent affinity for osteoblasts facilitating their proliferation and maturation and upregulating osteogenic gene expression. Chitin and chitosan hybrid porous scaffolds have shown remarkable in vitro outcomes by supporting chondrocytes growth, attachment, and proliferation while maintaining their morphology, as well as good in vivo results, proving their potential as solutions for cartilage regeneration.

However, maximizing the applications of those matrices requires more investments in vitro/in vivo studies and investigations of their mechanisms of action concerning their structural characteristics. In addition, NACOS and COS, hydrolyzed products of chitin and chitosan, have demonstrated to be also suitable for potential biological applications since they exert an excellent antioxidant and antimicrobial effect.

Some reported advances on chitin and chitosan-based nanosystems as promising therapeutical tools for delivering therapeutic moieties to target sites of infection suggested that it will also have an encouraging role in the infectious disease treatment.

Overall, chitin and chitosan-based systems are recognized biocompatible materials to be used in medical devices, where promising studies and recent products envisioning wound dressing, guided bone repair, cartilage regeneration, and infection diseases, among others, have been reported. Despite this, challenges remain related to the lack of translation of those systems to the market, and more investment is needed to overcome this limitation.

Acknowledgments The authors especially acknowledge financial support from Portuguese FCT (PD/BD/135247/2017, SFRH/BPD/93697/2013 and CEECIND/01306/2018). This work is also financially supported by Ph.D. program in Advanced Therapies for Health (PATH) (PD/00169/2013), FCT R&D&I projects with references PTDC/BII-BIO/31570/2017, PTDC/CTM-CTM//29813/2017, and R&D&I Structured Projects with reference NORTE-01-0145-FDER-000021.

References

Abdelrahman T, Newton H. Wound dressings: principles and practice. Surgery (Oxford). 2011;29 (10):491–5.

Adhikari HS, Yadav PN. Anticancer activity of chitosan, chitosan derivatives, and their mechanism of action. Int J Biomater. 2018;2018:2952085.

Aguilar A, Zein N, Harmouch E, Hafdi B, Bornert F, Offner D, et al. Application of chitosan in bone and dental engineering. Molecules. 2019;24(16):3009.

Alves NM, Mano JF. Chitosan derivatives obtained by chemical modifications for biomedical and environmental applications. Int J Biol Macromol. 2008;43(5):401–14.

Antabe R, Ziegler BR. Diseases, emerging and infectious. In: International Encyclopedia of Human Geography. Amsterdam: Cambridge, MA; 2020. p. 389–91.

Anthony J, Brennecke J, Holbrey J, Maginn E, Mantz P, Trulove A, et al. Physicochemical properties of ionic liquids. In: Wasserscheid P, Welton T, editors. Ionic liquids in synthesis. Weinheim: Wiley-VCH; 2002. p. 41–126.

Arnold ND, Brück WM, Garbe D, Brück TB. Enzymatic modification of native chitin and conversion to specialty chemical products. Mar Drugs. 2020;18(2):93.

Barber PS, Griggs CS, Bonner JR, Rogers RD. Electrospinning of chitin nanofibers directly from an ionic liquid extract of shrimp shells. Green Chem. 2013;15(3):601–7.

Bastiaens L, Soetemans L, D'Hondt E, Elst K. Sources of chitin and chitosan and their isolation. In: van den Broek L, Boeriu C, editors. Chitin and chitosan: properties and application. Hoboken: Wiley; 2019. p. 1–34.

Beck SC, Jiang T, Nair LS, Laurencin CT. Chapter 2: Chitosan for bone and cartilage regenerative engineering. In: Jennings JA, Bumgardner JD, editors. Chitosan based biomaterials volume 2. Amsterdam: Woodhead Publishing; 2017. p. 33–72.

Chang S-H, Lin H-TV WG-J, Tsai GJ. pH effects on solubility, zeta potential, and correlation between antibacterial activity and molecular weight of chitosan. Carbohydr Polym. 2015;134:74–81.

Chang S-H, Wu C-H, Tsai G-J. Effects of chitosan molecular weight on its antioxidant and antimutagenic properties. Carbohydr Polym. 2018;181:1026–32.

Chen L, Zhu C, Fan D, Liu B, Ma X, Duan Z, et al. A human-like collagen/chitosan electrospun nanofibrous scaffold from aqueous solution: electrospun mechanism and biocompatibility. J Biomed Mater Res A. 2011;99A(3):395–409.

Chen H, Cui S, Zhao Y, Zhang C, Zhang S, Peng X. Grafting chitosan with Polyethylenimine in an ionic liquid for efficient gene delivery. PLoS One. 2015;10(4):e0121817.

Chen J, Xie F, Li X, Chen L. Ionic liquids for the preparation of biopolymer materials for drug/gene delivery: a review. Green Chem. 2018;20(18):4169–200.

Chitin Market Size Chitin Market Size. With impact of domestic and global market top players, share, future challenges, revenue, demand, industry growth and top players analysis to 2026 [press release]. 2020.

Ciejka J, Wolski K, Nowakowska M, Pyrc K, Szczubiałka K. Biopolymeric nano/microspheres for selective and reversible adsorption of coronaviruses. Mater Sci Eng C. 2017;76:735–42.

Dai T, Tanaka M, Huang Y-Y, Hamblin MR. Chitosan preparations for wounds and burns: antimicrobial and wound-healing effects. Expert Rev Anti-Infect Ther. 2011;9(7):857–79.

Daraghmeh NH, Chowdhry BZ, Leharne SA, Al Omari MM, Badwan AA. Chapter 2: Chitin. In: Brittain HG, editor. Profiles of drug substances, excipients and related methodology, vol. 36. Amsterdam: Academic Press; 2011. p. 35–102.

Deepthi S, Jayakumar R. Prolonged release of TGF-β from polyelectrolyte nanoparticle loaded macroporous chitin-poly(caprolactone) scaffold for chondrogenesis. Int J Biol Macromol. 2016;93:1402–9.

Dong L, Wichers HJ, Govers C. Beneficial health effects of chitin and chitosan. In: Boeriu CG, editor. Chitin and chitosan: properties and applications. Hoboken: Wiley; 2019. p. 145–67.

El Knidri H, Belaabed R, Addaou A, Laajeb A, Lahsini A. Extraction, chemical modification and characterization of chitin and chitosan. Int J Biol Macromol. 2018;120:1181–9.

Gomes J, Silva S, Reis R. Exploring the use of choline acetate on the sustainable development of α-chitin-based sponges. ACS Sustain Chem Eng. 2020;8:13507–16.
Hajji S, Younes I, Ghorbel-Bellaaj O, Hajji R, Rinaudo M, Nasri M, et al. Structural differences between chitin and chitosan extracted from three different marine sources. Int J Biol Macromol. 2014;65:298–306.
Hirayama H, Yoshida J, Yamamoto K, Kadokawa J-I. Facile acylation of α-chitin in ionic liquid. Carbohydr Polym. 2018;200:567–71.
Hu W, Liu M, Yang X, Zhang C, Zhou H, Xie W, et al. Modification of chitosan grafted with collagen peptide by enzyme crosslinking. Carbohydr Polym. 2019;206:468–75.
Jing X, Mi H-Y, Wang X-C, Peng X-F, Turng L-S. Shish-kebab-structured poly(ε-caprolactone) nanofibers hierarchically decorated with chitosan–poly(ε-caprolactone) copolymers for bone tissue engineering. ACS Appl Mater Interfaces. 2015;7(12):6955–65.
Kievit FM, Florczyk SJ, Leung MC, Veiseh O, Park JO, Disis ML, et al. Chitosan–alginate 3D scaffolds as a mimic of the glioma tumor microenvironment. Biomaterials. 2010;31(22):5903–10.
Kim S. Competitive biological activities of chitosan and its derivatives: antimicrobial, antioxidant, anticancer, and anti-inflammatory activities. Int J Polymer Sci. 2018;2018:1708172.
Kumar PTS, Abhilash S, Manzoor K, Nair SV, Tamura H, Jayakumar R. Preparation and characterization of novel β-chitin/nanosilver composite scaffolds for wound dressing applications. Carbohydr Polym. 2010;80(3):761–7.
Kuo Y-C, Ku IN. Cartilage regeneration by novel polyethylene oxide/chitin/chitosan scaffolds. Biomacromolecules. 2008;9(10):2662–9.
Li S, Tian X, Fan J, Tong H, Ao Q, Wang X. Chitosans for tissue repair and organ three-dimensional (3D) bioprinting. Micromachines (Basel). 2019;10(11):765.
Li B, Elango J, Wu W. Recent advancement of molecular structure and biomaterial function of chitosan from marine organisms for pharmaceutical and nutraceutical application. Appl Sci. 2020;10(14):4719.
Liu L, Zhou S, Wang B, Xu F, Sun R. Homogeneous acetylation of chitosan in ionic liquids. J Appl Polym Sci. 2013;129(1):28–35.
LogithKumar R, KeshavNarayan A, Dhivya S, Chawla A, Saravanan S, Selvamurugan N. A review of chitosan and its derivatives in bone tissue engineering. Carbohydr Polym. 2016;151:172–88.
Lotfi G, Shokrgozar MA, Mofid R, Abbas FM, Ghanavati F, Baghban AA, et al. Biological evaluation (in vitro and in vivo) of Bilayered collagenous coated (Nano electrospun and Solid Wall) chitosan membrane for periodontal guided bone regeneration. Ann Biomed Eng. 2015;44:2132–44.
Loutfy SA, Elberry MH, Farroh KY, Mohamed HT, Mohamed AA, Mohamed EB, et al. Antiviral activity of chitosan nanoparticles encapsulating curcumin against hepatitis C virus genotype 4a in human hepatoma cell lines. Int J Nanomedicine. 2020;15:2699–715.
Milewska A, Chi Y, Szczepanski A, Barreto-Duran E, Liu K, Liu D, et al. HTCC as a highly effective polymeric inhibitor of SARS-CoV-2 and MERS-CoV. bioRxiv. 2020. https://doi.org/10.1101/2020.03.29.014183.
Mine S, Izawa H, Kaneko Y, Kadokawa J-I. Acetylation of α-chitin in ionic liquids. Carbohydr Res. 2009;344(16):2263–5.
Moreno-Vásquez MJ, Valenzuela-Buitimea EL, Plascencia-Jatomea M, Encinas-Encinas JC, Rodríguez-Félix F, Sánchez-Valdes S, et al. Functionalization of chitosan by a free radical reaction: characterization, antioxidant and antibacterial potential. Carbohydr Polym. 2017;155:117–27.
Mututuvari TM, Harkins AL, Tran CD. Facile synthesis, characterization, and antimicrobial activity of cellulose-chitosan-hydroxyapatite composite material: a potential material for bone tissue engineering. J Biomed Mater Res A. 2013;101(11):3266–77.
Muzzarelli RAA. Chitins and chitosans for the repair of wounded skin, nerve, cartilage and bone. Carbohydr Polym. 2009;76(2):167–82.
Muzzarelli RAA. Biomedical exploitation of chitin and chitosan via Mechano-chemical disassembly, electrospinning, dissolution in imidazolium ionic liquids, and supercritical drying. Mar Drugs. 2011;9(9):1510–33.
Nasiri B, Mashayekhan S. Fabrication of porous scaffolds with decellularized cartilage matrix for tissue engineering application. Biologicals. 2017;48:39–46.

Nitta S, Komatsu A, Ishii T, Ohnishi M, Inoue A, Iwamoto H. Fabrication and characterization of water-dispersed chitosan nanofiber/poly(ethylene glycol) diacrylate/calcium phosphate-based porous composites. Carbohydr Polym. 2017;174:1034–40.
Panjapheree K, Kamonmattayakul S, Meesane J. Biphasic scaffolds of silk fibroin film affixed to silk fibroin/chitosan sponge based on surgical design for cartilage defect in osteoarthritis. Mater Des. 2018;141:323–32.
Park BK, Kim M-M. Applications of chitin and its derivatives in biological medicine. Int J Mol Sci. 2010;11(12):5152–64.
Park JK, Chung MJ, Choi HN, Park YI. Effects of the molecular weight and the degree of deacetylation of chitosan oligosaccharides on antitumor activity. Int J Mol Sci. 2011;12:266–77.
Patrick SB, Julia LS, Robin DR. A "green" industrial revolution: using chitin towards transformative technologies. Pure Appl Chem. 2013;85(8):1693–701.
Pighinelli L. Methods of chitin production a short review. Am J Biomed Sci Res. 2019;3:307–14.
Pillai CKS, Paul W, Sharma CP. Chitin and chitosan polymers: chemistry, solubility and fiber formation. Prog Polym Sci. 2009;34(7):641–78.
Preethi Soundarya S, Haritha Menon A, Viji Chandran S, Selvamurugan N. Bone tissue engineering: scaffold preparation using chitosan and other biomaterials with different design and fabrication techniques. Int J Biol Macromol. 2018;119:1228–39.
Qiu J, Wang J. Chitin in rubber based blends and micro composites. In: Visakh PM, editor. Rubber based bionanocomposites: preparation. Cham: Springer International Publishing; 2017. p. 71–107.
Rasouli R, Barhoum A, Bechelany M, Dufresne A. Nanofibers for biomedical and healthcare applications. Macromol Biosci. 2019;19(2):1800256.
Roseti L, Parisi V, Petretta M, Cavallo C, Desando G, Bartolotti I, et al. Scaffolds for bone tissue engineering: state of the art and new perspectives. Mater Sci Eng C. 2017;78:1246–62.
Salah R, Michaud P, Mati F, Harrat Z, Lounici H, Abdi N, et al. Anticancer activity of chemically prepared shrimp low molecular weight chitin evaluation with the human monocyte leukaemia cell line, THP-1. Int J Biol Macromol. 2013;52:333–9.
Saravanan S, Leena RS, Selvamurugan N. Chitosan based biocomposite scaffolds for bone tissue engineering. Int J Biol Macromol. 2016;93:1354–65.
Schroeder JE, Mosheiff R. Tissue engineering approaches for bone repair: concepts and evidence. Injury. 2011;42(6):609–13.
Shamshina JL. Chitin in ionic liquids: historical insights into the polymer's dissolution and isolation. A review. Green Chem. 2019;21(15):3974–93.
Shamshina JL, Gurau G, Block LE, Hansen LK, Dingee C, Walters A, et al. Chitin–calcium alginate composite fibers for wound care dressings spun from ionic liquid solution. J Mater Chem B. 2014;2(25):3924–36.
Sharma M, Mukesh C, Mondal D, Prasad K. Dissolution of α-chitin in deep eutectic solvents. RSC Adv. 2013;3(39):18149–55.
Shen Y, Dai L, Li X, Liang R, Guan G, Zhang Z, et al. Epidermal stem cells cultured on collagen-modified chitin membrane induce in situ tissue regeneration of full-thickness skin defects in mice. PLoS One. 2014;9(2):e87557.
Silva SS, Motta A, Rodrigues MT, Pinheiro AFM, Gomes ME, Mano JF, et al. Novel Genipin-cross-linked chitosan/silk fibroin sponges for cartilage engineering strategies. Biomacromolecules. 2008;9(10):2764–74.
Silva SS, Duarte ARC, Carvalho AP, Mano JF, Reis RL. Green processing of porous chitin structures for biomedical applications combining ionic liquids and supercritical fluid technology. Acta Biomater. 2011;7(3):1166–72.
Silva SS, Santos TC, Cerqueira MT, Marques AP, Reys LL, Silva TH, et al. The use of ionic liquids in the processing of chitosan/silk hydrogels for biomedical applications. Green Chem. 2012;14(5):1463–70.
Silva SS, Duarte ARC, Oliveira JM, Mano JF, Reis RL. Alternative methodology for chitin–hydroxyapatite composites using ionic liquids and supercritical fluid technology. J Bioact Compat Polym. 2013a;28(5):481–91.
Silva SS, Duarte ARC, Mano JF, Reis RL. Design and functionalization of chitin-based microsphere scaffolds. Green Chem. 2013b;15(11):3252–8.

Silva SS, Mano JF, Reis RL. Ionic liquids in the processing and chemical modification of chitin and chitosan for biomedical applications. Green Chem. 2017;19(5):1208–20.
Silva SS, Gomes JM, Rodrigues LC, Reis RL. Marine-derived polymers in ionic liquids: architectures development and biomedical applications. Mar Drugs. 2020;18(7):346.
Singh R, Shitiz K, Singh A. Chitin and chitosan: biopolymers for wound management. Int Wound J. 2017;14(6):1276–89.
Sofi HS, Ashraf R, Beigh MA, Sheikh FA. Scaffolds fabricated from natural polymers/composites by electrospinning for bone tissue regeneration. Adv Exp Med Biol. 2018;1078:49–78.
Synowiecki J, Al-Khateeb NA. Production, properties, and some new applications of chitin and its derivatives. Crit Rev Food Sci Nutr. 2003;43(2):145–71.
Tan YN, Lee PP, Chen WN. Microbial extraction of chitin from seafood waste using sugars derived from fruit waste-stream. AMB Express. 2020;10(1):17.
Tao F, Cheng Y, Shi X, Zheng H, Du Y, Xiang W, et al. Applications of chitin and chitosan nanofibers in bone regenerative engineering. Carbohydr Polym. 2020;230:115658.
Uto T, Idenoue S, Yamamoto K, Kadokawa JI. Understanding dissolution process of chitin crystal in ionic liquids: theoretical study. PCCP. 2018;20(31):20669–77.
Varlamov V, Il'ina A, Shagdarova B, Lunkov A, Mysyakina I. Chitin/chitosan and its derivatives: fundamental problems and practical approaches. Biochem Mosc. 2020;85:154–76.
Vicente FA, Bradić B, Novak U, Likozar B. α-Chitin dissolution, N-deacetylation and valorization in deep eutectic solvents. Biopolymers. 2020;111(5):e23351.
Vishwanath V, Pramanik K, Biswas A. Optimization and evaluation of silk fibroin-chitosan freeze-dried porous scaffolds for cartilage tissue engineering application. J Biomater Sci Polym Ed. 2016;27(7):657–74.
Wang Z, Zheng L, Li C, Zhang D, Xiao Y, Guan G, et al. A novel and simple procedure to synthesize chitosan-graft-polycaprolactone in an ionic liquid. Carbohydr Polym. 2013;94(1): 505–10.
Wang W, Meng Q, Li Q, Liu J, Zhou M, Jin Z, et al. Chitosan derivatives and their application in biomedicine. Int J Mol Sci. 2020;21(2):487.
Wu Y, Sasaki T, Irie S, Sakurai K. A novel biomass-ionic liquid platform for the utilization of native chitin. Polymer. 2008;49(9):2321–7.
Xie J, Peng C, Zhao Q, Wang X, Yuan H, Yang L, et al. Osteogenic differentiation and bone regeneration of iPSC-MSCs supported by a biomimetic nanofibrous scaffold. Acta Biomater. 2016;29:365–79.
Younes I, Rinaudo M. Chitin and chitosan preparation from marine sources. Structure, properties and applications. Mar Drugs. 2015;13(3):1133–74.
Younes I, Ghorbel-Bellaaj O, Chaabouni M, Rinaudo M, Souard F, Vanhaverbeke C, et al. Use of a fractional factorial design to study the effects of experimental factors on the chitin deacetylation. Int J Biol Macromol. 2014;70:385–90.
Zhang Z, Li C, Wang Q, Zhao ZK. Efficient hydrolysis of chitosan in ionic liquids. Carbohydr Polym. 2009;78(4):685–9.
Zhang H, Penninger JM, Li Y, Zhong N, Slutsky AS. Angiotensin-converting enzyme 2 (ACE2) as a SARS-CoV-2 receptor: molecular mechanisms and potential therapeutic target. Intensive Care Med. 2020;46(4):586–90.
Zhao J, Han W, Chen H, Tu M, Huan S, Miao G, et al. Fabrication and in vivo osteogenesis of biomimetic poly(propylene carbonate) scaffold with nanofibrous chitosan network in macropores for bone tissue engineering. J Mater Sci Mater Med. 2012;23(2):517–25.

Chitosan

12

Anabelle Camarotti de Lima Batista, Weslley de Souza Paiva, and Francisco Ernesto de Souza Neto

Contents

Abstract

Chitosan has inspired several researchers around the world due to its use versatility, mainly due to the presence of the amine group in its structure. Initially, the research used animal chitosan because it is cheaper to obtain. However, research groups and companies have been investigating the advantages of using fungal chitosan. Its production can be sustainable since it can use residues from the agricultural sector or industry as a culture medium. There are several applications, especially in the biomedical area, due to the standardization of production and side effects reduction, particularly allergens and immune system sensitizers.

A. C. de Lima Batista (✉)
Agriculture Department, CCHSA, Federal University of Paraiba, Bananeiras, PB, Brazil

PROFBIO, CCEN, Federal University of Paraiba, João Pessoa, PB, Brazil

W. de Souza Paiva
RENORBIO, Federal University of Rio Grande do Norte, Natal, RN, Brazil

F. E. de Souza Neto
Nova Esperança, College of Mossoro, Mossoró, RN, Brazil

J. M. Oliveira et al. (eds.), *Polysaccharides of Microbial Origin*,
https://doi.org/10.1007/978-3-030-42215-8_14

Considering the advantages and descriptions of successful applications mentioned here, we hope that new research groups will be interested in working with fungal chitosan for the biomedical area, among others.

Keywords

Fungal chitosan · Biotechnology apply · Zygomycetes · Deacetylation degree · *Aspergillus niger*

1 Fungi Chitosan

Among the microbial polysaccharides previously described, fungal chitosan also appears as a biotechnological product with great potential for commercialization and profit in the international market. Its physicochemical and biological characteristics give it an advantage in terms of the different possibilities of application and conjugation with other molecules, which can be natural or synthetic. Its use in synergy also favors its use in biomedical applications and brings advantages compared to chitosan produced from animal chitin.

Thus, considering its importance, this chapter will explain the production, extraction, and purification of fungal chitosan, some of its physical-chemical and biological characteristics, and its application in areas of biotechnology directly or indirectly related to regenerative medicine.

1.1 Production

Fungi have a cell wall composed mainly of a network of interconnected molecules consisting of proteins, glucans, chitin, chitosan, lipids, and polyphosphates that may have their quantity and quality altered due to the environmental conditions and intrinsic characteristics of their species (Campos-Takaki et al. 1983; Paiva et al. 2021). Among these compounds, chitosan stands out for being associated with increased wall integrity, favoring protection against high temperatures, and cell inhibitors to which the fungal strain may be subjected (Baker et al. 2007). The high degree of deacetylation in chitosan's cell wall interferes with its flexibility, and the low degree interferes with its solubility (Franca et al. 2011).

Chitosan production in fungi is linked to the amount of chitin and the action of the enzyme chitin deacetylase (EC 3.5.1.41) on nascent or established chitin residues directly on the cell wall (Fig. 1) (Campos-Takaki et al. 1983; Fesel and Zuccaro 2016). This enzymatic synthesis of chitosan was first described by Araki and Ito (1974) in Mucor rouxii (Zygomycetes), demonstrating that about 14% of chitin deacetylase is associated with particles from cellular fractions, 49% is associated with a soluble fraction of the supernatant, and about 37% is extracellular (Araki and Ito 1974). Complementing the chitosan synthesis route, Davis and Bartnicki-Garcia (1984) found strong evidence that chitin deacetylase activity is highly efficient in

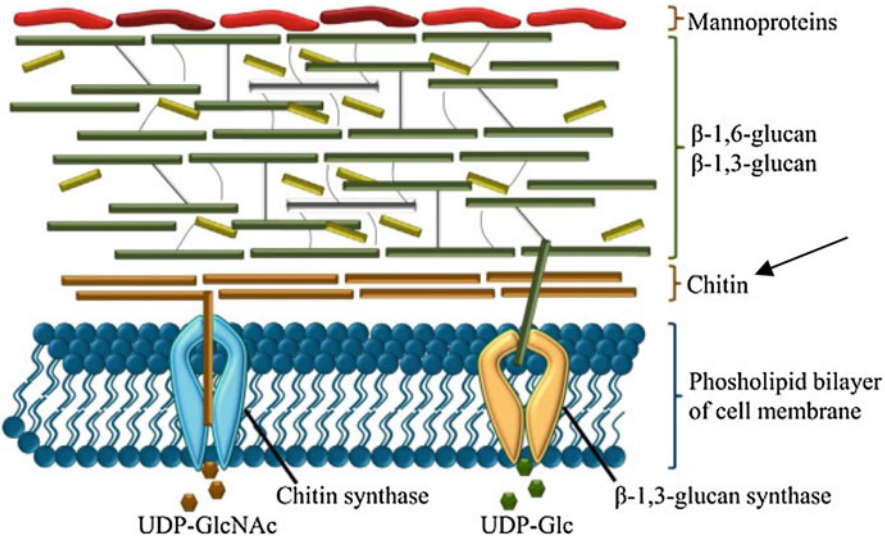


Fig. 1 Schematic overview of fungal cell wall composition. Chitin is represented by brown lines located close to the cell membrane. Chitin is synthesized by transferring *N*-acetylglucosamine residues from uridine diphosphate-*N*-acetylglucosamine (UDPGlcNAc; brown hexagon) to a growing fiber that is shuttled through the cell membrane by the transmembrane chitin synthase (light blue). In this preformed chitin, there is a little incidence of chitin deacetylase action to form chitosan. (Reprinted from Figure 1, Fesel PH, Zuccaro A. β-glucan: Crucial component of the fungal cell wall and elusive MAMP in plants. Fungal Genet Biol 90:53–60, 2016, with permission from Elsevier. Licensed by CC BY-NC-ND (https://creativecommons.org/licenses/by-nc-nd/4.0/)). Licensed by CC BY-NC-ND (https://creativecommons.org/licenses/by-nc-nd/4.0/))

residues of a growing chitin chain, and it shows low action on preformed chitin molecules. These findings suggest that changes in the culture medium in which fungi grow can directly influence chitosan production (Batista et al. 2020; Yuan et al. 2021). The literature also explains that in all cases, the optimum temperature for enzyme activity is 50 °C while optimum pH varies from 4.5 to 8.5 (Jeraj et al. 2006).

At the moment, Mucorales is the most representative and studied order as it contains most species that produce chitosan for commercial application, especially those belonging to the genus Mucor and Rhizopus. This commercial interest is since significant levels of beta-D-Glucan cannot be detected on their cell wall, the ease of growth of these organisms in submerged systems, and their ability to use different substrates as a source of carbon and nitrogen. Other orders have been reported and described as chitosan producers (Table 1); however, some accumulate beta-D-Glucan, for example, *Aspergillus* sp. and *Agaricus* sp. The presence of beta-D-glucan makes the purification process more expensive for subsequent application in the biomedical field (Fesel and Zuccaro 2016).

The production based on the cultivation conditions described in Table 1 is guided by the chitosan synthesis route in fungi, more precisely, in the enzymes related to its production. Although the description of these enzymes occurred almost 50 years ago,

Table 1 Chitosan production by species from different orders depends on the cultivation conditions. These conditions could interfere directly with yield and quality of the chitosan produced

Species	Cultivation conditions	Physical-chemical characteristic	References
Syncephalastrum sp. (Mucorales)	Corn steep liquor	DD 88.14%; Low molecular weight	Batista et al. (2020)
Rhizopus stolonifer (Mucorales)	YPD	DD 84%	Paiva et al. (2017)
Aspergillus niger (Eurotiales)	Not described	Not described	Kitozyme ®
Agaricus bisporus (Agaricales) and *Aspergillus niger* (Eurotiales)	Not described	DD $\geq$ 90%; low molecular weight (for both)	ChiBio ®

YPD media (Yeast Extract 10 g; Peptone 20 g; Dextrose 20 g per liter) (Davis and Bartinicki-Garcia 1984); Kitozyme (https://www.kitozyme.com/en/); ChiBio (https://www.chibiotech.com/)

Table 2 Substrates were directly influencing in enzyme action of chitin synthase and chitin deacetylase extracted from different fungi. In all cases, they positively interfere. Other authors checked the positive influence of substrates in chitosan production in vivo and reported that it depends on the concentration and the fungus species

Influence	Substrate	Reference
In chitin deacetylase	Zn^{2+}; Mn^{2+}; Co^{2+}; Ca^{2}	Jaworska and Konieczena (2001)
In chitin synthase	Trypsin; Mn^{2+}; Co^{2+}; Mg^{2+}	Shehata et al. (2018)
On chitosa production	Co^{2+}; Chitin; Fe^{2+}	Jaworska and Konieczena (2001), Shehata et al. (2018)

it was only in the last decade that the substrates influencing their action were described. Jaworska and Konieczena (2001), among others, mainly report the influence of ions on the synthase and deacetylase chitin enzyme action, with caveats for the concentrations to be added so that there is no decrease in chitosan production (Table 2). In vivo analysis of the influence of these substrates on chitosan production showed that Co^{2+} and chitin depend on the concentration present in the culture medium and the fungal species (Jaworska and Konieczena 2001; Batista et al. 2014). These ions have also been described as important for manipulating the physicochemical properties of chitosan (Jaworska and Konieczena 2001; Shehata et al. 2018).

1.2 Extraction and Isolation

Although some authors report that there is a process of extracting chitosan from crustaceans and insects (Mohan et al. 2020; Rasweefali et al. 2021), its nomenclature is not entirely correct since extraction processes occur when the compound is taken directly from the source. Here, there is only chitin extraction, a polymer present in these organisms, crustaceans, and insects, in the approximate proportions of 20%–30% and 10%–15%, respectively (Spranghers et al. 2017).

The extraction process occurs from the fungi cell wall, where chitosan is formed from the enzymatic deacetylation of chitin residues. In these organisms, the structure of chitosan was first described in 1954 by Kreger using the X-ray technique. At that time, they analyzed the cell wall of the yeast *Phycomyces blakesleeanus* (Kreger 1954). In the nineteenth century, scientists White et al. (1979) developed a methodology that allowed chitosan isolation from fungal mycelium. Inspired by this study, several scientists started developing adaptations to improve the efficiency of this process (Synowiecki and Ali-Khateeb 1997; Hu et al. 1999). Among these, the scientist Hu et al. (1999), at the end of the twentieth century, developed an extraction protocol that is currently widely used in experiments with fungal chitosan. This protocol describes each stage to be performed from the cultivation period to specific pH values, essential data for successful extraction. Modifications in this protocol have been made to adapt the process to each strain, to the culture medium, and general conditions of the laboratory (Yuan et al. 2021; Kitozyme ®; ChiBio ®). By using the most specific extraction process for each situation, researchers can obtain a higher yield of fungal chitosan, as well as lower production costs. In this way, they intend to compete commercially with the chitosan obtained from the shell of crustaceans (Ghormade et al. 2017).

Despite the amount of chitosan extracted from the cell wall of fungi, it is difficult to exceed 20% of dry biomass, profitability considered economically viable for some companies in different countries (Kitozyme ®; ChiBio ®; InvivoGen ®; Inbiose ®). For companies, production costs versus application area have been advantageous, especially when the application area is biomedical. For this field, the chitosan produced needs to be in a high degree of purification, and the physicochemical and biological properties must be as stable as possible for the production line to be continuous. Another factor to be analyzed is the production of chemical residues generated when producing fungal chitosan and producing animal chitosan. The fungal chitosan production is considered ecologically friendly (Ghormade et al. 2017; Batista et al. 2020; Paiva et al. 2021).

1.3 Purification and Production Advanced Processes

Purification of fungal chitosan occurs, in particular, for separating residual proteins, lipids, and glucans from the matrix, and the literature frequently focuses on removing glucan residues from this matrix. These methodologies are mainly used when producing chitosan from strains not belonging to the *Zygomycetes* class (see Sect. 1.1), as is the case for *Aspergillus* sp. (Heux et al. 2000).

Chitosan obtained from *Zygomycetes* strains, in general, have proteins and lipids removed during the process of extraction by an alkali solution, and from this substrate, modifications are made to increase the yield. Naghdi et al. (2014) suggest using 0.1 N H_2SO_4 at 25 °C/30 min to remove phosphate groups before precipitating the purified chitosan from *Rhizopus oryzae*. For the chitosan obtained from *A. niger*, the suggested purification requires washing with NaOH solutions (solution 1 - 0.1 M; solution 2 - 0.5 M) and chloroform/methanol (2/1 v/v) (Heux et al. 2000).

Nevertheless, regardless of the purification treatment conducted while extracting the fungal chitosan, there is a suggestion for checking the purity of the produced chitosan, where the chitosan powder is solubilized into a solution of sulfuric acid and sodium nitrite. In this solution, chitosan is converted into 2,5-Anhydro-D-mannose and gaseous nitrogen. The percentage of conversion is an indication of the purity of the fungal chitosan (Mohammadi et al. 2012).

2 Biochemical and Biological Characteristics

Chitosan is the usual and widely studied deacetylated form of chitin, which has three distinct polymorphs. These polymorphs have been described in different species and could appear without a standard. Cabib et al. (1988) found γ-chitin while Ifuku et al. (2011) found α-chitin in the fungi cell wall. These authors described these polymorphs in different fungi species, and the fungi are capable of producing chitosan on their walls in all polymorphs.

Structurally, chitosan is a polysaccharide heteropolymer formed from the deacetylation of some glucopyranose residues present in the chitin chain (Annu et al. 2017). It has 2-acetamido-2-deoxy-D-glucopyranose and 2-amino-2-deoxy-D-glucopyranose units linked by β- (Ahmed et al. 2018; Almutairi et al. 2020; Alshubaily 2019; Alshubaily and Al-Zahrani 2019) linkage. However, this molecule is only considered chitosan when more than 50% of the polymer residues are present in the form of 2-amino-2-deoxy-D-glucopyranose (Campos-Takaki 2005; Yuan et al. 2021). It is important to mention that the 50% proportion of the number of amino residues in the chain is still controversial for some authors, who prefer to consider chitosan only proportions above 75% (Li et al. 1997).

As a result of this structure, chitosan has physical, chemical, and biological properties that define it as an advantageous polymer for the most diverse biotechnological applications.

2.1 Physicochemical Properties

Chitosan can be evaluated from different physical and chemical properties; however, the most relevant are those that best define its biotechnological application. The degree of deacetylation, molecular weight, crystallinity, thermogravimetry, and solubility are described and characterized below.

2.1.1 Degree of Deacetylation

The determination of the degree of deacetylation (DD) can be performed using different techniques, including infrared spectroscopy (FIT-IR) and proton nuclear magnetic resonance (^{1}H NMR) (Brugnerotto et al. 2001; Martínez-Camacho et al. 2010). Its measurement is essential because its value will interfere with the chemical bonds between chitosan and different adjuvants and other compounds that direct it to the area to be applied, in addition to interfering with other chemical properties (Franca et al. 2011; Yuan et al. 2021). However, the DD value is highly influenced

by the analytical method used for its measurement (Khan et al. 2002). For this reason, attention should be paid to the description of the method used when determining the DD for commercial chitosan purposes. Among the mentioned above, FIT-IR and ^{1}H NMR are the most applied techniques.

The Fourier-transform infrared spectroscopy (FT-IR) analysis is used to obtain the degree of deacetylation as a function of the applied equation (Table 3). For this technique, different researchers propose equations according to the approximate rate of amide I (A1655 – A1630), amide II (A1560), hydroxyl groups (A3450 – A3430), and presence of the groups −OH, $-NH_2$, −CO to A1320 (Dong et al. 2001; Khan et al. 2002; Brugnerotto et al. 2001).

This technique has demonstrated high safety and low cost as advantages and does not suffer interference from pH value or the degree of hydration of the sample. However, changes in the accuracy of the results have been described when chitosan from different chitin polymorphs was analyzed (Brugnerotto et al. 2001).

Another important technique for determining the degree of deacetylation is the Hydrogen Nuclear Magnetic Resonance (H-NMR) which requires expensive equipment, and the sample must be completely solubilized to avoid reading errors (Yang and Montgomery 2000). However, similarly to FIT-IR, it needs a small amount of sample without ultra-purification.

The H-NMR is indicated for chitosan characterization with a possible high degree of deacetylation once it allows a more accurate analysis comparing to FT-IR or any other technique (Dung et al. 1994). Its determination is based on NMR spectra integrals, being the most common based on the H-1 liquid state spectrum. Several equations have been described to calculate the DD, especially for the proton spectrum in the liquid state of ^{1}H-NMR (Table 4).

By using this technique, it is possible to analyze chitosan when solubilized in a deuterated solvent, which can be D2O, when chitosan is soluble in water (Rinaudo et al. 1992) or acid chemical agents when it is in its original composition: CD3COOD (acetic acid deuterium) and DCl (chloride deuterium) (Dung et al. 1994); D2O/DCl (deuterium oxide/chloride deuterium) (Lavertu et al. 2003); CD3OOD (Kumirska et al. 2010).

Through the NMR-H1 spectrum, the characteristic signals for chitosan are described in the anomeric region, with H-1 for glucosamine δ ~ 4.9 and H-1 for N-acetylglucosamine δ ~ 4.6. The remaining protons of the H-2 ring of glucosamine residues are shifted to lower values (δ ~ 3.2) because of the adjacent amino group. The characteristic signal of protons in the *N*-acetyl group, from *N*-acetylglucosamine, is at δ ~ 2.1 (Kumirska et al. 2010).

Table 3 Proposed equations for analyzing the degree of deacetylation of chitosan based on Fourier-transform infrared spectroscopy (FTIR) analysis

Equation	References
$A_{1320}/A_{1420} = 0.3822 + 0.03133$ DA	Brugnerotto et al. (2001)
[(Abs1655/Abs3450) x 100]/1.33	Domszy and Roberts (1985)
A1560/A3430 = − 0.0057 DD + 0.7375	Dong et al. (2001)
DD = 100 − [(A1655/A3450) x 115]	Baxter et al. (1992)

Table 4 The used equations to determine the degree of acetylation (DA) by analyzing the proton spectrum ^{1}H-NMR in the liquid state (Ahmed et al. 2018). ICH3(A) degree of intensity of the presence of CH3 residues, and IH2-H6 (A + D), represents the sum of proton intensities from H2 to H6; (Almutairi et al. 2020) IH1(D) represents the intensity of the deacetylated monomer, and ICH3 (A) the peak intensity of the three protons in the acetyl group. According to the author, this formula is indicated for chitosan with a degree of deacetylation above 90%; (Alshubaily 2019) IH1 (D) represents the intensity of the deacetylated monomer and IH1(A) the intensity of the acetylated monomers. According to the author, this equation is not indicated for chitosan with a high degree of deacetylation, because the acetylated peak will not be visible in the spectrum

	Equation	Reference
1	$\%DA = \{[(1/3) * I_{CH3(A)}]/[(1/6) * I_{H2-H6(A+D)}]\} * 100$	Hirai et al. (1991)
2	$\%DA = 100 - \{I_{H1(D)}/[I_{H1(D)} + (I_{CH3(A)})/3]\} * 100\}$	Lavertu et al. (2003)
3	$\%DA = 100 - [I_{H1(D)}/(I_{H1(D)} + I_{H1(A)})] * 100\}$	Lavertu et al. (2003)

In addition to presenting varying degrees of deacetylation, chitosan molecules can also be of different sizes, with oligosaccharides being the most used for biomedical applications (Shehata et al. 2018; Luo et al. 2014; Liu et al. 2017). Low weight chitosan has MW <50 kDa, medium weight has 50–150 kDa, and high weight has >150 kDa (Tavaria et al. 2013).

In a production line, the size of the chitosan molecule can change according to environmental characteristics to which the fungus is subjected (Batista et al. 2020). Another possibility for obtaining chitosan with different molecular weights is by applying physical, chemical, or enzymatic techniques to break the glycosidic bridges that connect the N-glucosamine residues. This break will result in poligomers and oligomers of different sizes. According to Benchamas et al. (2021), it is better to use these techniques when chitosan has a degree of deacetylation above 80% (DD $\geq$ 80%).

For the profitable formation of these oligosaccharides, the used technique should include environmental-friendly methods, low production cost, high degree of purification, and to be easily organizable in the production line at the industrial level (Benchamas et al. 2021). Among the proposed techniques, the one that uses enzymes has been the most adequate, but they have a high cost to be used on a large scale.

Similar to the degree of deacetylation, the molecular weight can be measured by different techniques. Through viscometry analysis, the weight can be calculated from the relation to the intrinsic viscosity. Using this technique, chitosan solutions vary in concentration from 0.25 to 6.0 g/L (w/v) in a solvent formed by 0.3 M acetic acid/0.2 M sodium acetate (Rinaudo et al. 1993). The formed solutions are passed through Ubbelohde capillary viscometer in a water bath at 25 ° C, and the molecular weight is determined according to the Mark-Houwink-Sakurada equation (Martínez-Camacho et al. 2010; Rinaudo et al. 1993):

$$[\eta] = \kappa MV^{a} \quad (1)$$

In this equation, [η] is the intrinsic viscosity, MV is the viscosity-average molecular weight, and "κ" and "a" are empirical constants that depend on the polymer, solvent, and temperature. For that reference, the value of "κ" is 3.04 × 10−5 and "a" 1.26 (Rinaudo et al. 1993).

The crystallinity of chitosan molecules was first described by Clark and Smith (1937) by measuring the structure of chitosan using X-ray diffraction and determining the density of electrons within the crystal. This density can be influenced by intramolecular hydrogen bonding and hydrogen bonds in adjacent parallel chains (Yui et al. 1994). The density is given as a percentage and measured using the parameters $\lambda = 1542$Å, with range scanning of 4°–50° in intervals of 0.02°/min (Batista et al. 2020).

According to the literature, this crystallinity varies slightly considering the total percentage of deacetylated groups present in the molecules. These deacetylated groups prevent the formation of intermolecular hydrogen bonds and break the lateral packaging of the original chitin chains (Li et al. 1997). For this reason, the crystallinity of chitosan is a factor dependent on the original chitin polymorph (Yen and Mau 2007; Kumirska et al. 2010) (Fig. 2).

Thermal gravimetric analysis (TGA) makes it possible to identify the changes that heating can cause over time in the total mass of chitosan. By using TGA, it is possible to establish the temperature range in which chitosan composition is constant, the temperature at which starts to decompose, to follow the time of the dehydration, oxidation, combustion, and decomposition reactions (Canevaloro Jr 2004). Complementing the analysis, Denari and Cavalheiro (2012) paid attention to

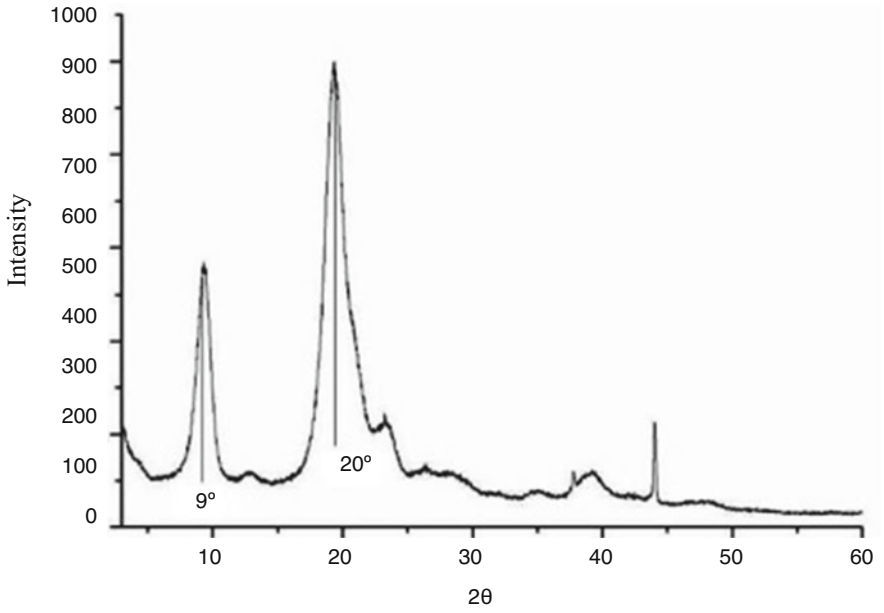


Fig. 2 X-ray diffraction spectrum analysis of chitosan extracted from *Cunninghamella elegans*. Diffractometer Shimadzu XRC6000, Kyoto, Japan, with copper tube (1 = 1.54 Å). The voltage and current used were 40KV and 30 mA, respectively. The measurements were performed in the range of 3–50 °C with a scanning rate of 1 °C/minute in steps of 0.02 °C. Here are marked characteristic points of crystallinity for chitosan. (Paiva et al. 2021)

factors that can influence the response, such as intramolecular nature, thermal conductivity, particle size, and reaction heat. The standards for the analysis vary slightly depending on the manufacturer of the equipment to be used. Approximately 10 mg of the sample is used, with a temperature variation of 0 °C–900 °C, heating rate of 10 °C/min, and under a constant flow of dry nitrogen gas (Batista et al. 2020).

Chitosan has a pKa around 6.0–6.5, characterizing this polysaccharide with a protonated amine group ($–NH_2$) when the pH of the solution is below this value. Chitosan is solubilized in acidic pH and this depends on physicochemical properties such as molecule size, degree of deacetylation, and crystallinity (Chiappisi and Gradzielski 2015). Another factor that interferes with solubility is the organization by ordering acetylated and amine residues. The greater the quantity of the amine/acetyl sequences, in that order, the greater the chain flexibility; while the higher quantity of acetyl/acetyl, acetyl/amine, and amine/amine sequences higher the stability of the chain through the interchain hydrogen bridges (Skovstrup et al. 2010).

2.2 Biological Properties

Among the chitosan biological properties mentioned, low toxicity, high biodegradability, and biocompatibility are the ones that can be modified depending on the chitosan molecular weight and degree of deacetylation (Wang et al. 2020). These properties are common regardless of the origin of the chitosan (animal or fungal) but each chitosan presents particularities. Animal chitosan has in its origin allergenic proteins associated with its structure, for example, the tropomyosin. Because of this associated molecule, a more expensive and careful purification of animal chitosan should be performed before applied in the biomedical area (Ghormade et al. 2017).

Fungal chitosan also has particularities, such as the presence of carbohydrate glucan in its wall structure; however, this molecule is not present in all fungal species. This carbohydrate has been described as a cytokine expression stimulator, among other molecules that activate the immune system in animals (Kozlowska et al. 2020). Also, when combined with chitosan, it can induce strong immune reactions. Glucan is also related to fungal infections diagnostic because it stimulates the immune system precisely (Jong et al. 2010).

The literature shows that glucan is present in species of the genus *Aspergillus* sp. These have been widely used by biotechnological companies that sell fungal chitosan and its derivatives (Heux et al. 2000; Kitozyme ®; ChiBio ®). However, there are not significant amounts of glucan in the wall of species of the class Zygomycetes (Ruiz-Herrera and Ortiz-Castellanos 2019), a widely used genus in research in Brazil for the application of chitosan in different areas (Batista et al. 2018).

3 Biotechnology Applications

Chitosan has been widely used in the most diverse biotechnological applications, as an example, in tissue engineering and the transport of medicines, due to its unique characteristics (see Sect. 2).

Table 5 Some biotechnology applications of fungal chitosan in the last 5 years in biomedical areas

Application potential	References
Antidiabetic; antioxidant; antibacterial; drug delivery system; antimycotic; antitumor	Sathiyaseelan et al. (2020), Alshubaily and Al-Zahrani (2019), Alshubaily (2019), El Rabey et al. (2019), Chien et al. (2016), Almutairi et al. (2020)

These characteristics occur mainly because chitosan has a cationic group in carbon 2 of its glucopyranose framework and because it can be of different sizes, referencing the properties of deacetylation degree and molecular weight, respectively. In the last decade, these two properties have been guiding some of the main biotechnological applications in the biomedical area (Table 5).

In addition to the different products from both chitosan (animal and fungal), there are several patents for applying these molecules in different areas of biotechnology. This fact is promising for the consumer market. Although the patents of INPI-BR (National Institute of Industrial Property), Espacenet (European Patent Office), and WIPO (World Intellectual Property Organization) are mostly on animal chitosan, we have noticed, in the last 5 years, an increase in the number of patent applications on fungal chitosan, especially for the biomedical area

These applications in the biomedical area that follows the characterizations described by Irastorza et al. (2021) as necessary for the polymer or composite include low *in vitro* immune reaction (Sathiyaseelan et al. 2020), the generation of nontoxic products (Islam et al. 2020); it can be sterilized without losing the characteristics and be commercially viable. Observing these characteristics, companies that produce fungal chitosan commercialize products for the most diverse biomedical applications, such as hemostatic dressing, health supplements, excipient for pharmaceutics products, chemotherapeutic agents, among others (Kitozyme ®; ChiBio ®).

Below we describe some of the most common applications in the scientific literature, in the last decade, considering **only fungal chitosan**.

3.1 Applications and Uses in the Pharmaceutical Industry

Studies in the pharmaceutical area are composed of highly challenging and expensive processes because when reaching the final stages of clinical trials, most drugs are unable to achieve favorable efficacy. This fact usually occurs due to the drug's inability to reach its target, leading to inadequate distribution of chemical formulations and consequently to the appearance of many side effects (Islam et al. 2017). To try to help with this problem, Yuan et al. (2021) initiated tests for the production of deuterated fungal chitosan directly from the cultivation of filamentous fungi and yeasts in a culture medium containing a deuterated carbon source. That's way it would be possible to detect chitosan along its path through the body *in vivo* tests. The use of fungal chitosan has been intensively explored by some researchers since it is possible to work with characteristics that add advantages to the entire chain of production, commercialization, and consumption of the drug.

In the pharmaceutical area, some works stand out for bringing innovations with potential for application to the consumer market. Rizeq et al. (2019) reveal a series of results that show that chitosan-based nanomaterials have been gaining notoriety due to satisfactory results. This study highlights the importance of chitosan-based nanoparticles in the delivery of drugs and genes, as well as in the therapeutic delivery for cancer, for wound healing, and as bactericidal agents. Through the data, it is possible to understand that by making some chemical modifications via hydroxyl and amino groups, new nanoparticles can be improved concerning stability and biocompatibility, increasing their effectiveness for different actions.

The interest in drug release research is due to a growing concern in the use of antibiotics in the accumulation of synthetic substances in the body, in addition to an increase in the rate of selection of microorganisms resistant to commonly used drugs. For this reason, different groups of researchers around the world have been testing chitosan as a bactericidal and fungicidal agent. Initially, there was exclusivity in the use of animal chitosan; however, in the last 5 years, there was an increase in the use of fungal chitosan as a bactericidal and fungicidal agent (Batista et al. 2011; Alshubaily and Al-Zahrani 2019).

Regarding the antimicrobial activity, four main mechanisms are proposed, regardless of the chitosan origin, since the activity has been mainly related to its degree of deacetylation and molecular weight. For the antimicrobial action to occur, some studies show a connection with the degree of deacetylation above 75% and low molecular weight (Amorim et al. 2003; Paiva et al. 2014; Martinez-Camacho et al. 2010):

1. Formation of polyelectrolyte complexes: chitosan has a positive residual surface charge present in the chain. These charges are due to the presence of an amine group that selectively binds to the cell surface of microorganisms, favoring changes in membrane permeability with consequent disruption, which may result in inhibition or cell death. This event leads to the entry of calcium ions as has been reported (Fig. 3).

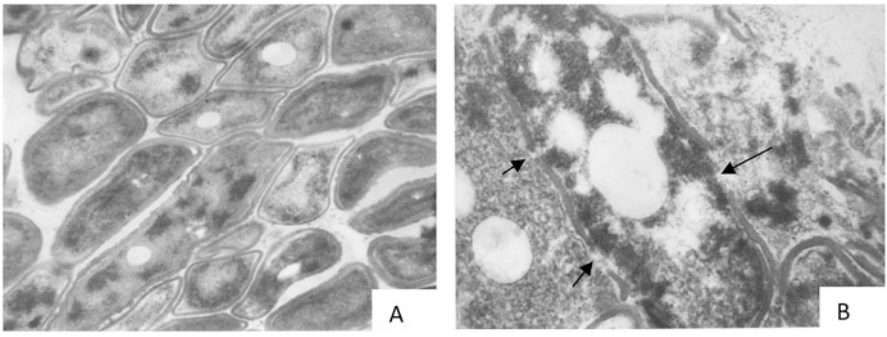


Fig. 3 Scanning electron microscopy of interaction between fungi chitosan and *Escherichia coli*. The arrows indicate rupture of cell wall. (**a**) untreated amplified 12,000x; (**b**) treated in presence of 0.5% of chitosan dissolved in 0.5% of acetic acid (v/v) 30,000x (Batista et al. 2011)

2. Chelation of ions necessary for the functioning of enzymes aimed at maintaining cellular integrity and passing information, thus, compromising cell growth.
3. Interaction of low molecular weight chitosan with microbial cell DNA and interference in mRNA activities, affecting protein synthesis.
4. The fungicidal mechanism involves penetration of chitosan molecule inside hyphae fungus body, followed by disruption of the enzyme structure, essential for fungus growth.

Along with research on antimicrobial activity, there are studies on drug delivery about the composite formed based on chitosan. Alshubaily and Al-Zahrani (2019) used the fungal chitosan extracted from *Cunninghamella elegans* to transport ceftriaxone (semi-synthetic antibiotic widely applied for treating several bacterial infections). They formed nanoconjugates by using the ionic reticulation method. Their results showed that fungal chitosan/ceftriaxone nanoparticles were crosslink and form particles with sizes of 56 nm, a charge of 54.37%, and 79.43% reticulation efficiency. Thus, scientists confirmed the efficiency of the antibacterial activity of the fungal chitosan/ceftriaxone nanocomposite against three strains of *Staphylococcus aureus* (resistant to methicillin), using disk diffusion and scanning electron microscope images.

Chitosan has also been used against fungal strains resistant to antimycotics already commercialized. For example, Alshubaily (2019) reports that chitosan nanocomposites extracted from *Aspergillus niger*, associated with costus extract (*Saussurea costus*), act against *Candida* spp. strains, especially those resistant to fungicides; El Rabey et al. (2019) used fungal chitosan extracted from *Amylomyces rouxii* conjugated with fluconazole against *Candida parapsilosis* and *Candida glabrata* strains and obtained increased antimycotic activity when using the nanoconjugate.

In addition to the efficient discoveries of the drug delivery systems and antimicrobial activity of fungal chitosan, researchers have been trying to assess the effectiveness of the antitumor (Chien et al. 2016; Almutairi et al. 2020), antioxidant, and antidiabetic (Sathiyaseelan et al. 2020) activity.

Chien et al. (2016) demonstrate a significant improvement in the anticancer activity of compounds formed from fungal chitosan compared with those from animal chitosan. Almutairi et al. (2020) produced fungal chitosan nanocomposites from *Amylomyces rouxii* and curcumin, an active substance in the Curcuma (*Curcuma longa*) that increases anticancer availability and bioactivity. It was possible to increase the antitumor activity on some types of colon cancer and human lung adenocarcinoma after 96 h of exposure to this nanocomposite. Sathiyaseelan et al. (2020) increased the antidiabetic, antioxidant, and antibacterial activity of a system that synthesized silver nanoparticles encapsulated by fungal chitosan extracted from *Cunninghamella elegans*. Also, good biocompatibility and less cytotoxicity in healthy cells have been proved compared to cancer cells.

In addition to the direct action reported by the authors above, chitosan deserves special mention because it also acts indirectly on tumor cells, and it has been reported by:

1. Increased Caspase-3 protein expression, which induces cell apoptosis (Liu et al. 2017)
2. Increased production of antioxidant enzymes, suppression of expression of pro-inflammatory cytokines, and production of nitric oxide (Nam et al. 2007)
3. Negative regulation of matrix metalloproteinase-2 (MMP-2) expression, indicating a possible suppression of the cancer cell metastasis process (Luo et al. 2014)

The enzyme immobilization technique applied in the cosmetic, food, and pharmaceutical industries is another interesting characteristic. As an example, Amorim et al. (2003) used fungal chitosan as a film to perform lipase immobilization. In this experiment, the degree of deacetylation of fungal chitosan was 88.9%. This characteristic provided a smaller number of acetamide clusters (reactive points), which favored a decrease in the molecule density and enzyme immobilization. As a result, it was found 47% of the initial catalytic activity after four reaction cycles and efficiency statistically compared to the use of animal chitosan.

3.2 Applications and Uses in Biomedical Fields

Following the advancement of research worldwide, the interest and use of fungal chitosan in medicine are also increasing. Currently, the majority of works still use animal chitosan for biomedical applications, but fungal chitosan has been gaining ground mainly because of companies producing their products in the area and selling chitosan powder for manufacturing by others (Ding et al. 2020; Kitozyme ®; ChiBio ®).

Here, it is essential to emphasize that chitosan has effective properties in the manufacture of dressings, production of hydrogels and membranes, scaffold construction, and ideal structures for cell regeneration. These frameworks are usually built for tissue engineering and have compatible physicochemical and biological properties, allowing good adhesion of new cells to them. The properties required for an effective framework include biodegradability, biocompatibility, biomineralization, porosity, expansion capacity, protein absorption, wettability, and mechanical strength, all of them present in fungal chitosan materials (Ahmed et al. 2018; Paiva et al. 2021).

Sathiyaseelan et al. (2017, 2018) revealed the potential of fungal chitosan from *Cunninghamella elegans* in the formation of nanocomposites added with Aloe vera extract to obtain a product for wound dressing applications. The nanocomposite produced showed satisfactory results against different pathogenic bacteria in vitro, and cell viability against human dermal fibroblasts (HDF cells) was proved in vitro. The results were significantly important and complemented the following year with other cell lines and associating the release of tetracycline hydrochloride antibiotic.

Also, the literature describes the importance of fungi chitosan as a catalyst in the wound healing process, stimulating inflammatory cells, macrophages, and fibroblasts, thus speeding up the inflammatory phase. Its effectiveness is even more evident in mitigating diabetic damage, as it starts the proliferative phase earlier (Matica et al. 2019).

4 Conclusion

The use of chitosan, fungal or animal, has several advantages for biotechnological processes and has been highly demanded in the biomedical industry. Even with the large-scale use of animal chitosan, the advantages of applying fungal chitosan are noticeable, especially for the biomedical area. It can be produced in an environmentally friendly way and does not require expensive purification processes. Although the presence of glucan in extracts of some filamentous fungi is reported, the literature describes members of the Zygomycetes class as low glucan and good chitosan producers.

Thus, considering the advantages and descriptions of successful applications mentioned here, we hope that new research groups will be interested in working with fungal chitosan for the biomedical area, among others.

References

Ahmed S, Annub AAA, Sheikh J. A review on chitosan centred scaffolds and their applications in tissue engineering. Int J Biol Macromol. 2018;116:849–62.

Almutairi FM, El Rabey HA, Tayel AA, et al. Augmented anticancer activity of curcumin loaded fungal chitosan nanoparticles. Int J Biol Macromol. 2020;155:861–7.

Alshubaily FA. Enhanced antimycotic activity of nanoconjugates from fungal chitosan and *Saussurea costus* extract against resistant pathogenic Candida strains. Int J Biol Macromol. 2019;141:499–503.

Alshubaily FA, Al-Zahrani MH. Appliance of fungal chitosan/ceftriaxone nanocomposite to strengthen and sustain their antimicrobial potentiality against drug resistant bactéria. Int J Biol Macromol. 2019;135:1246–51.

Amorim RVS, Melo ES, Carneiro-da-Cunha MG, et al. Chitosan from *Syncephalastrum racemosum* used as a film support for lípase immobilization. Bioresour Technol. 2003;89:35–9.

Annu SA, Ahmed S, Ikram S. Chitin and chitosan: history, composition and properties. In: Ahmed S, Ikram S, editors. Chitosan: derivatives, composites and applications. Beverly: Scrivener Publishing LLC; 2017.

Araki Y, Ito E. A pathway of chitosan formation in *Mucor rouxii*: enzymatic deacetylation of chitin. Biochem Biophys Res Commun. 1974;55:669–75.

Baker LG, Specht CA, Donlin MJ, Lodge JK. Chitosan, the Deacetylated form of chitin, is necessary for cell wall integrity in *Cryptococcus neoformans*. Eukaryot Cell. 2007;6:855–67.

Batista ACL, Dantas GC, Santos J, et al. Antimicrobial effects of native chitosan against opportunistic gram-negative bacteria. Microbiol J. 2011;1:105–12.

Batista ACL, Bandeira MGL, Souza Neto FE, et al. Obtenção de quitosana fúngica crescida em meio alternativo constituído com farinha de carapaça de camarão. Revista Saúde e Ciência On Line. 2014;3:11–7.

Batista ACL, Souza Neto FE, Paiva WS. Review of fungal chitosan: past, present and perspectives in Brazil. Polímeros [online]. 2018;28:275–83.

Batista ACL, Melo TBL, Paiva WS. Economic microbiological conversion of agroindustrial waste to fungi chitosan. Acad Bras Cienc. 2020;92:e20180885.

Baxter A, Dillon M, Taylor K, et al. Improved method for i.r. determination of the degree of N-acetylation of chitosan. Int J Biol Macromol. 1992;4:166–9.

Benchamas G, Huang G, Huang S, et al. Preparation and biological activities of chitosan oligosaccharides. Trends Food Sci Technol. 2021;107:38–44.

Brugnerotto J, Lizardi J, Goycoolea FM, et al. An infrared investigation in relation with chitin and chitosan characterization. Polymer. 2001;42:3569–80.
Cabib E, Bowers B, Sburlati A, et al. Fungal cell wall synthesis: the construction of a biological structure. Microbiol Sci. 1988;5:370–5.
Campos-Takaki GM. The fungal versatility on the copolymers chitin and chitosan production. In: Dutta PK, editor. Chitin and chitosan opportunities and challenges. Allahabad: Dubey Printers and Graphics; 2005. p. 69–94.
Campos-Takaki GM, Beakes GW, Dietrich SM. Electron microscopic X-ray microprobe and cytochemical study of isolated cell walls of mucoralean fungi. Trans Br Mycol Soc. 1983;80: 536–41.
Canevaloro SV Jr. Técnicas de Caracterização de Polímeros. ArtLiber Associação Brasileira de Polímeros. SP: São Carlos; 2004.
Chiappisi L, Gradzielski M. Co-assembly in chitosan–surfactant mixtures: thermodynamics, structures, interfacial properties and applications. Adv Colloid Interf Sci. 2015;220:92–107.
Chien RC, Yen MT, Mau JL. Antimicrobial and antitumor activities of chitosan from shiitake stipes, compared to commercial chitosan from crab shells. Carbohydr Polym. 2016;138:259–64.
Clark GL, Smith AF. X-ray diffraction studies of chitin, chitosan and derivatives. J Phys Chem. 1937;40:863–79.
Davis LL, Bartinicki-Garcia S. Chitosan synthesis by the tandem action of chitin synthetase and chitin deacetylase from *Mucor rouxii*. Biochemist. 1984;23:1065–73.
Denari GB, Cavalheiro ETG. Princípios e aplicações de análise térmica. São Carlos: IQSC/ USP; 2012.
Ding S, Wang Y, Li J, et al. Progress and prospects in chitosan derivatives: modification strategies and medical applications. J Mater Sci Technol. 2020; https://doi.org/10.1016/j.jmst.2020.12.008.
Domszy JG, Roberts G. Evalution of infrared spectroscopic techniques for analyzing chitosan. Macromol Chem Phys. 1985;186:1671–7.
Dong Y, Xu C, Wang J, et al. Determination of degree of substitution for N-acylated chitosan using IR spectra. Sci China Ser B. 2001;44:216–24.
Dung P, Milas M, Rinaudo M, et al. Water soluble derivatives obtained by controlled chemical modifications of chitosan. Carbohydr Polym. 1994;24:209–14.
El Rabey HA, Almutairi FM, Alalawy AL, et al. Augmented control of drug-resistant Candida spp. via fluconazole loading into fungal chitosan nanoparticles. Int J Biol Macromol. 2019;141:511–6.
Fesel PH, Zuccaro A. β-glucan: crucial component of the fungal cell wall and elusive MAMP in plants. Fungal Genet Biol. 2016;90:53–60.
Franca EF, Freitas LCG, Lins RD. Chitosan molecular structure as a function of N-acetylation. Biopolymers. 2011;95:448–60.
Ghormade V, Pathan EK, Deshpande MV. Can fungi compete with marine sources for chitosan production? Int J Biol Macromol. 2017;104:1415–21.
Heux L, Brugnerotto J, Desbrieres J, et al. Solid state NMR for determination of degree of acetylation of chitin and chitosan. Biomacromolecules. 2000;1:746–51.
Hirai A, Odani H, Nakajima A. Determination of degree of deacetylation of chitosan by 1H NMR spectroscopy. Polym Bull. 1991;26:87–94.
Hu KJ, Yeung KW, Ho KP, et al. Rapid extraction of high-quality chitosan from mycelia of *Absidia glauca*. J Food Biochem. 1999;23:187–96.
Ifuku S, Nomura R, Morimoto M, et al. Preparation of chitin nanofibers from mushrooms. Materials. 2011;4:1417–25.
Irastorza A, Zarandona I, Andonegi M, et al. The versatility of collagen and chitosan: from food to biomedical applications. Food Hydrocoll. 2021; https://doi.org/10.1016/j.foodhyd.2021.106633.
Islam S, Bhuiyan MAR, Islma MN. Chitin and chitosan: structure, properties and applications in biomedical engineering. J Polym Environ. 2017;25:854–66.
Jaworska MM, Konieczna E. The influence of supplemental components in nutrient medium on chitosan formation by the fungus Absidia orchidis. Appl Microbiol Biotechnol. 2001;56:220–4.

Jeraj N, Kunic B, Lenasi H, et al. Purification and molecular characterization of chitin deacetylase from Rhizopus nigricans. Enzym Microb Technol. 2006;39:1294–9.
Jong MAWP, Vried LEM, Theelen B, et al. C-type lectin Langerin is a β-glucan receptor on human Langerhans cells that recognizes opportunistic and pathogenic fungi. Mol Immunol. 2010;47: 1216–25.
Khan TA, Peh KK, Ch'ng HS. Reporting degree of deacetylation values of chitosan: the influence of analytical methods. J Pharm Pharmacol. 2002;5:205–12.
Kozlowska E, Brzezinska-Blaszczyk E, Rasmus P, et al. Fungal β-glucans and mannan stimulate peripheral blood mononuclear cells to cytokine production in Syk-dependent manner. Immunobiology. 2020;225:151985.
Kreger DR. Observations on cell walls of yeast and some other fungi by X-ray diffraction and solubility tests. Biochim Biophys Acta. 1954;13:1–9.
Kumirska J, Czerwicka M, Kacznski Z, et al. Application of spectroscopic methods for structural analysis of chitin and chitosan. Mar Drugs. 2010;8:1567–636.
Lavertu M, Xia Z, Serreqi AN, et al. A validated 1H NMR method for the determination of the degree of deacetylation of chitosan. J Pharm Biomed Anal. 2003;32:1149–58.
Li J, Revol JF, Marchessault RH. Effect of degree of deacetylation of chitin on the properties of chitin crystallites. J Appl Polym Sci. 1997;65:373–80.
Liu L, Xin Y, Liu J, et al. Inhibitory effect of chitosan oligosaccharide on human hepatoma cells in vitro. Afr J Tradit Complement Altern Med. 2017;14:272–7.
Luo Z, Dong X, Ke Q, et al. Downregulation of CD147 by chitooligosaccharide inhibits MMP-2 expression and suppresses the metastatic potential of human gastric cancer. Oncol Lett. 2014;8: 361–6.
Martínez-Camacho AP, Cortez-Rocha MO, Ezquerra-Brauer JM. Chitosan composite films: thermal, structural, mechanical and antifungal properties. Carbohydr Polym. 2010;82:305–15.
Matica MA, Aachmann FL, Tøndervik A, et al. Chitosan as a wound dressing starting material: antimicrobial properties and mode of action. Int J Mol Sci. 2019;20:5889.
Mohammadi M, Zamani A, Karimi K. Determination of glucosamine in fungal cell walls by high-performance liquid chromatography (HPLC). J Agric Food Chem. 2012;60:10511–15.
Mohan K, Ganesan A, Muralisankar T, et al. Recent insights into the extraction, characterization, and bioactivities of chitin and chitosan from insects. Trends Food Sci Technol. 2020;105:17–42.
Naghdi M, Zamani A, Karimi K. A sulfuric-lactic acid process for efficient purification of fungal chitosan with intact molecular weight. Int J Biol Macromol. 2014;63:158–62.
Nam KS, Kim MK, Shon YH. Inhibition of proinflammatory cytokine induced invasiveness of HT-29 cells by chitosan oligosaccharide. J Microbiol Biotechnol. 2007;17:2042–5.
Paiva WS, Souza Neto FE, Batista ACL. Avaliação da atividade antibacteriana da quitosana fúngica. Perspect Online, Biol Saúde. 2014;13:37–43.
Paiva WS, Souza Neto FE, Batista ACL. Characterization of polymeric biomaterial chitosan extracted from *Rhizopus stolonifer*. J Polym Mater. 2017;34:115–21.
Paiva WS, Souza Neto FE, Paiva ES, Batista ACL. Fungal chitosan as membranous material modified by atmospheric plasma. Res Soc Dev. 2021;10:9210111543.
Rasweefali MK, Sabu S, Sunooj KV, et al. Consequences of chemical deacetylation on physico-chemical, structural and functional characteristics of chitosan extracted from deep-sea mud shrimp. Carbohydr Polym Technol Appl. 2021;2:100032.
Rinaudo M, Le Dung P, Gey C, et al. Sustituent distribution on O,N-carboxymethylchitosa by 1H and 13C n.m.r. Int J Biol Macromol. 1992;14:122–8.
Rinaudo M, Milas M, Le Dang P. Characterization of chitosan: influence of ionic strength and degree of acetylation on chain expansion. Int J Biol Macromol. 1993;15:281–5.
Rizeq BR, Younes NN, Rasool K, et al. Synthesis, bioapplications, and toxicity evaluation of chitosan-based nanoparticles. Int J Mol Sci. 2019;20:5776–800.
Ruiz-Herrera J, Ortiz-Castellanos L. Cell wall glucans of fungi. A review. Cell Surf. 2019;5:100022.

Sathiyaseelan A, Shajahan A, Kalaichelvan PT. Fungal chitosan based nanocomposites sponges – an alternativemedicine for wound dressing. Int J Biol Macromol. 2017;104:905–1915.

Sathiyaseelan A, Saravanakumar K, Mariadoss AVA, et al. Biocompatible fungal chitosan encapsulated phytogenic silver nanoparticles enhanced antidiabetic, antioxidant and antibacterial activity. Int J Biol Macromol. 2020;153:63–710.

Shehata AN, El Aty AA, Darwish DA, et al. Purification, physicochemical and thermodinamic studies of antifungal chitinase with production of bioactive chitosan-oligosaccharide from newly isolated Aspergillus griseoaurantiacus KX010988. Int J Biol Macromol. 2018;107:990–9.

Skovstrup S, Hansen SG, Skrydstrup T. Conformational flexibility of chitosan: a molecular modeling study. Biomacromolecules. 2010;11:3196–207.

Spranghers T, Ottoboni M, Klootwijk C, et al. Nutritional composition of black soldier fly (*Hermetia illucens*) prepupae reared on different organic waste substrates. J Sci Food Agric. 2017;97:2594–600.

Synowiecki J, Ali-Khateeb NAAQ. Mycelia of *Mucor rouxii* as a source of chitin and chitosan. Food Chem. 1997;60:605–10.

Tavaria FK, Costa EM, Pina-Vaz I, et al. A quitosana como biomaterial odontológico: estado da arte. Braz J Biom Eng. 2013;29:110–20.

Wang W, Xue C, Mao X. Chitosan: structural modifications, biological activity and application. Int J Biol Macromol. 2020;164:4532–46.

White SA, Farina PR, Fulto I. Production and isolation of chitosan from *Mucor rouxii*. Appl Environ Microbiol. 1979;38:323–8.

Yang BY, Montgomery R. Degree of acetylation of heteropolysaccharides. Carbohydr Res. 2000;323:156–62.

Yen MT, Mau JL. Selected physical properties of chitin prepared from shiitake stipes. LWT–Food Sci Technol. 2007;40:558–63.

Yuan Y, Li H, Leite W, et al. Biosynthesis and characterization of deuterated chitosan in filamentous. Carbohydr Polym. 2021;257:117637.

Yui T, Imada K, Okuyama K. Molecular and crystal structure of the anhydrous form of chitosan. Macromolecules. 1994;27:7601–5.

An Insight into Pullulan and Its Potential Applications

13

C. Nagendranatha Reddy, Bishwambhar Mishra, Sanjeeb Kumar Mandal, Dinesh Chand Agrawal, and Chandana Kruthiventi

Contents

C. N. Reddy · B. Mishra (✉) · S. K. Mandal · C. Kruthiventi
Department of Biotechnology, Chaitanya Bharathi Institute of Technology, Hyderabad, Telangana, India
e-mail: nagendranath_biotech@cbit.ac.in

D. C. Agrawal
Department of Biotechnology, Faculty of Engineering and Technology, Rama University, Kanpur, Uttar Pradesh, India

J. M. Oliveira et al. (eds.), *Polysaccharides of Microbial Origin*,
https://doi.org/10.1007/978-3-030-42215-8_15

Abstract

Pullulan is the one of the most potent biocompatible polymer, which is basically synthesized by the *Aureobasidium pullulans*. This polymer appears to be a linear α-glucan of maltotriose units with occasional branching of glucosyl or maltosyl substitution. The employment and application of pullulan in biomedical and tissue engineering field is emerging owing to its biocompatible, nontoxic, non-immunogenic, and inert nature. It can be derivatized via various chemical reactions to increase its utility in the field of pharmaceuticals. In addition, pullulan and its derivatives have photographic, lithographic, and electronic applications in the biomedical instrumentation. This chapter provides comprehensive information about "pullulan" considering its microbial sources, biosynthesis aspects, characterization, and functionalization. It also highlights the various applications of pullulan and its derivatives in the pharmaceutical and biomedical fields.

Keywords

Polysaccharide · Pullulan · *Aureobasidium pullulans* · Surface modification · Application

1 Introduction

Pullulan is a microbial exopolysaccharide, which is synthesized by yeast like fungus *Auereobacidium pullulans* also known as Black yeast. Pullulan was first isolated by Bernier (1958) but Bauer (1983) made observations on extracellular polymer formation by *A. pullulans.* This glucan like exopolysaccharide was named as pullulan by Bender et al. (1959). Later in the 1960s there were several experiments and studies about this novel exopolysaccharide and they characterized to discover its structure. Pullulan is a linear homopolysaccharide and is described as α (1, 6) linked maltotriose units. The chemical formula of pullulan was given as $(C_6H_{10}O_5)_n$ by the elemental analysis. The structure of pullulan has been illustrated by Fig. 1. In the early 1960s, scientists discovered that pullulan is a linear α -D glucan possessing (1, 4) and (1, 6) linkages in a ratio of 2:1 due to which it is highly flexible (Mishra and Varjani 2019). Unlike most of other microbial dextrans, which are synthesized extracellularly, pullulan is synthesized intracellularly and released out. The development of pullulan occurs as the cell type shifts from mycelia to unicellular form, with the highest development corresponding to the yeast form.

Studies have reported that there are many potential applications of pullulan and its good film-forming properties. Pullulan can form transparent thin films, which are oil resistant and impermeable to oxygen. It can also be used as a coating, packaging

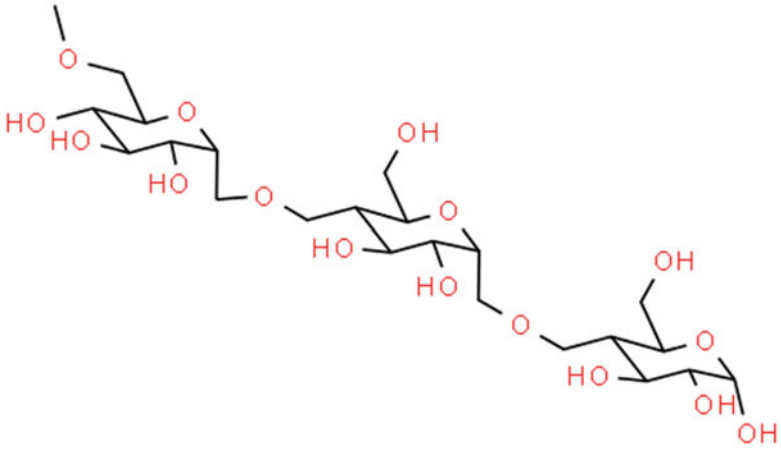


Fig. 1 General structure of pullulan, where three glucose units are connected by an α-1,4 glycosidic bond and consecutive maltotriose units are connected by α-1,6 glycosidic bond

material, and as a sizing agent for paper. Pullulan-coated papers also decompose easily and do not contaminate the environment. In low-calorie food formulations, it can be used as a starch replacer, which is expected to be efficient. There are various applications such as it is used in cosmetic emulsions and in other industries. In addition, its adhesive properties may be used as binders and stabilizers in food pasta, denture adhesives, and tobacco and as adhesives to attach nuts to cookies addition. Because of its water retention and film-forming properties, it can result in an interesting moisturizing and protective ingredient for cosmetic lotions, powders, facial packs and protective packs, hair wash director, and hair dressings. Pullulan, therefore, being resistant to mammalian amylase and providing low calories, is used to manufacture low-calorie food products as a substitute for starch and acts as a prebiotic to encourage the growth of beneficial bifido bacterium (Mishra et al. 2011). Pullulan used as an edible coatings helps to delay dehydration and respiration, improves the quality of the food matrix, retains volatile flavor compounds, and reduces microbial activity. Active films can also interact with food and the environment, enhance quality and safety, and absorb and/or release compounds. Examples of active films include those that act as oxygen and carbon dioxide scavengers, moisture absorbers, gas emitters, and gas absorbers. Some of the most innovative, active films are those designed to control antimicrobial release. Pullulan is suitable for all consumer groups because of its nonanimal origin nature. It is a slow digesting macromolecule, which is colorless, odorless and hence it is used as a very low calorie food additive. This is used as a texturizer and glazing agent in chewing gum and bubble gum. It is also used as foaming agent in milk-based desserts (Mishra et al. 2016).

Despite a large number of valuable applications, the major constraint prevailing on the use of pullulan is its cost, which is three times higher than the price of other polysaccharides such as dextran and xanthan. Engineering innovations or improved

production strains, particularly with reduced melanin production could be beneficial to improve the economics of the production, thereby opening new avenues for pullulan utilization. The average molecular weight of pullulan ranges between 4.5×10^4 and 6×10^5 Da and are greatly affected by cultivation parameters. The molecular weight distribution and average molecular weight are critical for pullulan's bioactivities, such as immune-modulatory activity and chemical releasing capability (Shu et al. 2007). Although many researchers studied regarding pullulan biosynthesis, the mechanism is still unclear. The employment and application of pullulan in biomedical and tissue engineering field is emerging owing to its biocompatible, nontoxic, non-immunogenic and inert nature. As comparison to dextran, the degradation rate of pullulan in blood serum is much quicker. The microbial pullulan act as the promising biomaterial that is currently used for the packaging of readily oxidized food materials, controlled drug delivery, tissue engineering, and can function as artificial molecular chaperones. In addition, pullulan and its derivatives have photographic, lithographic, and electronic applications in the biomedical instrumentation.

This chapter gathers comprehensive information about "pullulan" considering its microbial sources, biosynthesis aspects. It also discusses about the characterization, and functionalization of pullulan. In the last part, it describes various applications of pullulan and its derivatives in the pharmaceutical and biomedical fields (Mishra et al. 2011).

2 Physicochemical Properties of Pullulan

Pullulan is a water soluble exopolysaccharides, which acts like a model to study the behavior of some aqueous polysaccharides. Dry pullulan is white to off-taste, odorless powder, which forms a viscous nonhygroscopic solution when dissolved in water at 5–10%. It starts to decompose at 250 °C and chars at 280 °C. Pullulans are insoluble in organic solvents dimethylsulfoxide and formamide and is hygroscopic in nature. It has low viscosity when compared to other polysaccharides and it has a stable aqueous form. As it is soluble in water, pullulan can be used as drug carrier and helps in controlled release of drug in plasma. The molecular weight of pullulan was estimated to be in range of 5000–9,000,000 g/mol with straight unbranched chain. Later, the solution properties were studied by photon correlation spectroscopy (PCS), partial specific volume, intrinsic viscosity, and sedimentation equilibrium measurements. These were carried out on eight fractions of pullulan with different molecular weights and narrow molecular weight distributions. Physical properties and some characteristics of pullulan are summarized in (Table 1) as per the information available in the literatures (Singh and Saini 2008).

The solution of pullulan with water remains unaffected by pH changes and heat. This property made its applications as plasma substitute in blood. The mechanical properties and rheological properties of pullulan solution is a function of molecular weight. The glossiness of the film made up of pullulan also depends upon these rheological variations. In many cases, pullulan is given preferences over dextran because of its branching pattern and presence of α-1,6-linked glucoses exclusively. These α-1,6-linked bonds make a randomly coiled structure in the pullulan structure

Table 1 Physical properties and characterization of pullulan

S.L No.	Parameters	Specifications
1	Solubility	High solubility in water, not soluble in organic solvents, soluble mainly in ethanol, substitution with ether and ester make pullulan insoluble in water and soluble in organic solvent
2	Appearance and properties	Dry white powder, tasteless and odorless, nontoxic, edible
3	pH	5.0–7.0
4	Film forming	Low oxygen permeability, thermally stable, dissolves fast in water
5	Viscosity	Stable viscous solution, low viscosity compared to other polysaccharides
6	Biodegradability	Degraded by microbial enzymes, pullulan and isopullulan fermented to short chain fatty acids by bacteria, degradation faster than other dextran
7	Adhesiveness	Intensively adhesive, adheres to foods
8	Moisture retention	10–15% moisture content

and extremely flexible polymer. The flow property of the polymeric solution of pullulan always depends upon the molecular weight. Xiao et al. (2012) had reported about the Newtonian-like flow behavior of pullulan at low concentration less than 4%. When this solution is mixed with other polysaccharides, it exhibits viscoelastic properties. Recently, some researchers could enhance the electro-spinnability of materials by decreasing electrical conductivity and surface tension (Soto et al. 2019). A dynamic rheological behavior is also found in pullulan solutions and its blending mixture with alginate. Here, the blend was found to exhibit average relaxation time and longer projecting inter-chain network formation. One of the very peculiar characters is the oxidation of pullulan in various conditions. Due to the Coulombic repulsion, it behaves like polyelectrolyte in its dilute solution. As a result, swelling of polymer chain was induced. This was evident from an increased viscosity upon dilution (a typical polyelectrolyte like behavior), attributable to increased coil dimension (Table 2).

The flow property of pullulan can be calculated by applying the following formula.

$$t = k\gamma^{n}$$

Where τ is the shear stress (Pa), γ the shear rate (s^{-1}), k the flow consistency index (Pa s^{n}), and n is the flow behavior index. It was found that, when the concentration of pullulan solution is lower than 8% w/v, it possesses a Newtonian behavior. In contrast, it possesses pseudoplastic behavior at its concentration more than 8% w/v. The major cause of pseudoplastic behavior is due to separation and alignment of exopolymers from each other in a single pullulan unit in a shear field. This makes a decrement of viscosity of the pullulan solution and eventually come to a constant value.

Table 2 Summary of pullulan Infrared (IR) Spectroscopy data

Reference	IR-Spectroscopy Assignment of Pullulan								
	O H stretching	C H stretching	O C-O stretching	C O-H bending	C O-C stretching	C O-C stretching	α D-Glucopyran-ose	α-(1,6) Glycosidic linkage	α- (1,4) Glycosidic linkage
Wang et al. (2014)	3283.94	2922.79	1648.38	1363.21	NA	1013.46	851.48	931.99	755.63
Sugumaran et al. (2013)	3388	2911	1656	1395	1177	1090	848	968	795
Singh et al. (2009)	3391.7	2926	1648.1	1368.6	1156.7	1024.8	851.4	NA	NA
Cheng et al. (2010)	3431	2928	NA	1645–1650	1154–1160	NA	850	929	755
Singh et al. (2009)	3369.2	2925.1	1655.1	1370.1	1156.3	1020.7	851.8	1018.64	753.7
Choudhury et al. (2011)	3397.9	2927.9	1653.1	1418.4	1154.7	NA	847.5	1018.7	755.3
Thirumavalavan et al. (2009)	3386	2929	1654	1342	1125	1032	860	NA	NA
Mishra et al. (2019)	3448	2926	1641	1384	NA	NA	867.97	992	779.24

The emulsifying activity (EA) and emulsion stability (ES) can be calculated by using the following formula

$$\text{EA}\ (\%) = (\text{Volume of the emulsion phase}/\text{Total volume of system}) \times 100$$
$$\text{ES}\ (\%) = (\text{The remaining emulsified layer volume}/\text{The initial emulsified layer volume}) \times 100$$

The emulsifying activity of pullulan is 58% at 25 °C. The emulsion stability for pullulan is more stable (88%) at low temperature. The emulsifying capability of pullulan is due to the presence of peptide molecules in the culture medium obtained along with the pullulan.

3 Fermentative Production of Pullulan

The industrial-scale production of pullulan via the process of fermentation is done by utilizing specific strains that are nonpathogenic under particular environments (Table 3).

3.1 Microbial Sources of Pullulan

One among the widely used strains in commercial scale for production of pullulan is *Aureobasidium pullulans* because of its maximum yield and better properties of produced pullulan. *A. pullulans* is a yeast-like polymorphic fungus that occurs in all environments like soils, fresh water, wood, rock, and tissues of animals and plants. It is pathogenic to plants while nonpathogenic to humans, but there are few strains of

Table 3 Comparison of molecular weight of pullulan biosynthesized by various strains of *A. pullulans* with respect to different types of carbon and nitrogen sources

S.L No	Source organism	Carbon source	Nitrogen source	pH	Molecular weight	Reference
1	*A. pullulans* SZU 1001	Glucose	Yeast extract and $(NH_4)_2SO_4$	6.8	5.74×10^6	Yu et al. (2012)
2	*A. pullulans* CCTCC M 2012259	Glucose	Yeast extract and $(NH_4)_2SO_4$	6.8	3.09×10^6	Zan and Zou (2013)
3	*A. pullulans* NCIM 1049	Jack fruit seed	Yeast extract and $(NH_4)_2SO_4$	NA	1.17×10^6	Sugumaran et al. (2013)
4	*A. pullulans* CJ001	Sucrose	$(NH_4)_2SO_4$ and yeast extract	5.5	2.7×10^5	Chen et al. (2012)
5	*A. pullulans* MTCC2670	Asian palm kernel	Sodium nitrite	3.2	8.44×10^6	Sugumaran et al. (2014)
6	*A. pullulans* MTCC2670	Cassava bagasse	Sodium nitrite	3.8	9.8×10^6	Sugumaran et al. (2014)

A. pullulans that can cause pathogenic effect and cause health adversities (Singh et al. 2019). The isolates of *A. pullulans* produce various enzymes such as amylases, proteases, esterases, pectinases, hemicellulases, etc. (Singh and Saini 2008). The growth cycle of *A. pullulans* was investigated to report the production of pullulan from hyphal cells, blastospores in submerged fermentation, etc. (Singh et al. 2019). Chi et al. (2009) has reported the significance of *A. pullulans* in various engineering fields and its utilization of biological metabolites. Few studies illustrated the production of nonidentical pullulans (composition and structure) by various *A. pullulans* strains. *A. pullulans*, apart from polysaccharide pullulan, produces a dark pigment called melanin, which in turn provides microbial resistance to phagocytosis in the host and also produced discoloration of the polysaccharide. There are various physicochemical techniques (adsorption using activated charcoal, solvents, and salts) for removal of melanin from the fermented media but the cost should be taken into consideration. Hence, to decrease capital investment, the strains have to be mutated or metabolism should be engineered or new strains have to be identified. However, care has to be taken to retain the ability of strain to produce pullulan with good quality of viscosity, distribution of molecular weight, and other physical properties. Apart from *A. pullulans*, the other potent strains capable of producing pullulan are *Rhodosporidium paludigenum*, *Aspergillus japonicus*, *Rhodotorula bacarum*, *Teloschistes flavicans*, *Cryphonectria parasitica*, *Cyttaria darwinii*, *Cyttaria harioti*, and *Tremella mesenterica*. The pullulan produced from different microorganisms and species may vary because of diverse proportions of component glycosidic linkages (1:1 and 2:1) and presence of maltotriose and maltotetraose sequences in the polysaccharide backbone (Singh and Saini 2008).

In order to improve the pullulan productivity by *A. pullulans*, the strain has to be studied by performing mutation and also by metabolic engineering. Few studies reported the production of enhanced productivity of pullulan, higher molecular weight pullulan, reduced pigmentation, etc. Apart from *A. pullulans*, other strains such as *Aspergillus japonicus*-VITSB1, *Aureobasidium mousonni* (NCIM 1226), etc., were mutated by using UV rays and Ethyl methane sulfonate (EMS) mutagenesis for high yield and superior quality of pullulan (Mishra and Suneetha 2014). For metabolic engineering, the different factors, viz., ATP/ADP ratio, integrating desired gene into genomic DNA, knock out of PKSIII (Polyketide Synthase III) gene, etc., were reported to increase the pullulan productivity and reduce melanin production (Haifeng et al. 2016).

3.2 Mechanism of Pullulan Synthesis

Pullulan is a highly hydrated exopolysaccharide layer produced by microbes in order to protect themselves from desiccation, predators, and aid in diffusion. Pullulan is produced inside the cell and it is secreted into the medium through β-glucan layer as loose, slimy, and amorphous layer. The precursor's production in the microbe will increase the rate of pullulan formation. Biochemically, pullulan comprises maltotriose units connected by α-1,4glycosidic bond, whereas consecutive maltotriose units are

connected to each other by α-1,6 glycosidic linkages. The linkage has significant properties of structural flexibility and enhanced solubility of pullulan (Dailin et al. 2019). Pullulanase, an enzyme, acts on the α-1,6 glycosidic linkages and hydrolyzes them converting to maltotriose. Hence, pullulan is considered as a linear polysaccharide with maltotriose units connected by α-1,6-linkages.

The pullulan biosynthesis in *A. pullulans* is a multistep biochemical reaction. The biosynthesis of pullulan is done through arbitration of sugar-nucleotide-lipid carrier intermediates related with the cell membrane portion. The exact mechanism of pullulan biosynthesis has not been properly implicit due to the intricate characteristics of the microbe that produces pullulan. The buildup of carbohydrate remains in the cell facilitates pullulan production during the early phases of fermentation. The key enzymes involved in the production of pullulan are α-phosphoglucomutase, uridine diphosphate glucose pyrophosphorylase (UDPG-pyrophosphorylase), and glucosyltransferase. Various carbon sources extending from simple to multifaceted substrates are utilized for the synthesis of pullulan by *A. pullulans*. Pullulan can be produced from carbon source (sucrose) with participation of respective enzymes, ATP and UPDG. α-phosphoglucomutase and UDPG-pyrophosphorylase are the crucial enzymes that convert the simple carbon (glucose) to UDP-glucose, which is a key precursor for pullulan production. The enzyme hexokinase aids in the formation of glucose-6-phosphate from glucose, which in turn is transformed to glucose-1-phosphate by α-phosphoglucomutase enzyme. The formed glucose-1-phosphate is transformed to UDP-glucose by the help of enzyme UDPG-pyrophosphorylase. The glucosyl 1-phosphate attained from UDP-glucose binds with lipid hydroperoxide thereby yielding Phosphodiester Bridge. The D-glucose present in UDP-glucose combines with other glucose units resulting in the production of isomaltosyl residue. The biochemical reaction occurs between the isomaltosyl and lipid-linked glucose to form isopanosyl residue, which will further yield the pullulan polysaccharide by the polymerization of isopanosyl residues with the help of glucosyltransferase enzyme (Sugumaran and Ponnusami 2017). The overall biosynthesis process is classified into three main stages: formation of phosphodiester bond from UDP-glucose, formation of isomaltose units, and isopanosyl molecule production in the last stage, which further forms pullulan chains.

The different strains of *A. pullulans* behave differently with respect to medium provided. The two varieties of *A. pullulans,* viz., var. pullulans and var. aubasidani showed significant changes in molecular features, nutritive composition, and exopolysaccharide structures synthesized by them. The nitrogen source, ammonium sulfate, will stimulate the synthesis of pullulan by *A. pullulans* var. pullulans, while aubasidani is synthesized by *A. pullulans* var. aubasidsani with sodium nitrate as optimal nitrogen source.

3.3 Upstream and Downstream Processing for Pullulan

The upstream processing concern with the fermentation of pullulan, takes care of early fermentation process, viz., microbial strain selection, inoculum preparation,

media and process parameters optimization, oxygen requirement, cell morphology, inducers and inhibitors, growth kinetics, etc., to achieve maximum product yield. There are multiple parameters that directly impact the pullulan biosynthesis. The process parameters and fermentation broth components need to be optimized with respect to different strains of *A. pullulans* used as the seed as they vary substantially with regard to their growth, yield, and cell morphology. The fermentation media's high viscosity and pigmentation (melanin) are the two hindrances faced during the process of pullulan biosynthesis. During the process of submerged fermentation, the characteristics of liquid media changes significantly as the change in viscosity is observed. Primarily, the media trails the Newtonian behavior with low viscosity (close to pure water), which further changes to Non-Newtonian due to upsurge in viscosity as the exopolysaccharide is produced (Singh and Saini 2008).

At the end of fermentation, the resulting media contains polymer that is produced and released outside the cell, cells and related debris, unutilized residual media components, etc. In order to recover the polymer of interest with high purity and quality, numerous downstream processing steps, viz., dilution, biomass separation, separation of exopolysaccharide from melanin and other proteins, precipitation, purification, freeze drying, etc., have to be followed with proper care. Biomass separation from the fermented media can be done using the cross-flow membrane filtration or by centrifugation (30 min; 4 °C, and 10,000 rpm). The metabolites recovery is considerably persuaded by time of leaching, agitation speed, temperature and pH, solvent to solid weight ratio, etc. Melanin is eliminated using activated charcoal, salt, and solvent addition and treating with H_2O_2. The issue of melanin separation can be eliminated by regulating the metabolism of the microbe or by using the strains that are modified to produce no melanin or comparatively less melanin. Pullulan recovery can be done by following the precipitation by using the organic solvents. The precipitation of pullulan from the solution is done by the solution of organic solvent and supernatant in the volume ratios 2:1 to 3:1. The pullulan recovered can be further purified by using resins (ion exchange) and filtration (ultrafiltration). The harvested pullulan will be dried in an oven at 80 °C and mechanically ground into powder for further application (Fig. 2).

3.4 Factors Influencing Pullulan Production

The pullulan fermentation process is influenced by various media and other process parameters. The factors that may influence the effective performance of fermentation process for enhanced productivity of pullulan are fermentation media composition, fermentation type and time, configuration, bioreactor design, microbial consortia, moisture content, particle size, cell morphology, operational temperature, pH, light intensity, oxygen profile, etc. Some of these parameters that can influence the overall process are summarized underneath and given in the Fig. 3.

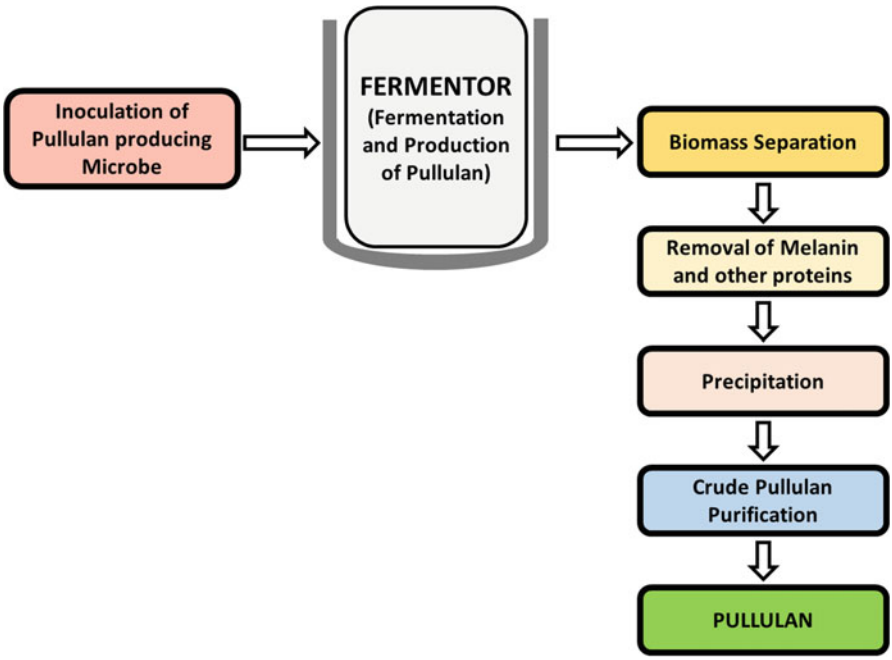


Fig. 2 Schematic representation of pullulan downstream processing

3.4.1 Fermentation Media Supplements

Carbon Source

Pullulan can be produced from various sources of carbon ranging from simple to complex substrates. In order to cut down the cost, waste material is used as effective substrate for synthesis of pullulan. The strain *A. pullulans* is capable of growing on various substrates including different types of wastes, viz., agricultural, coconut by-product, food industry, jaggery, hydrolyzates of potato starch, peat hydrolysate, starch, olive oil, carob pod, rice hull hydrolysate, etc. The yield of pullulan is directly proportional to substrate conversion rate. The amount of pullulan produced is directly proportional to the type of substrate used. Sucrose, as carbon source, has been extensively studied as it produces higher yields when compared to other sources. While the medium with xylose or lactose depicts lower cell activity along with low pullulan productivity. With maltose as a carbon substrate, the wild fungi propagated rigorously but the pullulan produced was extensively low. Excess glucose (>5%) may also show inhibition effect on pullulan synthesis.

Nitrogen Source

The ammonium ion (NH_4^+) has substantial part in pullulan synthesis and diminution of nitrogen is considered as an indication for pullulan production. It is reported that ammonium ions may apply its impact as an influencer of enzyme activity and

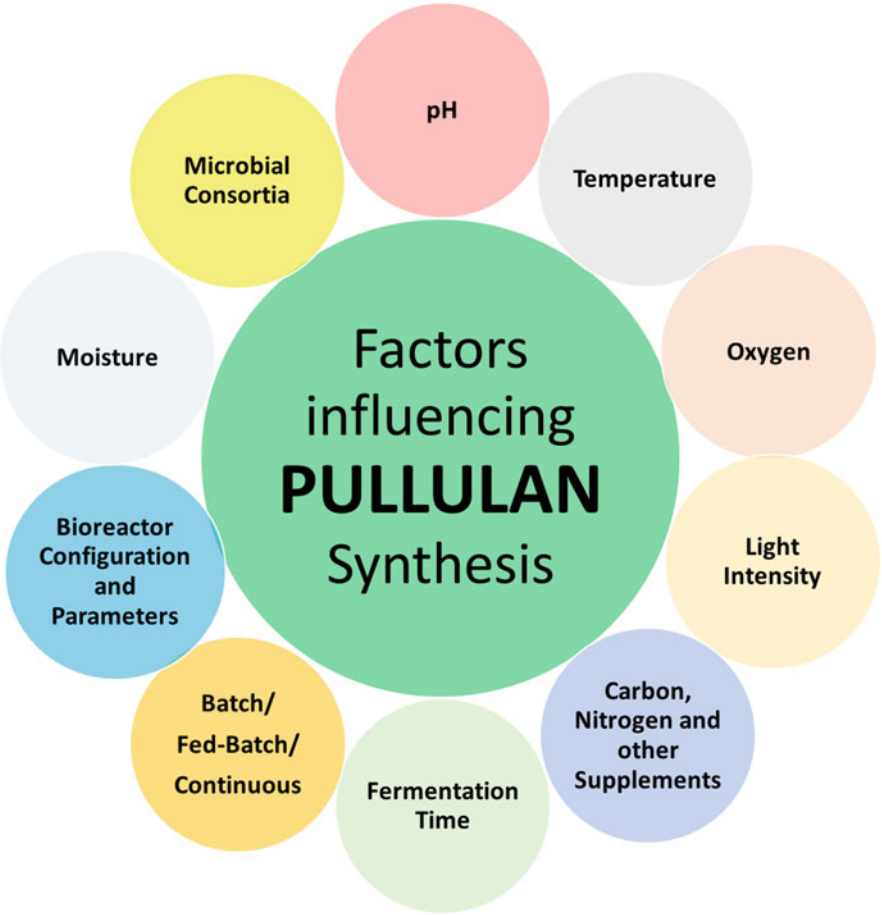


Fig. 3 Factors that influence the effective pullulan synthesis

controller of carbon flow. The surplus nitrogen source to the process does not enhance the exopolysaccharide yield but it can increase the biomass growth. With higher nitrogen concentration at the initial stages, the yield of pullulan is decreased as the enzyme activity is noticed at the later pullulan fermentation stages. As per the reports, 10:1 C/N ratio is the utmost favorable state for synthesis of exopolysaccharide. In many studies, the combined source of ammonium sulfate and yeast extract had been used for pullulan production. Apart from simpler nitrogen forms, multifaceted nitrogen sources were also used as substitute and this in fact augment synthesis of pullulan.

Supplements

Along with carbon and nitrogen sources in the fermentation broth, various supplements, viz., mineral salts, inducers, inhibitors, precursors, etc., should be monitored

for improved pullulan synthesis in submerged fermentation. The variety of sources (C and N) and also various supplements play a significant role in microbial production of pullulan. Uracil, a predecessor for UDP-glucose, has substantial part on biopolymer and biomass synthesis, and uridine phosphorylase (UPase) activity. By the UPase activity and addition of uracil (5 mM), the rate of pullulan production increased to 49.07 g/L. Other supplements, viz., olive oil and tween 80 were also used for enhancing the biopolymer yield. The biopolymer yield was extremely enriched by adding tween 80 in the medium. The influence of various iodoacetic acid concentrations was assessed to evaluate the function of key enzymes that are involved in biopolymer production. Few studies portrayed the effect of soya bean oil as nitrogen source for production of biopolymer.

3.4.2 Fermentation Type

Numerous studies evaluated the effect of fermentation type, i.e., batch, fed-batch, and continuous for the increased efficiency of pullulan production. The difficulty of suppression effect caused by higher substrate concentrations could be evaded by sporadic supply of limiting substrate to the medium. A study was conducted to evaluate the sucrose suppression effect on pullulan production under fed-batch mode and observed the production of 58 g/L exopolysaccharide. However, the fed-batch mode showed increased productivity till certain time and did not show substantial yield upsurge after adding sucrose. Moreover, the fed-batch mode showed little decrease in pullulan concentration after 7 days of cultivation.

Several studies depicted the usage of continuous mode for the production of pullulan. Reports suggested the increased exopolysaccharide production relatively for a long time without any problems. But, the dilution rate was extremely low at the continuous mode of operation. Rate of dilution is a critical parameter that influences biopolymer synthesis in chemostat. Literature reports show that the utilization of chemostat system enhanced the pullulan productivity even at low dilution rates. The continuous processes of fermentation in combination with enhanced cell biomass are feasible for long-term production.

3.4.3 Bioreactor Configuration and Operation

The various factors that highly influence the polymer synthesis in submerged fermentation include the broth composition and behavior at different agitation speeds, sturdy air provision, and low shear rate, etc., thereby providing optimal conditions for the growth of microbes. All the above-mentioned parameters can be controlled in the bioreactor. Hence, bioreactor design and operation play a significant role in enhanced efficiency of pullulan production. The advancement of novel and innovative fermentation reactors will aid in high productivity. Different bioreactors, viz., reciprocating plate bioreactor, were developed to suit the fermentation process and yield good productivity of pullulan. Reactor configuration, viz., biofilm and suspended culture also affect the performance of biological system and regulate the process. The carriers for biofilm configuration had been widely utilized to immobilize the strain. In spite of numerous advantages with biofilm configuration, the

formation of substrate agglomeration and other factors like low void volume, aeration rate, etc., affected the metabolite production.

3.4.4 Fermentation Time

The amount of pullulan produced and its yield varies with fermentation time. Reports suggest that the fermentation time for achieving maximum yield of pullulan changes with respect to different operating conditions and microbial cultures. Hence, the optimal time for production of high pullulan yields varies from 48 h to 5.36 days depending much on the microbial cultures and operational parameters (Sugumaran and Ponnusami 2014).

3.4.5 Microbial Culture

Another important factor that affects the pullulan productivity is the type of microbial culture. As reported in the previous literature, *A. pullulans* is the highest pullulan-producing strain ever reported among wild strains. Apart from *A. pullulans*, there are other strains also that have the ability to synthesize pullulan. *Aureobasidium melanogenum* TN1-2 strain isolated from natural honey was used to synthesize pullulan. The low productivity obtained with *A. pullulans* when maltose was used as carbon source was overcome by using the mutant strain. The mutant strains aided in performing reactions in large scale with optimal conditions. Other mutant strains aided in producing high molecular weight pullulan, increasing the cell growth along with reduction in melanin pigmentation. Coculturing of pullulan-producing strain, *A. pullulans* SH 8646, and an insulin degradation strain, *Kluyveromyces fragile* ATCC 52466 provide the carbon source for *A. pullulans* by the hydrolyzation of inulin by the enzyme inulase synthesized by *K. fragile*. The efficiency of fermentation (FE) indicates that the presently used mutant strains of *A. pullulans* are nearly indistinguishable with regard to their polymer synthesizing activity (Mishra et al. 2018).

3.4.6 Oxygen Intensity

The provision of air to the pullulan production is one of the critical parameters as the microbe does not grow or produce pullulan under anaerobic conditions. Concentrated air provision during fermentation leads to enhanced pullulan production as pullulan-producing cells accumulate in the medium. As viscosity of medium upsurge swiftly, the oxygen demand intensifies as polymer elaborates during submerged fermentation. Hence, optimum DO (dissolved oxygen) concentration and agitation speed are required for obtaining maximum yield of pullulan. The drawbacks associated with intense aeration during pullulan production include lower molecular weight polymer as the vital enzyme for defining the molecular weight, α-amylase, will be altered and also formation of excess foam due to high viscous nature of medium with pullulan. Another report suggested the enhancement in pullulan productivity at low constant dissolved oxygen as it has decreased shear rate. The increase in partial air pressure improves the oxygen rate of transfer to the microorganism thereby synthesizing higher exopolysaccharide.

3.4.7 pH

The pH of the fermentation medium not only influences the yield of pullulan but also affects the cultivation time, metabolite molecular weight, and morphology of the *A. pullulans*. The ideal pH will increase the action of the enzyme, UDPG pyrophosphorylase. As per the reports, the pullulan productivity increases with the pH from 2.5 to 5.5 but thereafter decreases. The pullulan production is suppressed by the lower pH conditions, but the amount of insoluble glucan production is elevated. The optimal pH of 5.5 to 7.5 varies with respect to strain and the mutated strains of *A. pullulans* showed good productivity at pH 4 and 5.9 at different conditions. At near-neutral pH conditions, the microbes produce high molecular weight (MW > 2,000,000) pullulan. The ideal pH values for biopolymer synthesis are varied due to the diverse operating conditions and microorganisms used.

3.4.8 Temperature

Like pH, the temperature also varies with respect to the strain used for pullulan production and it is one of the most influential parameters for biopolymer synthesis. The ideal temperature varies from 25 to 37 °C with respect to different strains. Studies reported the isolation of strain that tolerates high temperature (42 °C) and that which does not produce melanin. The synthesis of pullulan is evaluated by using two-stage temperatures for higher cell growth and pullulan synthesis. The lower temperature of 26 °C reinforced the unicellular biomass growth of *A. pullulans*.

4 Characterization of Pullulan

4.1 Structural Characterization

The cationic groups in pullulan can be determined by Fourier-transform infrared spectroscopy (FTIR) and proton nuclear magnetic resonance (pNMR). Centered on the absorption of infrared (IR) energy by unique functionality groups/linkages in the sample with a specific wave number, the structure of the compound could be predicted. Chemical bonding of biopolymers, stretching and functional groups, can be easily identified by FTIR spectroscopy. The peaks at 856 cm^{-1} and 1156 cm^{-1} are due to α- glycosidic linkage between individual glycoside residues and D-glucose in pyronose form. The absorption peak intensity was recorded at 931 cm^{-1} and 764 cm^{-1} confirming the presence of α-1,4 glycosidic linkage and α-1,6 glycosidic linkage, respectively. The hydroxyl proton present in the samples exhibit the signal between 4.5 and 5.6 ppm using proton NMR spectra. In the purified sample, the pyranose is identified by the absence of signals ranging from 82 to 88 ppm. The presence of anomeric carbon α-1,6 is observed on the peak at 100.1 ppm.

One-dimensional proton NMR spectra of the pullulan exhibits signals integrated for the total of 23 protons. The signals arrived in the downfield region between 3 and 5 ppm infers proton carrying carbon atoms attached to an electronegative atom, which is a characteristic chemical environment of a carbohydrate moiety. Total integration for 23 protons infers that the repeating unit of the carbohydrate polymer contains three

monosaccharide units. In order to characterize and identify the composition of the pullulan, the pullulanase hydrolysis study has been also performed (Mishra and Varjani 2019). Generally, pullulanase hydrolyses α-1,6 glycosidic linkage of branched chain of pullulan and releases the reducing sugars with one –OH free group in each case. The extent of hydrolysis percentage (%) was calculated as follows.

$$\text{Hydrolysis } (\%) = \frac{\text{Amount of reducing sugar released after the hydrolysis}}{\text{Amount of pullulan}} \times 100$$

4.2 Molecular Weight

The molecular weight of pullulan is greatly affected by the initial pH of the medium, the form of strain used, the quality and composition of substrate used, the composition of medium, and the harvesting time. Weight-average molecular weight and number-average molecular weight can be determined by size exclusion chromatography coupled to multi-angle laser light scattering (MALLS). The molecular weight depends upon the various parameters like (a) composition of substrate (b) initial pH (c) types of microbial strain (d) composition of the media, and (e) time of fermentation. The average molecular weight of pullulan is ranged from 362 to 480 kDa. The molecular weight is ranged from 100 to 200 kDa. The molecular weight of pullulan is higher that biosynthesized from agro wastes than that of synthetic medium. The polydispersity index (ratio of weight-average molecular weight and number average molecular weight) for pullulan ranges from 2.1 to 4.1. The variation in polydispersity index depends upon variation in metabolic pathway and cell morphology (Singh et al. 2009). The medium composition like nitrogen source, carbon source, phosphate, and NaCl affect the molecular weight during fermentative production of pullulan. The molecular weight of the pullulan was found to be varied depending upon the concentration of soybean pomace in a study. In a similar way, ammonium ions play a major role in the production of high molecular weight pullulan as compared to nitrate ions. In many cases, the molecular weight of pullulan has been decreased in the due course of fermentation time due to coproduction of pullulanase and α-amylase that degrades the maltotriose subunits present in the pullulan.

4.3 Thermal and Mechanical Properties

Thermal property is an important parameter for exopolysaccharides, which is analyzed by Thermogravimetric Analysis-Differential scanning calorimetry (TGA-DSC). It specifies the decomposition point for pullulan with respect to temperature. Singh and Saini (2008) had observed that the decomposition for pullulan was found to be in the range of 250–280 °C. The decomposition property of pullulan also depends upon the substrate that is used for it biosynthesis and the type of strain used for this. It was found to be 245 ° C and 296.72 ° C, respectively, that was synthesized by *A. pullulans* by utilizing Asian Palm kernel and Cassava

bagasse, respectively (Sugumaran and Ponnusami 2017). The molecular weight, compositions, and structure also affect the decomposition behavior of pullulan. In many cases, the substitution of any groups (-H or –OH) of pullulan with other groups also changes the thermal property due to intermolecular interaction. Incorporating nanofibrillated cellulosic substances to pullulan also enhances its decomposition point to 370 °C. The mechanical property of pullulan is the essential factor to be considered for its applications in pharmaceuticals and food industries. The molecular weight also affects its mechanical properties and tensile strength. The tensile strength can be modulated by the substitution of any groups. For example, substitution with nanocellulosic substances enhances the tensile strength where with acetylated pullulan (pullulan acetate) was found to possess lower tensile strength.

As per the X-ray diffraction study, pullulan is amorphous in nature. Here, the intensity level at the 2-theta value of 20 to 30 denoted the amorphous nature of the pullulan. It was also found that, there was no shifting of the band found in the X-ray power diffractometer graph.

5 Surface Derivatization Approaches for Pullulan

5.1 Polyionic Derivatives

Polyionic derivatives are formed due to mixing of two oppositely charged particles. When charged molecules of opposite natures are mixed in the aqueous solution, nanoparticles can be created. The size, charge, and effectiveness of the nanoparticles depend upon amount of polyelectrolyte relatively. The polyelectrolytic effect of chemically modified pullulan based upon its charges has been illustrated in Fig. 4. There are various ionizable groups like carboxylate, sulfate, and trialkyl ammonium that regulate the viscosity of pullulan solution at different levels. The ionic strength of the ionizing groups and formed polyelectrolyte can control the viscosity of pullulan solution (Spatareanu et al. 2014).

The derivatives of pullulan can also be complexed with oppositely charged polymeric groups to prepare a hybrid polyelectrolyte membrane (Jafari et al. 2019). Generally, anionic modifications (sulfation or carboxylation) can create negatively charged pullulan, while cationic modification (Diethyl amino ethyl or polyethylenimine) creates positively charged pullulan. Cationic derivatives of pullulan serve as the vector for delivering DNA and specific genes.

Cationic derivative of pullulan act as the immunotherapeutic agents in cancer treatment due to their more interaction with the tumor cells. Scientists report that cationic pullulan-nanoparticles can act as anticancer molecules for multidrug-resistant malignant cells even without application of drugs. Here, these nanoparticles irritate the cell membrane through electrostatic stress. When pullulan is modified with sulfide groups, drug delivery can be made easily by disulfide linkages formed through reduction. Attachment of cysteine to the derived pullulan-PEI has enhanced the uptake of plasmid DNA and the drug (doxorubicin) into the cancer cells.

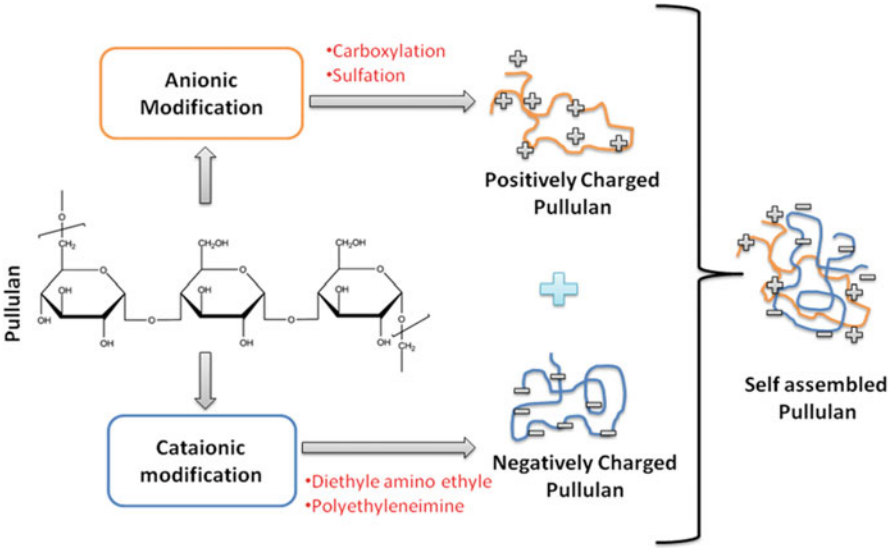


Fig. 4 Schematic representation to illustrate the derivatization of pullulan through cationic and anionic substitution

5.2 Cross-Linking

Pullulan is highly hydrophilic and due to this, it creates an obstacle for certain application. Cross-linking method can be useful to address this problem. In most of the cases, with regard to pullulan film the cross-linking could enhance the swelling, permeability, water absorption, and tensile strength. Pullulan-based hydrogels can be formed by the application of cross-linking processes. Researchers had developed a cross-linked-pullulan/Gelatin gel by alkyne-azide cycloaddition that exhibited unique mechanical properties (Highly stable; Not melted with heating up to 50 °C) (Han and Lv 2019).

This can be possible by stabilized configuration of -OCO-, -OCONH-, and -OCOO- linkages in the cross-linked pullulan as illustrated by Fig. 5. There are various reports dealing with the cross-linking of pullulan using commercially available cross-linkers. The properties of the final cross-linked product depend upon the concentration of the pullulan and the cross-linker. Various studies have been performed in order to optimize the concentration of cross-linkers. Sodium trimeta phosphate used as cross-linker with pullulan has been studied extensively with different concentrations. Enhancing the concentration of photo initiator in the reaction, cross-linking density also increased in pullulan. However, retardation of the reaction was observed in higher concentration of photo initiator (Tiwari and Bahadur 2019).

5.3 Hydrophobic Modification

Pullulan can be modified hydrophobically in the form of highly ordered self-assembled structure in aqueous system to impart plasticity for developing functional

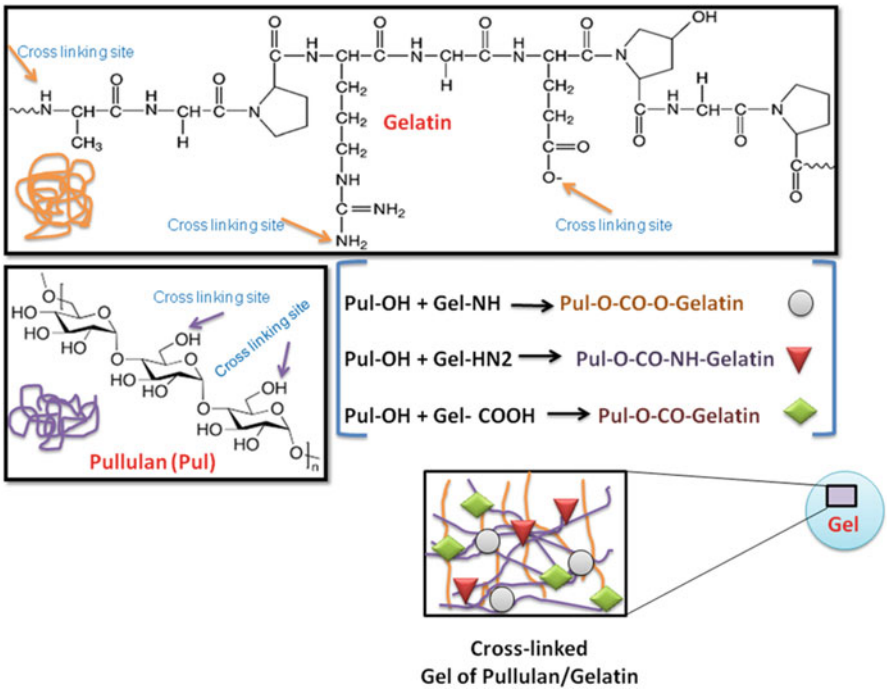


Fig. 5 Schematic representation for creating cross-linked pullulan with gelatin and gellan

biomaterial for various applications. Based upon the chemical composition and block length, different forms of nanoaggregates like vesicles, micelles, and worm-like micelles are formed .These kinds of formulations have been used for target drug delivery system for both small molecules and large molecules. Special types of recognition factors have been tagged with this formulation in order to target them to specific set of cells. As explained by Wang et al. (Wang et al. 2014), due to steric hindrance, aggregation number of these derivatives of pullulan is relatively low. Pullulan was modified with reacting 1-ethyl-3-(3 dimethyl amino propyl) carbodiimide hydrochloride and 4- dimethyl amino pyridine with the application of α-tocopheryl succinate. Applications of cholesterol to convert hydrophilic pullulan to hydrophobic have been described in so many literatures. The schematic representation of the formation of hydrophobic pullulan with cholesterol has been illustrated in Fig. 6.

The hydrophobic domains of cholesterol groups form nanogels of size 20–30 nm with hydrophilic outer shell formed by pullulan (Fig. 6). As per the published report, these nanogels were used as carrier for enzymes, vaccines, drugs, molecular chaperon, and therapeutic proteins (Fujioka-Kobayashi et al. 2012). The surfactants play a major role in association and dissociation of these gels. It was reported that sodium dodecyl sulfate (SDS) helps in the self-aggregation of modified pullulan. A transition from macroscopic gel-to-sol occurred as excess SDS is added. Addition of more SDS triggers the solubilization of hydrophobic groups resulting in reduction of viscosity of the solution.

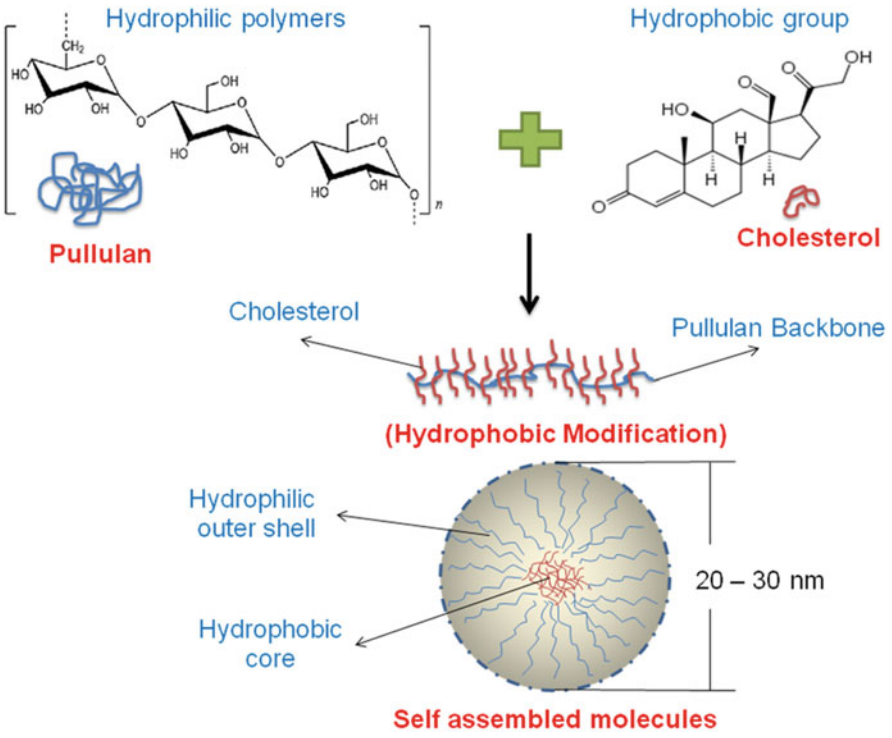


Fig. 6 Schematic representation for developing hydrophobic pullulan through application of cholesterol

5.4 Grafting

The performance and range of applications of polysaccharides, specifically involving melt processing methods can be improved by grafting process. This can be carried out by "grafting from" and "grafting onto" strategies, which produce a macromolecular architecture with multiple side chains (molecular brushes) and a linear backbone. The former involves attachment of pre-synthesized polymers containing complementary functional groups. Despite its simplicity, it cannot produce dense polymer brushes, as the attachment of the incoming chains is hindered by the steric congestion of the chains previously attached to the side.

A substantial decrease in the response rate was identified as a result of the increase in side chain conversion. On the other hand, "grafting from" requires the covalent attachment of a monomer over the polymer backbone and its subsequent polymerization as a side chain (Lepoittevin et al. 2019). The monomer acts as an active center and initiates chain growth via free radical or redox-initiated polymerization (Fig. 7). The efficiency of the grafting can be determined by initiator density, which is comparatively higher in "grafting from" procedure due to its small size of initiator molecule. Molecular weight and weight distribution of the product can be

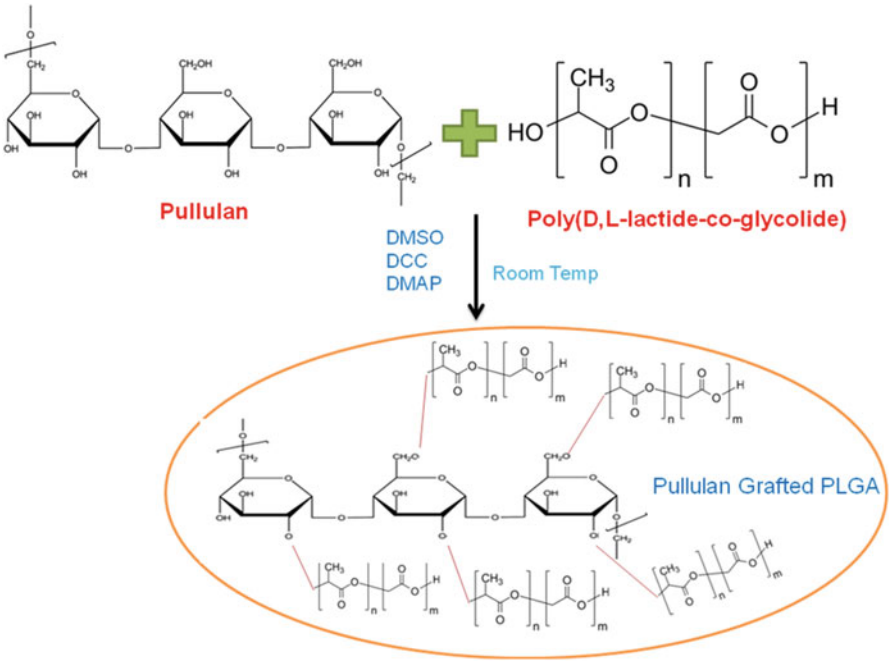


Fig. 7 Schematic representation for making grafted pullulan-PLGA with application of poly (D, L-lactide-co-glycolide) (Note: *DMSO* Dimethyle sulfoxide, *DCC* N,N′-Dicyclohexylcarbodiimide, *DMAP* 4-Dimethylaminopyridine)

controlled by varying the reaction conditions. Knowing the total number and size of graft polymers through characterization could be difficult but they act as good multiphase polymer systems. Graft polymers are widely used in drug delivery, tissue engineering, and biosensing. The solubility in solvents such as methanol and dimethyl sulfoxide can be expanded by grafting of polyethylene glycol (PEG) on pullulan. On other hand, grafting of poly (methyl acrylate) residues decreases its hydrophilicity. However, there is limited amount of acrylate grafting application in biomedical due to its nonbiodegradability under physiological conditions. Since the grafting of poly (N-isopropylacrylamide) (pNIPAM) undergoes sharp phase transition at physiological conditions the intensive tests are done to introduce temperature sensitivity in polysaccharides. Poly-N-isopropylacrylamide (pNIPAM)-grafted pullulan displayed a reversible phase transition at about 32 °C thereby producing nanoaggregates in aqueous system. Nanoparticles showed a high loading efficiency for indomethacin due to strengthened hydrogen bonding and hydrophobic interactions between the drug and pNIPAM grafts. The dissociation of hydrogen bonds at high temperatures enabled the controlled drug release (Constantin et al. 2017).

Fundueanu and coworkers reported the attachment of thermo- and pH-sensitive units onto pulllulan microspheres. pNIPAM-co-acrylamide grafts were inserted for thermo-responsive units. Remaining –OH groups were linked with pH-sensitive

groups (succinic anhydride). The sharp volume phase transition was maintained by the microspheres at low values of exchange capacities at both below and above pKa of carboxylic acid. Akiyoshi's group integrated the self-assemblage of collagen hybridizing peptide (CHP) with thermo-responsive behavior of pNIPAM to develop botryoidal clusters of CHP/pNIPAM nanogels. They have observed that the solution concentration and relative amount of components determines the nanogel morphology. It revealed two distinct volume transitions over 20–55 °C temperature window. A decrease in hydrodynamic radii from 93 to 57 nm as the temperature increased from 20 to 35 °C, followed by a sharp increase from 57 nm to 90 nm at 55 °C. Authors attributed the phase change of CHP-pNIPAM nanogels to its botryoidal assembly, which is different from the assembly of block copolymers that possess distinct temperature-sensitive blocks. Individual units in this structure were held together by cholesteryl cross-linking points (Morimoto et al. 2007).

6 Recent Applications and Uses in Biomedical and Pharmaceutical Fields

6.1 Pharmaceutical Formulations

Pullulan, often available in powdered form, can also be manufactured in thin film format. These films have many applications in the health-care industries, pharmaceutical, and food. Listerine® mouth freshener is the most common and commercially effective product in pullulan films. Pullulan can be used in pharmaceuticals, such as in pill and capsule coatings, including formulations for sustained release (Dubey 2018). *Ulmus davidiana* var. *Japonica* (UD) has historically been used to treat various types of illnesses, including skin wounds and inflammation, with Korean medicine. Compared to pullulan-only gel film, UD gel films showed good thermal stability and better mechanical properties with outstanding swelling performance and favorable skin adhesiveness.

The latest method mixes the essential ingredients in current vaccines with a sugar gel where they remain viable for 8 weeks or more, even at high temperatures. The method produces light, effective, and compact doses that, according to the researchers, would be perfect for shipping Ebola vaccine to affected African regions, for example. Consequently, framework only adds marginal costs to the preparation of a vaccine and eliminates almost all transportation costs, which can account for 80% of the revenue of inoculation. Mixing the vaccines and sugars (pullulan and trehalose) is almost as simple as pouring cream and sugar into coffee, the researchers conclude. At McMaster, the storage device was developed by chemical engineers who had already proven its utility in other applications, such as an edible coating that can improve the shelf life of fruit and vegetables. To adapt the technology to the vaccines, the engineers partnered with colleagues from health sciences across campus who specialize in virology and immunology. The new vaccine storage method suspends the active components of the vaccine in a thin, single-dose jar filled with a sugar-gel mixture that dries into the vaccine for sealing. Clinicians then

reconstitute and administer the vaccine with water as they usually would to patients. The researchers have proved the viable approach of using two experimental vaccines such as herpes simplex virus and influenza virus, inoculate and research the exposure of viruses to mice since the immune response of mice is similar to that of humans. The US Food and Drug Administration has already licensed the products in the storage medium, thereby simplifying the marketing route (Leung et al. 2019).

Based on a mixture of trehalose/pullulan, protein-loaded orodispersible films (ODFs) were prepared by air-and freeze-drying. Depending on the outstanding trehalose-stabilizing protein capacity and pullulan film-forming capability, these two carbohydrates were chosen. Three model proteins were mounted onto ODFs. Ovalbumin was used to analyze the impact of protein incorporation on mechanical properties, time of disintegration, uniformity of weight, and thickness of ODF. The stability of the proteins has been tested by lysozyme and β-galactosidase. The loading of ovalbumin did not have a noticeable effect on the mechanical properties of freeze-dried ODFs, while the introduction of ovalbumin into air-dried ODFs resulted in a large decrease in tensile strength. Lysozyme stability was not affected by the trehalose/pullulan ratio, whereas β-galactosidase stability improved with increased trehalose/pullulan ratios. Likewise, for process stability over airdrying, freeze-drying proved to be advantageous, while for storage stability, the opposite was observed. In addition, ODFs based on trehalose/pullulan are promising for potential protein delivery via the oral cavity from a technical point of view (Tian et al. 2018).

The inclusion of therapeutic proteins in a sugar glass matrix is known to increase protein stability, but when exposed to elevated relative humidity (RH), safety is sometimes lost. It was hypothesized that the use of disaccharide and polysaccharide binary glasses may be beneficial for the stability of proteins, particularly under these conditions. Different amounts of polysaccharide pullulan were then used in glasses of freezed-dried trehalose. When subjected to elevated RH, the presence of pullulan above 50% weight in these homogeneous blends prevented trehalose crystallization. The β-galactosidase protein model incorporated into pullulan/trehalose blends tested for storage stability for up to 4 weeks showed superior pure trehalose activity at 30 °C/0 percent RH, while pullulan/trehalose blends showed the highest stability at 30 °C/56 percent RH. Therefore, binary pullulan and trehalose glasses can provide excellent protein stability under storage conditions, such as high RH and high temperatures, which can occur in activity (Teekamp et al. 2017).

Hussain et al. (2017), have synthesized using the 1,10-carbonyldiimidazole (CDI) reagent activating green carboxylic acid. In order to prepare aspirin-imidazolide at room temperature for 24 h, the aspirin was first reacted with CDI, which reacted in situ with predissolved pullulan, and the reaction was then followed by nitrogen at 80 °C for 24 h. Using ^{1}H NMR spectroscopy, the degree of aspirin substitution on pullulan (DS 0.32–0.40) was determined. The elevated charge and purity of the covalent content have been verified by spectroscopic techniques. Thermal research has shown that the new macromolecular prodrugs (MPDs) of aspirin are thermally more stable than pure aspirin. As analyzed by transmission electron microscopy (TEM), the amphiphilic pullulan-aspirin conjugates self-assembled in nanoparticles

at a solvent interface within the range of 500–680 nm without further structural changes. This novel pullulan-aspirin can theoretically be safe stomach prodrugs combined with masked functional group COOH.

6.2 Tissue Engineering

In medical applications, biomimetic hydrogels are structurally and mechanically identical to the extracellular matrix (ECM) and have several possibilities. Nevertheless, new methods of controlling hydrogel feature resolution, biofunctionalization, and mechanical properties are required to create synthetic microenvironments that mimic the cell growth and differentiation effects of natural tissue niches. As a cell-adhesive, hydrogel possesses three-dimensional (3D) photo-printing in size ranging from macro- to micro-scale dimensions, and tunable mechanical properties. This could produce multiscale printed scaffolds that are found to be inert and biologically compatible with cell adhesive proteins for cell adhesion.

Using natural polysaccharide pullulan, cross-linked with sodium trimetaphosphate (STMP) and combined with collagen, a three-dimensional (3D) scaffold was manufactured to build a polymeric network. Collagen has been derived from the skin of unexplored puffer fish (*Lagocephalus inermis*). The cross-link took place at alkaline pH at room temperature to give translucent, transparent, and soft hydrogels. Swelling tests showed remarkable properties of water absorption with a swelling ratio of up to 320%, an ideal feature for the hydrogel for a moist wound healing environment. SEM research revealed that there was a closely interconnected porous structure of the collagen blended pullulan hydrogels. On NIH3T3 fibroblast cell lines, the MTT assay showed that the prepared hydrogels were 100% biocompatible with increased cell adhesion and proliferation. The hydrogels promoting angiogenesis in the chick chorioallantoic membrane were studied in the Chick Chorioallantoic Membrane (CAM) assay. The highly porous hydrogels of collagen-pullulan, a promising biomaterial for wound healing applications, have been successfully developed with substantial biological output in vitro and in vivo (Iswariya et al. 2016).

In tissue engineering and regenerative medicine, a multifunctional biomaterial with the ability to bind to hard tissues, such as bones and teeth, is required for medical and dental applications. Phosphorylated-pullulan (PPL) is able to bind hydroxyapatite to the bones and teeth. PPL has been used for bone engineering as a novel biocompatible material. First, an in vitro assessment of the mechanical properties of PPL showed that composites of both PPL and PPL-Linked-β-tricalcium phosphate (β-TCP) have higher shear bond strength than existing clinical use products, including polymethylmethacrylate cement (PMMA) and α-tricalcium phosphate cement (α-TCP), Biopex-R. In addition, the composite compressive strength of PPL/β-TCP was substantially higher than Biopex-R. Next, PPL/β-TCP in vivo composite osteoconductivity was investigated in a murine intramedullary injection model. Bone formation, which was even more visible at 8 weeks, was observed 5 weeks after injection of PPL/β-TCP composite; and now no bone

formation was found after injection of PPL alone. Later, PPL/β-TCP was composed of an ulnar rabbit bone defect model, and bone formation similar to that induced by Biopex-R was observed. At 4 weeks, PPL/β-TCP composite implantation induced new bone growth, which was shockingly obvious at 8 weeks. By comparison, after 8 weeks, Biopex-R remained isolated from the surrounding bone. In a model of pig vertebral bone defects, PPL/β-TCP composite-treated defects were almost entirely replaced by new bones, while PPL alone failed to induce bone formation. Taken together, these results demonstrate that PPL/β-TCP composites can be useful in bone engineering (Takahata et al. 2015).

Pullulan is a biologically compatible and efficient cytoadhesive medium for mesenchymal stem cell (MSC) tissue engraftment. Prolonged pullulan exposure has no detrimental effect on the morphology, viability, and differentiation capability of the cells. In the fibrillated surface of articular osteoarthritic cartilage, pullulan greatly increases the survival of MSCs. In the expression of a lectin transmembrane complex of type Dectin-2 C, pullulan induces upregulation. A pamidronate-pullulan conjugate has been shown to be involved in hydroxyapatite binding and accumulating in regenerating bone tissue (Liu et al. 2012).

6.3 Targeted Drug Delivery

Solid dispersion (SD) technique is the innovative method used to improve the solubility of BCS (Biopharmaceutical Classification System) Class II drugs. Throughout their solid state the drug was molecularly distributed in the SDs in a hydrophilic polymer. The major challenge in the development of the dosage form was the drug's poor aqueous solubility. In this study the technique of solid dispersion was used to improve solubility. Pioglitazone is an oral antidiabetic medication, which is used in the treatment of type II diabetes mellitus and is used as a model drug for solubility enhancement testing. The SDs were prepared using the kneading method, solvent evaporation method, and combining method of solvent evaporation with kneading process. The solid dispersions are formulated using various drug ratios: polymer (1:1, 1:1.5 and 1:2). In fact, the results obtained show that the solubility decreases as the polymer concentration decreases. It was concluded from this that natural hydrophilic polymer is important for the improvement of the solubility of Biopharmaceutical Classification System (BCS) class II drug (Kulkarni et al. 2019).

6.4 Gene Delivery

Doxorubicin (DOX) is an important antitumor agent, which is commonly used. However, its therapeutic use is limited due to its side effects, including anti-apoptotic defense of cancer cells caused by DOX-induced autophagy and deleterious effects on normal tissues. In order to enhance the anticancer effect of DOX by blocking the autophagy mechanism mediated by the Beclin1 protein, a new folate (FA)-decorated

amphiphilic bifunctional pullulan-based copolymer (named FPDP) was developed as an effective nano-carrier for the co-delivery of Beclin1's DOX and short hairpin RNA, a pivotal autophageal gene. In the FPDP molecules, pullulan was modified for the formation of micelles with lipophilic desoxycholic acid, the introduced branched polyethylenimine (PEI) of low molecular weight (1 kDa) was used to deliver shBeclin1, and folate (FA) was used as the target group for the tumor. FPDP micelles have an average diameter of 161.9 nm, sufficient storage stability, good biocompatibility, excellent DOX and shBeclin1 loading capacities, and a sustained drug release profile. Moreover, in vivo studies demonstrated superior antitumor efficacy compared to non-FR-targeted PDP and free DOX micelles of tumor-targeted FPDP/DOX/shBeclin1. These findings showed that Beclin1 DOX and shRNA co-delivery with FPDP micelles has the potential to overcome DOX shortcomings in clinical cancer therapy (Chen et al. 2018).

Using oxidized pullulan (oxPL) and a disulfide-containing poly(β-amino) ester (ssPBAE) for the effective delivery of genes and chemotherapeutic agents via polymer degradation that responds to intracellular pH and redox tumor status, a dual-responsive nanoparticles system was created. By means of the pullulan oxidation process, OxPL containing excess aldehyde groups was obtained. With diethylenetriamine, ssPBAE was modified and then grafted onto oxPL by Schiff's base reaction. Via the acid-cleavable hydrazone bond, DOX was bonded to ssPBAE-oxPL, resulting in ssPBAEoxPL-DOX with a DOX content of about 3.6%. SsPBAE-oxPL-DOX has shown in vitro hepatoma targeting properties and good condensing capacity of fluorescein-labeled oligoDNA (FAM-DNA) and plasmid DNA (pDNA) genes to some degree. There were non-sphere shapes and fairly uniform sizes of ssPBAE-oxPL-DOX/pDNA nanoparticles. The in vitro releases of DOX and pDNA from ssPBAEoxPL-DOX/pDNA nanoparticles showed strong pH-and redox-responsive properties. The nanoparticles of ssPBAE-oxPL-DOX/pDNA effectively inhibited cell proliferation in HepG2 hepatoma cells, and induced S-phase cell apoptosis and cell cycle arrest. Furthermore, ssPBAE-oxPL-DOX/FAM-DNA nanoparticles effectively delivered DOX and FAM-DNA to the nucleus and cytoplasm in HepG2 cells, respectively, and also showed distinct hepatoma targeting properties after injection in HepG2 tumor-bearing mice. In short, this innovative dual-responsive nanoparticle device has shown tremendous potential for combination gene therapy and chemotherapy for hepatoma (Wang et al. 2016).

A modern type of anticancer therapy has emerged as a combination treatment of drugs and therapeutic genes. A new amphiphilic bifunctional pullulan derivative (known as PSP) consisting of stearic acid and low molecular weight (1 kDa) branched polyethylenimine was prepared and characterized in this study as a nanocarrier for potential cancer therapies for drug and gene co-delivery. The drug loading content and encapsulation efficacy of PSP nanomicelles for DOX, an antitumor drug, was approximately 5.10% and 56.07%, respectively, and the continued release of DOX in PSP nanomicelles. The in vitro IC50 of PSP/DOX nanomicelles was significantly lower than the free DOX against MCF-7 cells. In addition, PSP nanomicelles effectively condensed DNA in gene transfection experiments to form compact structures and induced comparable rates of GFP gene expression to

Lipo2000 at N/P = 10. Compared with single DOX or p53 delivery, co-delivery of DOX and therapy gene p53 using PSP micelles showed higher cytotoxicity and induced higher apoptosis rates of tumor cells in vitro. Furthermore, PSP showed low cytotoxicity and good blood compatibility in the MTT and hemolysis assays. All in all, PSP nanomicelles have tremendous potential to provide hydrophobic anticancer drugs and therapeutic genes for enhanced cancer treatment at the same time (Chen et al. 2015).

A combination of excellent protection and adequate performance is what is desperately required in the development of a gene carrier network. For successful genetic photodynamic therapy, a straightforward and flexible synthesis of a cationic guanidine-decorated dendronized pullulan (OGG3P) was studied here. With a weight ratio of 2, OGG3P has been able to block DNA mobility. Nonetheless, G3P missing guanidine residues could not block DNA migration until disclosure of guanidation could facilitate DNA condensation at a weight ratio of 15. A zeta potential plateau (~ + 23 mV) of OGG3P complexes through unique guanidinium–phosphate interactions suggested that pullulan nonionic hydrophilic hydroxyl groups neutralize excessive detrimental cationic loads. Compared with that of native G3P in Hela and HEK293 T cells, there was no clear cytotoxicity and hemolysis, and also transfection efficiency improvement with respect to OGG3P. More precisely, the uptake efficiency between OGG3P and G3P complexes in Hela cells was found not to be significantly different. However, guanidation induced changes in the uptake pathway and contributed to the macropinocytosis pathway, which can be an important factor in improving transfection efficiency. OGG3P complexes achieved substantial improvements in the expression of KillerRed protein and in the development of ROS under irradiation following the introduction of a therapeutic pKillerRed-mem plasmid. Suppression of proliferation of the cancer cells caused by ROS was also reported. This study shows the possible development of dendronized pullulan decorated with guanidine as a robust nonviral gene carrier specifically designed to carry therapeutic genes (Zhou et al. 2018).

Magnetic nanoparticles have been used as efficient vehicles for the targeted delivery of therapeutic agents that can be controlled by the concentration and distribution of externally guided magnets to a particular part of the body. The functionalization, characterization, and synthesis of pullulan-spermine(PS) magnetic nanoparticles for medical applications is the subject of this study. Thermal decomposition of goethite (FeOOH) produced magnetite nanopowder in oleic acid and 1-octadecene; pullulan-spermine was deposited on the magnetite nanoparticles in the form of pullulan-spermine clusters. EGFP-p53 plasmid was loaded to transfer functional iron oleate into cells. The nanocomplexes have been tested for the efficacy of encapsulation and drug processing. FTIR studies demonstrated the existence of oleic acid and 1-octadecene in their nanopowder on oleate and authenticated the spermine–pullulan interaction. In the pullulan spermine-coated ironoleate (PSCFO) spectrum, the characteristic PS bands indicated that PS covered the surface of their onoleate particles. Magnetic tests found that the magnetic saturation of the PSCFO was lower compared to those on oleate nanopowder due to the presence of organic compounds in the former. In cytotoxicity studies performed using U87 cells as

glioblastoma cells found a survival rate of 92% at 50 mg/ml of plasmid-carrying PSCFO with an IC50 value of 189 mg/ml (Eslaminejad et al. 2016).

7 Future Perspectives

Despite a large number of valuable applications, the major constraint prevailing on the use of pullulan is its cost, which is three times higher than the price of other polysaccharides such as Dextran and Xanthan. Previous studies have addressed the melanin by-product in pullulan synthesis but its cost (25–30 USD/Kg) is even a greater problem. Engineering innovations or improved production strains, particularly with reduced melanin production, could be beneficial to improve the economics of the production, thereby opening new avenues for pullulan utilization. Pullulan does not have antimicrobial activity. The clear understanding of mechanism of pullulan biosynthesis is required to enhance the product quality and also to its study in Metabolic Engineering and Molecular Editing. The biochemistry of pullulan can answer the major down streaming and production problems.

A comprehensive review of pullulan production in relation to molecular properties, upstream genetic regulations, and downstream processes, including new bioreactor design, cultivation parameters, and applications, is still to be explored. Through further chemical modifications, pullulan can be a potential source in various industries as a novel bioactive derivative. In the last two decades, the main demand for pullulan has been in food applications. With the innovative technologies of modification and cultivation skills, the development of modified pullulan derivatives with distinctive material properties and pullulan with a particular molecular weight distribution can be achieved. As a result, an increase in the number of studies on pullulan applications in medical, environmental, and pharmaceuticals has been reported.

The demand for pullulan is increasing in cancer therapy with its related studies. The high bioactivity with some cytotoxic molecules is exhibited by the modified pullulan and it has known to form the complexes of those molecules. The gradual release of cytotoxic molecules is facilitated by accumulation of these conjugates at target sites. In many medical cosmetic industries other synthetic materials which produce CO_2 are replaced with pullulan as it does not have any adverse effects. It is useful to assess them for a more intensive use of this polymer applications related to personal care and cosmetics, not only as a revolutionary active ingredient, but also as a safe compound for biodegradable materials and packaging. There appears to be a robust demand for antiaging cosmetics. The personal care and cosmetic products should be packed in biodegradable containers in such a way they do not affect the environment. This opens a broad scope of using pullulan exopolysaccharide in dermatological and cosmetic sector to produce smart products.

The other emerging market is in biomedical engineering as pullulan has strong absorption ability. Safer innovative methods are being discovered for biosynthesis of pullulan. Numerous methods have been developed for the use of pullulan in drug delivery like in subcellular targeting, stimulus-responsive drug delivery systems, and

nanoplatforms. Pullulan derivative nanoparticles or gels have wide applications in drug delivery material and gene transfer in pharma and food industries. Various notable developments are happening with pullulan in stem cell therapy, imaging, cancer cell targeting, etc. Considering these aspects, there is potential future for pullulan in health care sector for the betterment of human race. The surface modification of pullulan can be done to widen its application. Pullulan's metallic stability is greatly decreased due to its hydrophilic nature. Pullulan hydrogels have non-fouling properties and are less studied in bone tissue culture applications. The future research can be oriented to provide surface support for cell adhesion and proliferation through osteogenesis in bone tissue culture applications.

8 Conclusion

The main advantage of polysaccharides produced from the microorganisms over the plant or algae is that, microorganism exhibits higher growth rate than plants and algae; moreover, they are more amenable to the manipulation and adjustment of the conditions for the enhancement of the growth and production. The progress of the researches in the area of fungal polysaccharides are also very less. The optimal conditions of process parameters for pullulan production are still debating.

References

Bender H, Lehmann J, Wallenfels K. Pullulan, ein extracelluläres Glucan von Pullularia pullulans. Biochim Biophys Acta. 1959;36:309–16. https://doi.org/10.1016/0006-3002(59)90172-6.

Chen J, Wu S, Pan S. Optimization of medium for pullulan production using a novel strain of *Auerobasidium pullulans* isolated from sea mud through response surface methodology. Carbohydr Polym. 2012;87:771–4. https://doi.org/10.1016/j.carbpol.2011.08.062.

Chen L, Wang X, Ji F, Bao Y, Wang J, Wang X, Guo L, Li Y. New bifunctional-pullulan-based micelles with good biocompatibility for efficient co-delivery of cancer-suppressing p53 gene and doxorubicin to cancer cells. RSC Adv. 2015;5:94719–31.

Chen L, Qian M, Zhang L, Xia J, Bao Y, Wang J, Guo L, Li Y. Co-delivery of doxorubicin and shRNA of Beclin1 by folate receptor targeted pullulan-based multifunctional nanomicelles for combinational cancer therapy. RSC Adv. 2018;8:17710–22.

Cheng KC, Catchmark JM, Demirci A. Enhanced pullulan production in a biofilm reactor by using response surface methodology. J Ind Microbiol Biotechnol. 2010;37:587–94. https://doi.org/10.13031/2013.29694.

Chi Z, Wang F, Chi Z, Yue L, Liu G, Zhang T. Bioproducts from Aureobasidium pullulans, a biotechnologically important yeast. Appl Microbiol Biotechnol. 2009;82:793–804. https://doi.org/10.1007/s00253-009-1882-2.

Choudhury AR, Saluja P, Prasad G. Pullulan production by an osmotolerant Aureobasidium pullulans RBF-4A3 isolated from flowers of Caesulia axillaris. Carbohydr Polym. 2011;83:1547–52. https://doi.org/10.1016/j.carbpol.2010.10.003.

Constantin M, Bucătariu S, Stoica I, Fundueanu G. Smart nanoparticles based on pullulan-g-poly (N-isopropylacrylamide) for controlled delivery of indomethacin. Int J Biol Macromol. 2017;94:698–708. https://doi.org/10.1016/j.ijbiomac.2016.10.064.

Dailin DJ, Low LZMI, Kumar K, Malek RA, Natasya KH, Keat HC, Sukmawati D, Enshasy HE. Agro-industrial waste: a potential feedstock for pullulan production. Biosci Biotechnol Res Asia. 2019;16:229–50. https://doi.org/10.13005/bbra/2740.

Dubey KK. Insights of microbial pullulan production: a bioprocess engineer assessment. Current Biotechnol. 2018;7:262–72.

Eslaminejad T, Nematollahi-Mahani SN, Ansari M. Synthesis, characterization, and cytotoxicity of the plasmid EGFP-p53 loaded on pullulan–spermine magnetic nanoparticles. J Magn Magn Mater. 2016;402:34–43.

Fujioka-Kobayashi M, Ota MS, Shimoda A, K-i N, Akiyoshi K, Miyamoto Y, Iseki S. Cholesteryl group-and acryloyl group-bearing pullulan nanogel to deliver BMP2 and FGF18 for bone tissue engineering. Biomaterials. 2012;33:7613–20. https://doi.org/10.1016/j.biomaterials.2012.06.075.

Haifeng L, Yunyi Z, Yunyun G, Yuanyang L, Xiaopu Y, Lifeng H. Characterization of UGPase from Aureobasidium pullulans NRRLY-12974 and application in enhanced pullula production. Appl Biochem Biotechnol. 2016;178:1141–53. https://doi.org/10.1007/s12010-0151934-2.

Han Y, Lv S. Synthesis of chemically crosslinked pullulan/gelatin-based extracellular matrix-mimetic gels. Int J Biol Macromol. 2019;122:1262–70. https://doi.org/10.1016/j.ijbiomac.2018.09.080.

Hussain MA, Abbas K, Lodhi BA, Sher M, Ali M, Tahir MN, Tremel W, Iqbal S. Fabrication, characterization, thermal stability and nanoassemblies of novel pullulan-aspirin conjugates. Arab J Chem. 2017;10:1597–603.

Iswariya S, Bhanukeerthi A, Velswamy P, Uma T, Perumal PT. Design and development of a piscine collagen blended pullulan hydrogel for skin tissue engineering. RSC Adv. 2016;6:57863–71. https://doi.org/10.1039/c6ra03578g.

Jafari A, Sun H, Sun B, Mohamed MA, Cui H, Cheng C. Layer-by-layer preparation of polyelectrolyte multilayer nanocapsules via crystallized miniemulsions. Chem Commun. 2019;55:1267–70. https://doi.org/10.1039/c8cc08043g.

Kulkarni A, Madane T, Aloorkar N, Mujumdar S. Enhancement of solubility of BCS class II drug (pioglitazone) using natural polymer (pullulan). Current Pharma Res. 2019;9:3321–34.

Lepoittevin B, Elzein T, Dragoe D, Bejjani A, Lemee F, Levillain J, Bazin P, Roger P, Dez I. Hydrophobization of chitosan films by surface grafting with fluorinated polymer brushes. Carbohydr Polym. 2019;205:437–46. https://doi.org/10.1016/j.carbpol.2018.10.044.

Leung V, Mapletoft J, Zhang A, Lee A, Vahedi F, Chew M, Szewczyk A, Jahanshahi-Anbuhi S, Ang J, Cowbrough B. Thermal stabilization of viral vaccines in low-cost sugar films. Sci Rep. 2019;9:1–11. https://doi.org/10.1038/s41598-019-44020-w.

Liu J, J-i J, Kawai Y, Aoki I, Tanaka C, Yamamoto M, Tabata Y. Preparation of polymer-based multimodal imaging agent to visualize the process of bone regeneration. J Control Release. 2012;157:398–405. https://doi.org/10.1016/j.jconrel.2011.09.090.

Mishra B, Suneetha V. Biosynthesis and hyper production of pullulan by a newly isolated strain of Aspergillus japonicus-VIT-SB1. World J Microbiol Biotechnol. 2014;30:2045–52. https://doi.org/10.1007/s11274-014-1629-9.

Mishra B, Varjani S. Evaluation of pullulan production by a newly isolated Micrococcus luteus. Indian J Exp Biol. 2019;57:813–20.

Mishra B, Vuppu S, Rath K. The role of microbial pullulan, a biopolymer in pharmaceutical approaches: a review. J Appl Pharm Sci. 2011;01:45–50.

Mishra B, Manikanta A, Zamare D. Preparation of Maltotriose syrup from microbial pullulan by using pullulanase enzyme. Biosci Biotech Res Asia. 2016;13:481–5.

Mishra B, Zamare D, Manikanta A. Selection and utilization of agro-industrial waste for biosynthesis and hyper-production of pullulan: a review. In: Varjani S, Parameswaran B, Kumar S, Khare S, editors. Biosynthetic technology and environmental challenges. Energy, environment, and sustainability. Singapore: Springer; 2018. p. 89–103. https://doi.org/10.1007/978-981-10-7434-9_6.

Morimoto N, Winnik FM, Akiyoshi K. Botryoidal assembly of cholesteryl– pullulan/poly (N-isopropylacrylamide) nanogels. Langmuir. 2007;23:217–23. https://doi.org/10.1021/la0616045.

Shu CH, Lin KJ, Wen BJ. Effects of culture temperature on the production of bioactive polysaccharides by Agaricus blazei in batch cultures. J Chem Technol Biotechnol. 2007;82:831–6. https://doi.org/10.1002/jctb.1748.

Singh R, Saini G. Pullulan-hyperproducing color variant strain of Aureobasidium pullulans FB-1 newly isolated from phylloplane of Ficus sp. Bioresour Technol. 2008;99:3896–9. https://doi.org/10.1016/j.biortech.2007.08.003.

Singh RS, Saini GK, Kennedy JF. Downstream processing and characterization of pullulan from a novel colour variant strain of Aureobasidium pullulans FB-1. Carbohydr Polym. 2009;78:89–94. https://doi.org/10.1016/j.carbpol.2009.03.040.

Singh RS, Kaur N, Kennedy JF. Pullulan production from agro-industrial waste and its applications in food industry: a review. Carbohydr Polym. 2019;217:46–57. https://doi.org/10.1016/j.carbpol.2019.04.050.

Soto KM, Hernández-Iturriaga M, Loarca-Piña G, Luna-Bárcenas G, Mendoza S. Antimicrobial effect of nisin electrospun amaranth: pullulan nanofibers in apple juice and fresh cheese. Int J Food Microbiol. 2019;295:25–32. https://doi.org/10.1016/j.ijfoodmicro.2019.02.001.

Spatareanu A, Bercea M, Budtova T, Harabagiu V, Sacarescu L, Coseri S. Synthesis, characterization and solution behaviour of oxidized pullulan. Carbohydr Polym. 2014;111:63–71. https://doi.org/10.1016/j.carbpol.2014.04.060.

Sugumaran KR, Ponnusami V. Downstream processing studies for pullulan recovery in solid state fermentation using asian palmyra palm kernelinexpensive substrate. Biotechnology. 2014;9(2):79–82.

Sugumaran K, Ponnusami V. Review on production, downstream processing and characterization of microbial pullulan. Carbohydr Polym. 2017;173:573–91. https://doi.org/10.1016/j.carbpol.2017.06.022.

Sugumaran K, Sindhu R, Sukanya S, Aiswarya N, Ponnusami V. Statistical studies on high molecular weight pullulan production in solid state fermentation using jack fruit seed. Carbohydr Polym. 2013;98:854–60. https://doi.org/10.1016/j.carbpol.2013.06.071.

Takahata T, Okihara T, Yoshida Y, Yoshihara K, Shiozaki Y, Yoshida A, Yamane K, Watanabe N, Yoshimura M, Nakamura M. Bone engineering by phosphorylated-pullulan and β-TCP composite. Biomed Mater. 2015;10:065009.

Teekamp N, Tian Y, Visser JC, Olinga P, Frijlink HW, Woerdenbag HJ, Hinrichs WL. Addition of pullulan to trehalose glasses improves the stability of β-galactosidase at high moisture conditions. Carbohydr Polym. 2017;176:374–80. https://doi.org/10.1016/j.carbpol.2017.08.084.

Thirumavalavan K, Manikkadan T, Dhanasekar R. Pullulan production from coconut by-products by *Aureobasidium pullulans*. Afr J Biotechnol. 2009;8:254–8.

Tian Y, Visser JC, Klever JS, Woerdenbag HJ, Frijlink HW, Hinrichs WL. Orodispersible films based on blends of trehalose and pullulan for protein delivery. Eur J Pharm Biopharm. 2018;133:104–11.

Tiwari S, Bahadur P. Modified hyaluronic acid based materials for biomedical applications. Int J Biol Macromol. 2019;121:556–71. https://doi.org/10.1016/j.ijbiomac.2018.10.049.

Wang J, Cui S, Bao Y, Xing J, Hao W. Tocopheryl pullulan-based self assembling nanomicelles for anti-cancer drug delivery. Mater Sci Eng C. 2014;43:614–21. https://doi.org/10.1016/j.msec.2014.07.066.

Wang D, Bian J, Wei G, Jiang M, Dong M. Simultaneously enhanced production and molecular weight of pullulan using a two-stage agitation speed control strategy. J Chem Technol Biotechnol. 2016;91:467–75. https://doi.org/10.1002/jctb.4600.

Xiao Q, Tong Q, Lim L-T. Pullulan-sodium alginate based edible films 2012 rheological properties of film forming solutions. Carbohydr Polym. 2012;87:1689–95.

Yu X, Wang Y, Wei G, Dong Y. Media optimization for elevated molecular weight and mass production of pigment-free pullulan. Carbohydr Polym. 2012;89:928–34. https://doi.org/10.1016/j.carbpol.2012.04.038.

Zan Z, Zou X. Efficient production of polymalic acid from raw sweet potato hydrolysate with immobilized cells of *Aureobasidium pullulans* CCTCC M2012223 in aerobic fibrous bed bioreactor. J Chem Technol Biotechnol. 2013;88:1822–7. https://doi.org/10.1002/jctb.4033.

Zhou J, Mohamed Wali AR, Ma S, He Y, Yue D, Tang JZ, Gu Z. Tailoring the supramolecular structure of guanidinylated pullulan toward enhanced genetic photodynamic therapy. Biomacromolecules. 2018;19:2214–26.

Scleroglucan and Schizophyllan

14

Microbial Polysaccharides of Functional Importance

Hafiz Ubaid ur Rahman, Waqas Asghar, and Nauman Khalid

Contents

Abstract

Microbial exopolysaccharides, particularly scleroglucan and schizophyllan, have shown outstanding potential for use in food and medical/pharmaceutical industries. Scleroglucan and schizophyllan are obtained from fungal sources and their exclusive physico-chemical attributes offer a wide range of applications as stabilizers, gelling agents, thickeners, carriers of nutraceuticals, drugs and other bioactive compounds, prebiotics, immunostimulants, immunomodulators, antimicrobial and antiviral agents, hypoglycemic and hypocholesterolemic substances, and excipients. Additionally, several potential applications of these exopolysaccharides are yet to be explored for the development of functional foods and commercial pharmaceuticals. This chapter provides a comprehensive overview about the structural description, sources, physicochemical properties,

H. U. u. Rahman · W. Asghar · N. Khalid (✉)
School of Food and Agricultural Sciences, University of Management and Technology, Lahore, Pakistan
e-mail: nauman.khalid@umt.edu.pk

J. M. Oliveira et al. (eds.), *Polysaccharides of Microbial Origin*,
https://doi.org/10.1007/978-3-030-42215-8_16

production, isolation, and recovery of scleroglucan and schizophyllan. Moreover, potential applications of these exopolysaccharides for the development of functional foods, edible coatings and delivery systems for drugs, nutraceuticals, and bioactive substances have been discussed in detail. Furthermore, various health-promoting aspects of scleroglucan and schizophyllan in controlling lifestyle-related disorders (hyperglycemia and hypercholesterolemia) have also been conversed in this treatise.

Keywords

Microbial exopolysaccharides · Scleroglucan · Schizophyllan · Biomedical applications · Functional foods

1 Introduction

Polysaccharides have been extensively distributed in the nature and are being obtained by all organisms of animal, plant, and microbial origin. These functional molecules have been associated with various biological functionalities such as cell wall formation (cellulose), energy storage (starch), and cellular communications (glycosaminoglycans) (Ates 2015). Polysaccharides are complex macromolecules having very high molecular weights reaching up to millions of Daltons. Generally, polysaccharides are classified as homo- and hetero-polymers, contain either anionic (uronic acid) or neutral sugars (pentoses and hexoses), and sometimes are linked with nonsugar components as conjugated molecules (Jindal and Khattar 2018).

Mostly, polysaccharides of industrial importance are obtained from plants or seaweeds; however, microbial polysaccharides have also been emerged as quite useful biopolymers due to their unique physico-chemical attributes. In addition, polysaccharides obtained from microbial origin have also been used as alternatives to natural gums such as carrageenan and Gum Arabic (Milani and Maleki 2012; Shit and Shah 2014). Some key advantages of using microbial polysaccharides over plant-based or synthetic polysaccharides are discussed below:

- Production of microbial polysaccharides is not influenced by regional or climatic factors.
- Recovery of microbial polysaccharides is a quite easy and quick process with special reference to optimization of the process and rate of production.
- Production of polysaccharides with required properties is easy through genetic modifications of microorganisms.
- The materials used as growth media for microorganisms are simple and safe.
- Production of microbial polysaccharides is quite cheap and environment friendly as food industrial wastes can also be used as microbial growth medium (Ladrat et al. 2014).

Microbial polysaccharides are generally classified as intracellular and extracellular polysaccharides; however, extracellular polysaccharides have been used more

extensively in the food processing industries as compared to the intracellular microbial polysaccharides (Patel et al. 2010). The extracellular polysaccharides of microbial origin are either attached on the cellular surfaces as sheath/capsules or can be secreted by microbial cells as slime (Madhuri and Prabhakar 2014). The polysaccharides have different structural characteristics depending upon their mode of cellular production. For example, the sheath exists in the form of an electron-dense, thin layer surrounding the microbial cells, or colonies, whereas the capsules are present in the form of a slimy and relatively thick layer on the cell surface having sharp outlines containing exudate particles. Additionally, the slime is released into the medium in the form of water-soluble materials (Li et al. 2001). The water-soluble microbial polysaccharides (slime) can be easily extracted/isolated from the medium as compared to the sheaths or capsules, therefore, have more attraction for the processors as compared to those microbial polysaccharides which remain attached on the cellular surfaces.

Microorganisms produce a wide range of polysaccharides. The list of polysaccharides obtained from various microorganisms along with their industrial applications is provided in Table 1. Accordingly, the present chapter is mainly focused on the physico-chemical properties, structural attributes, production, identification, isolation, characterization, and industrial applications of scleroglucan and schizophyllan with special reference to food and medicinal importance.

2 Structural Description

The main features associated with the primary structure of polysaccharides include presence of various sugars, phosphate or sulfate containing substitute groups, specific sequence of sugars, size of furanose or pyranose rings, α or β configurations, types of glycosidic linkages, and absolute configuration (d or l). Polysaccharides have different secondary structures, exhibiting a specific set of helix parameters. For instance, type-A helix has a ribbon-like structure which is a distinctive property of structural polysaccharides. These polysaccharides have β-(1,4) linkage and strong hydrogen bonding. Storage polysaccharides have type-B helical structure with less compactness and higher number of residues (8) per turn. The polysaccharides containing type-C helix have coil-like flexible structure which is formed by joining several monomers with β-(1,2) linkages (Venugopal 2011).

The two most common examples of exopolysaccharides of fungal origin are scleroglucan and schizophyllan. Both of these compounds are the examples of beta-glucans and these are also regarded as neutral exopolysaccharides. Scleroglucan is mainly produced by *Sclerotium rolfsii*, (Survase et al. 2007b), whereas schizophyllan is naturally produced by *Schizophyllum commune* (Zhang et al. 2013a). These fungal exopolysaccharides contain a branched backbone of β-(1→3)-D-glucopyranose molecule with β-(1→6)-D-glucopyranose residue located at every third glucose unit of the structure (Moscovici 2015). The structural configuration of both exopolysaccharides is quite similar; however, scleroglucan has

Table 1 Examples of microbial polysaccharides and their potential applications

Example of polysaccharide	Microbial source	Industrial applications
Glucan	*Saccharomyces cerevisiae*	Food thickener
Gellan	*Pseudomonas elodea*	Gelling agent in jams, jellies, glazes, frostings, and icings
Xanthan	*Xanthomonas campestris*	Oil recovery, pharmaceutical applications, cosmetics industry, gelling agent for ice creams, cheese spreads, and puddings
Levan	*Alcaligenes viscosus*	Functional biopolymer in food and pharmaceutical industries, food additive, skin moisturizer
Pullulan	*Aureobasidium pullulans*	Food additive to provide texture, inhibits fungal growth in foods
Alginate	*Azotobacter* and *Pseudomonas*	Stabilizer, thickening agent, gelling agent in dairy products, sauces, jams, and soups
Curdlan	*Alcaligenes faecalis*	Texture modifier in foods, viscosity improvement in foods, gelling agent
Emulsan	*Acinetobacter calcoaceticus*	Stabilizer, emulsifying agent
Dextran	*Leuconostoc mesenteroides*	Viscosity improvement in ice creams
Welan	*Alcaligenes* spp.	Thickening agent in beverages, salad dressings, jellies and dairy products, medical and pharmaceutical industry
Acetan	*Acetobacter xylinum*	Gelling agent in confectionary products
Kefiran	*Lactobacillus hilgardii*	Improves viscosity of foods
Scleroglucan	*Sclerotium*, *Sclerotinia*, and *Corticium*	Gelling agent, pharmaceutical industries, medical uses
Schizophyllan	*Schizophyllum commune*	Thickening agent, food preservative, pharmaceutical and medical applications

more intense branching as compared to schizophyllan with a glucose residue at every two β-1, 3-glucosyl units.

Scleroglucan is a homopolysaccharide having branched structure and it only gives D-glucose on hydrolysis. This polysaccharide mainly possesses a chain of 1→3-linked units of ß-D-glucopyranosyl. The chemical structure of scleroglucan was established through periodate oxidation and hydrolysis using selective glucanase enzymes. Hydrolysis of scleroglucan yields gentiobiose (one mole) for two moles of glucose, which affirmed the ratio of 1→3 and 1→6 glycosidic linkages. Additionally, methylation analysis is helpful in quantitative and qualitative determination of various types of glyosidic bonds present in the structure. Nuclear magnetic resonance (NMR) spectral analysis also confirmed the regular polymer structure corresponding to various types of glucose units. Moreover, light scattering technique has also been used for determining the molecular weights of scleroglucan. Dissolved chains of scleroglucan possess a rod-shaped triple helix structural conformation with

side groups of D-glucoside units which mainly prevent the aggregation of helices and provide structural integrity to the scleroglucan molecule (Brigand 2012).

Schizophyllan is a neutral exopolysaccharide produced by submerged culture of *Schizophyllum commune* (Zhang et al. 2013b). Studies involved in the enzymatic degradation have documented that the repeating unit of schizophyllan is comprised of three β-(1-3)-linked residues of D-glucopyranose residues having one β-(1-6) linkage. NMR spectral analysis also confirmed the structural configuration of schizophyllan with a regioselective cleavage of β-1,3 chain with β-l,6 side chains. The triple helix structural conformation of schizophyllan describes that the molecule has a rigid chain with rod-like structure having largest chain length (LpE200 nm). It has also been investigated that the rigid structure of schizophyllan is converted to a slightly flexible structure when the molecular weight exceeds 500 kDa. The triple helix is synthesized by convolution of three individual helices with β-(1-6) D-glucose side chains and the helix is stabilized by hydrogen bonding. Higher concentrations of schizophyllan develop a liquid crystalline phase (Zhang et al. 2013b). The mentioned structural properties are responsible for diverse applications of these microbial exopolysaccharides in food and medicinal industries.

3 Physicochemical Aspects

Microbial exopolysaccharides are ubiquitous in nature and composed of repeating units of sugar moieties attached to a lipid carrier. Functionality and properties of exopolysaccharides mainly depend on ecological niche of microorganisms and structural characteristics of polysaccharides. Additionally, the commercial applications of exopolysaccharides of microbial origin also depend on their physicochemical properties and mainly include food processing industries, pharmaceutical applications, detoxification, petrochemical industries, bioremediation, biotechnology, paint industry, etc. (Mishra and Jha 2013). This chapter is mainly focused on the two important exopolysaccharides of microbial origin: scleroglucan and schizophyllan. Hence, the physicochemical properties are also directed towards these two biomolecules. Gels produced from scleroglucan have vast stability as these gels can survive in the temperature, pH, and salt concentration ranges of 0–120 °C, 1–12.2, and 0–20%, respectively (Zhang et al. 2013b). These properties make scleroglucan a good candidate for various applications in food, pharmaceutical, and medical industries (Smelcerovic et al. 2008). Schizophyllan is a water-soluble and nonionic exopolysaccharide having wide applications such as thickening agent and food preservative in food industries, petroleum recovery, thickening substance in cosmetic creams and lotions, and pharmaceutical and medical applications. Some important properties of both exopolysaccharides have been discussed here.

3.1 Properties of Scleroglucan

Scleroglucan can be easily dissolved in cold and hot water. Addition of alkalis or acids does not impose significant effect on the properties of scleroglucan solution

over a pH range of 2.5–12. Moreover, the viscosity of the solution is not influenced by ionic species. The solution of scleroglucan is thermostable and possesses pseudoplastic behavior at higher concentrations. Scleroglucan also has significant emulsifying potential which makes it useful as stabilizer in food and cosmetic industries (Brigand 2012; Zhang et al. 2013a). The important characteristics of scleroglucan have been further elaborated as follows.

3.1.1 Properties of Scleroglucan Aqueous Solutions

Powdered or granular form of scleroglucan has the ability to disperse in water at all temperatures. However, scleroglucan powder must be added to the water slowly with high speed mechanical stirring to prevent lumping. Additionally, the rate of dissolution or dispersion can be increased by pretreatment of powder with alcohol. Likewise, the required maximum viscosity of the solution can be achieved in a shorter period of time by strong agitation while preparing the solution. Alternatively, the viscosity can be attained by keeping the solution for 24 h or heating the solution up to 90 °C followed by cooling. The pH range is also important to get desired viscous properties of the solution. The ideal pH range for viscosity development is 6.5–9. Studies have also shown that concentrated solutions have faster rates of viscosity development as compared to the diluted solutions (Brigand 2012).

3.1.2 Rheological Properties of Scleroglucan

Due to the high viscosity, scleroglucan solutions have high shear-thinning properties which provide good suspending characteristics to the solutions. The concentrated solutions of scleroglucan behave like pseudoplastic materials and have high practical yield. For example, 0.5% scleroglucan solution has the yield value of about 50 dynes/cm^2, while 1% solution contains a yield value of 150–200 dynes/cm^2. This high yield value along with shear-thinning characteristics of scleroglucan solution gives good pourability and suspending power to the solution. Additionally, high yield value of scleroglucan solutions is useful in stabilizing the emulsions. For instance, 0.25% solution containing equal parts of scleroglucan and a vegetable oil develops highly stable emulsion.

The viscous properties of scleroglucan solutions are not affected over a wide temperature range and remain constant between 15 and 90 °C. At lower temperature values, the scleroglucan solutions develop thermo-reversible gels. Furthermore, viscosity of scleroglucan solutions is not significantly affected at low pH values but pH value above 12.5 results in decreasing the viscosity due to the transition of triple helical structure to random coil-like structure. Scleroglucan is also quite stable in acidic medium and forms gels due to the breakdown of some glycosidic bonds and aggregation of triple helix. Moreover, at high electrolyte concentrations, scleroglucan solution flocculates and forms gels. Likewise, high alkaline conditions also result in the precipitation and formation of gels.

3.1.3 Physiological Properties of Scleroglucan

Bioefficacy studies based on animal modeling have described that intake of scleroglucan is not associated with any kind of toxicity, tissue pathology, or blood

abnormalities. Likewise, skin and eye tests did not exhibit any significant adverse effects on the skin or sensitization. Studies have also revealed that dietary administration of scleroglucan to the chicks is helpful in reducing the cholesterolemia and increasing the excretion of cholesterol and lipids. Additionally, scleroglucan also displays considerable antitumor capability.

3.2 Properties of Schizophyllan

3.2.1 Properties of Dilute Schizophyllan Solutions

Schizophyllan is fairly soluble in water and it dissolves in water as a triple helix with rod-like structure, whereas it is dispersed in the basic solutions like dimethyl sulphoxide solution in the form of single coil. It has also been studied that schizophyllan is completely dissolved in water at lower salt concentrations (0.01 M NaOH) without disturbing the helical structure and provides stability to the microgels. Dilute solutions of schizophyllan also show conformational transitions due to temperature variations. The conformational transitions at higher temperature are thermo-irreversible and mainly involve the dissociation of complex triple helical structure into random coils. Contrarily, the low-temperature conformational transitions are thermo-reversible and are associated with the dissociation of triple helices of schizophyllan aggregates or intramolecular conversion between type I and type II triple helices (Zhang et al. 2013b).

3.2.2 Behavior of Concentrated Schizophyllan Solutions

Generally, the shear rate at which the viscosity of a solution starts decreasing is lowered with increased concentration of the solution. It is reported that the dependence of molecular weight on the shear rate of schizophyllan solutions also becomes weaker with increased flexibility resulting due to the higher solution concentrations. However, the solutions containing rod-like structure of schizophyllan in the solution have stronger dependence on the molecular weight of the compounds as compared to the solutions containing flexible polymers of schizophyllan. Additionally, the behavior of the solutions is also changed as the concentration of schizophyllan is changed. For instance, at higher concentrations, schizophyllan produces liquid crystals with varied dependence of the viscosity on the concentration for different types of solutions. It is observed that at lower concentrations (2.33%), the solution remains isotropic in nature at ambient temperature, whereas at higher concentration (14.34%), the solution of schizophyllan shows cholesteric mesophase. It has also been investigated that the optical rotatory dispersion (ORD) of the concentrated schizophyllan solution is drastically changed upon cooling the solution to 5 °C due to the transition of isotropic phase to the cholesteric phase (Zhang et al. 2013b).

3.2.3 Gelation Behavior of Schizophyllan

Schizophyllan produces weak gels at lower temperatures, that is, below 6 °C (Zhang et al. 2013b). However, the addition of several compounds such as sorbitol or borax develops relatively stronger gels. Addition of borax provides strength to the

schizophyllan gels by making complexes between borate ions and hydroxyl groups of schizophyllan side chains. The rate of gel formation is directly related to the concentration of borax, salt, and schizophyllan in the solution, but inversely related to the temperature. In contrast to the schizophyllan-borate system, the gelation behavior of schizophyllan is quite different in case of sorbitol addition. It has been reported that addition of sorbitol leads to the reduced water mobility resulting in the aggregation of schizophyllan molecules to develop a three-dimensional network of gel. It has also been proposed that triple helical structure of schizophyllan is broken down in the presence of sorbitol and the broken chains are then re-associated through hydrogen bonding to form gel networks. Moreover, it has also been noticed that the temperature of the system is increased due to the increased concentration of the sorbitol. Another gelation mechanism of schizophyllan-sorbitol system involves the conformational transition of schizophyllan chains from type II to type I helix which increases the stiffness and diameter of the helices and reduces the mobility of water and schizophyllan molecules (Zhang et al. 2013b) resulting in chain enlargement and formation of strong gel network. These properties of the schizophyllan gels are helpful in developing the schizophyllan-protein co-gels and widening the applications of schizophyllan as gel metrices in food and pharmaceutical industries.

4 Production and Isolation of Scleroglucan and Schizophyllan

For the production microbial polysaccharides, the most extensively practiced method is fermentation due to its financial aspects and feasibility. Hence, optimization of the fermentation process for the production of exopolysaccharides of microbial origin is of prime importance and the cost of the production process can be significantly reduced by controlling several parameters such as temperature, pH, rate of agitation, concentration of oxygen, and compositional properties of culture medium. Additionally, the microbial strains used for the production of polysaccharides also influence their properties such as structural conformation, rheological and physicochemical attributes, and monomer composition (Lembre et al. 2012). Therefore, control of fermentation parameters and careful selection of feedstock and microbial strains are important to achieve the polysaccharides with required specifications.

4.1 Production and Isolation of Scleroglucan

Various species of *Sclerotium* such as *Sclerotium glucanicum*, *S. delphinii*, and *S. rolfsii* are involved in the extracellular production of scleroglucan (Farina et al. 2001; Survase et al. 2007). *Sclerotium* species are filamentous fungi and heterotrophic in nature. These fungi are also regarded as plant pathogens due to the oxalic acid producing ability which facilitates plant cell lysis. These fungal species possess several types of enzymes such as cellulases, arabinose, galactosidase, phosphatidase,

exo-galactanase, exo-mannase, and poly-galacturanase, etc. *Sclerotium* species contain light-colored mycelia, or black to brown-colored sclerotia which are resistant to biochemical and chemical degradation, but do not have the ability to sporulate. In liquid media, these organisms produce pellets having central capsules with extended hyphae. On solid media, aerial hyphae are produced and arranged in the mycelia. *Sclerotium* species produce scleroglucan which assists these organisms in the attachment to the surface of plants and also provides protection against harsh environmental conditions. Additionally, scleroglucan is also used by these organisms as energy source due to the activity of hydrolytic enzymes which degrade the biopolymer into glucose molecules. The optimum growth temperature for *Sclerotium* species is reported as 30 °C. Additionally, these organisms can tolerate lower sucrose (0.15–1.17 M) and salt (0.86 M) concentrations; however, higher concentrations cause growth inhibition (Farina et al. 1996).

4.1.1 Biosynthesis of Scleroglucan

The generic pathway for biosynthesis of scleroglucan involves three prime steps which include (a) uptake of the substrate, (b) intracellular production of polysaccharide, and (c) extrusion of the produced polysaccharide from the fungal cell. During the biosynthetic production of scleroglucan, glucose is firstly transferred to the cells by hexokinase enzyme and then phosphorylated by two enzymes, namely, phosphor-glucomutase (PGM) and phosphor-glucoisomerase (PGI). Afterwards, UDP-G (uridine diphosphate glucose) is produced due to the action of pyrophosphorylase (UGP) enzyme. UDP-G starts polymerization by reacting with the lipid carrier. The proposed schematics of the biosynthetic pathway of scleroglucan are illustrated in Fig. 1.

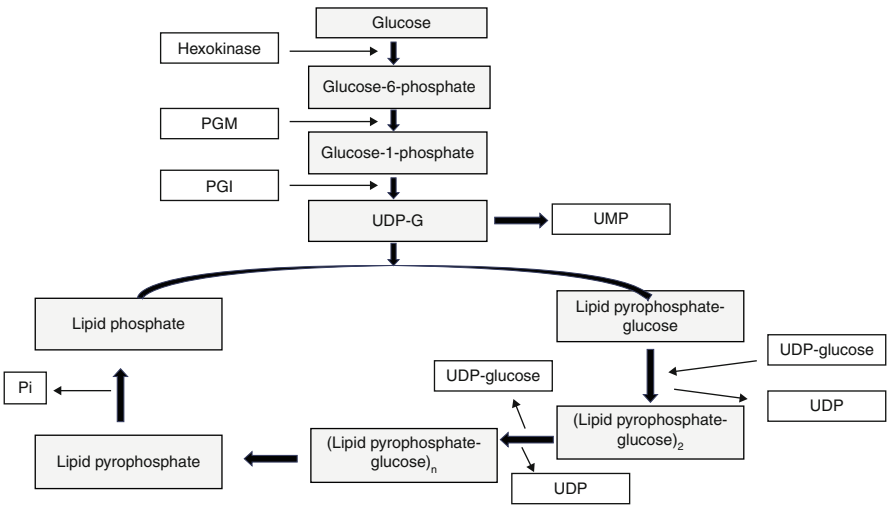


Fig. 1 Biosynthetic pathway of scleroglucan production

4.1.2 Fermentation Conditions for Scleroglucan Production

Generally, several factors influence the production of scleroglucan. These factors include preparation of inoculum, type of growth medium, environmental conditions, and byproduct formation. Additionally, the production of desired polysaccharide can also be enhanced by controlling the activities of undesirable enzymes and modifying the genetic determinants. The composition of growth medium is highly important for effective utilization of substrate by the microbial enzymes and transformation into the final product. Normally, the media with high carbon to nutrient (limiting nutrient) ratio is recommended for efficient production of polysaccharides. High yield fermentation processes generally convert 60–80% of consumed carbon source into crude biopolymer. The main nutrients needed by fungal cells include carbon, nitrogen, oxygen, phosphorus, potassium, sulfur, and magnesium. It has also been reported that addition of several precursor molecules improves the rate of polysaccharide synthesis by providing metabolic force. In general, nucleotide phosphate sugars improve the metabolic flux of exopolysaccharide production. Incorporating amino acids (L-lysine) and sugar nucleotides can be used as metabolic precursors for the production scleroglucan (Survase et al. 2007a).

Among various environmental factors, temperature is of prime importance to carry out the microbial activities smoothly. The optimum temperature for growth of culture is 28 °C (Wang and McNeil 1995a), whereas for the production of scleroglucan is 20–37 °C. It is also observed that the growth of culture is reduced below 28 °C due to excessive formation of oxalic acid which ultimately influences the yield of scleroglucan in an adverse way. Similarly, pH range also imposes significant effect on the growth culture as well as on scleroglucan production rate. The optimum pH value for effective fungal growth is 3.5, while production rate of scleroglucan is enhanced at pH value of 4.5. It is also documented that fermentative production of scleroglucan under these pH conditions results in 10% mitigation of byproduct formation (Wang and McNeil 1995b). Oxygen concentration also plays an important role in the growth of aerobic microorganisms. Studies have affirmed that high dissolved oxygen facilitates the growth and metabolic activities of *Sclerotium* species which promotes the reduction of carbon and formation of scleroglucan. Moreover, it has also been investigated that higher rate of aeration and agitation (600 rpm) facilitates the fungal growth by improved dissolution of oxygen and nutrient mixing; however, reducing the rate of agitation at later stage of fermentation enhances the scleroglucan synthesis because higher agitation can induce molecular degradation of the produced polysaccharide. The main byproduct during scleroglucan production is oxalic acid which in undesirable because oxalates block the biosynthetic pathway of scleroglucan production and need another purification step for its separation. Hence, pH adjustment is important to reduce the production of oxalic acid during fermentation. Generally, low pH values reportedly enhance the activity of oxalate decarboxylase enzyme which breaks down oxalate into carbon dioxide and formate and prevents the blockage of scleroglucan formation pathway.

4.1.3 Downstream Processing for Recovery of Scleroglucan

Optimizing all the fermentation conditions is not enough to ensure the good yield of scleroglucan, but another important step for obtaining high yield of scleroglucan is recovery of the product. The selection of suitable recovery method for exopolysaccharides mainly depends on several factors including properties of the organisms used for the production of exopolysaccharide and desired purity level of the product. Usually, the crude product is obtained by completely drying the whole fermentation broth. The actual product/polysaccharide can be separated by filtration or differential centrifugation. Additionally, drum drying or spray drying methods can be used for water and solvent removal. Sometimes, use of electrolytes is helpful in the precipitation of the polysaccharides by neutralization of the charges (Taurhesia and McNeil 1994).

In general, three different methods have been reported for the recovery of scleroglucan produced by fermentation process. A generalized illustration is provided in Fig. 2. The common step in all the three recovery processes is pretreatment of fermentation broth. Afterwards, the product has been recovered by different methods. The common pretreatment process includes neutralization of the fermentation broth with HCl or NaCl, three to fourfold dilution using distilled water, heat treatment for 30 min at 80 °C, homogenization of the diluted broth, and centrifugation (10,000 × g) for 30 min. After centrifugation, the pallets are obtained which are then subjected to washing process using distilled water followed by drying at 105 °C. The obtained supernatant is then further used for the recovery of final product, that is, scleroglucan.

The first method of scleroglucan recovery involves the cooling of supernatant to 5 °C followed by addition of isopropanol or ethanol (96%) for precipitation. The

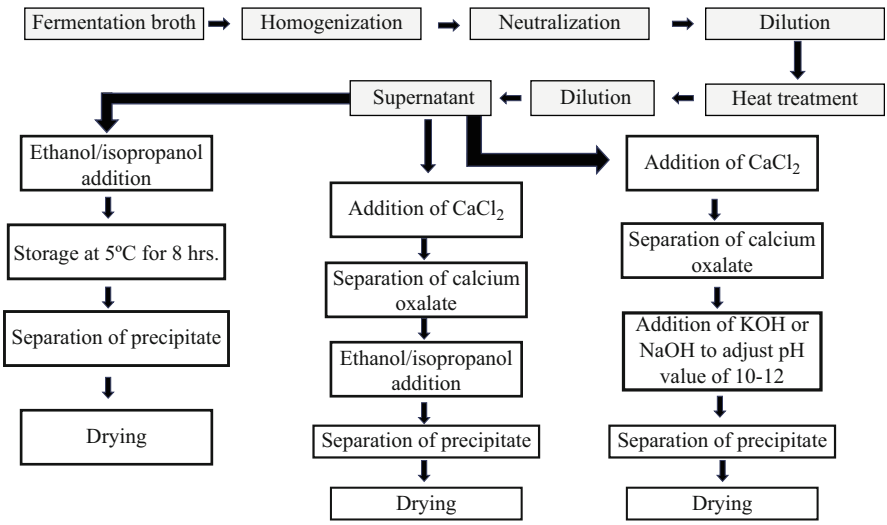


Fig. 2 Methods for recovery of scleroglucan

mixture is usually kept at this temperature for about 8 h to complete the precipitation of exopolysaccharide. After that, the scleroglucan is recovered through sieving and dissolved in distilled water. For better recovery, the crude mixture can be retreated with ethanol or isopropanol for reprecipitation. After that, the precipitated biopolymer can be freeze-dried or thermally dried at 55 °C for about 8 h. Finally, the product is milled to obtain whitish scleroglucan powder which is then packed for further marketing (Farina et al. 2001).

The second method used for the recovery of scleroglucan uses divalent cations such as calcium, iron, cobalt, magnesium, nickel, and manganese with organic solvent. Generally, calcium chloride is preferred for this purpose and can be used at concentration of 0.5–2.0%. Addition of calcium chloride to the fermentation broth results in the precipitation of calcium oxalate, which can be removed by filtration or centrifugation process. After that, 20–40% ethanol or isopropyl alcohol is added to the broth which results in further precipitation and the precipitates are again removed by filtration or centrifugation. The obtained scleroglucan is then purified through reprecipitation or rehydration processes.

In the third method of scleroglucan recovery, 0.5–2% calcium chloride is added to the fermentation broth and then metal hydroxides are added to the mixture to maintain the pH of system under alkaline conditions. After completion of the reaction, calcium oxalate precipitates are removed and metal hydroxides (sodium or potassium hydroxide) are added to adjust the pH value at 10–12. The polysaccharide will be precipitated under alkaline conditions which will then be removed by filtration or centrifugation. The purity of the product can be further increased by repeating the treatment.

4.2 Production and Isolation of Schizophyllan

The fungus *Schizophyllum commune* ATCC 38548 is reported to produce schizophyllan. The produced homoglucan is appeared in the form of gum which is attached with the microbial cell wall or secreted in the fermentation broth. Additionally, shear stress, applied through agitation generally, decreases the pellet growth and enhances the secretion of biopolymer from the fungal cell wall. However, studies have reported that too high shear force results in the damage of fungal hyphae production of schizophyllan. The resultant cellular debris is subjected to downstream processing for separation of the product. The growth medium used for bioreactor cultivation of *Schizophyllum commune* mainly consists of deionized water, glucose, yeast extract, KH_2PO_4, and $MgSO_4{\cdot}7H_2O$. The optimum temperature and pH values for cultivation of this fungus are 27 °C and 5.3 (initial pH value). Generally, cultivation of fungal culture is carried out using bioreactors in 42 L capacity vessel with a working volume of 30 L. The medium is sterilized and then 5% inoculum is added to start the growth and fermentation activities (Rau 2008). A generalized process for production and isolation of schizophyllan is depicted in Fig. 3.

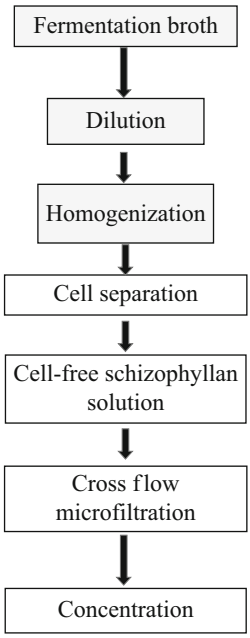


Fig. 3 Production and isolation of schizophyllan

5 Applications for Scleroglucan and Schizophyllan

Microbial exopolysaccharides (EPS) have found applications across a wide variety of domains, including the food industry, pharmaceuticals, biomedical solutions, and in the oil (petroleum) drilling operations. Being structurally versatile, these molecules exhibit diverse structural combinations, endowing them with unique properties. Conventional sources of polysaccharides included marine algae, higher plants, or animals, albeit with low resultant yields and high cost. However, microbial fermentation processes at commercial scale have emerged as a viable, industry significant, high yield, high purity, nontoxic, and biocompatible alternative (Survase et al. 2007) and consequently, have paved the way for greater utilization of these highly valuable biomolecules. Moreover, these microbial exopolysaccharides have a low environmental footprint and can be obtained from easily available and renewable natural resources (Survase et al. 2007a), as well as greater stability at elevated temperatures, and pH and salinity extremes (Brigand 2012; Jindal and Khattar 2018), thereby increasing their scope for utilization manifold.

Apart from the inherent thermal stability characteristics, the research on solutions of scleroglucan (SG) has revealed a pseudoplastic behavior with a high yield value, rendering these solutions with high suspending power and excellent pouring properties. Consequently, this high yield value enables scleroglucan to be highly effective at keeping particles in suspension, in both static and dynamic conditions, and

without any risk of sedimentation (Wüstenberg 2015). Scleroglucan (trade names include actigum, clearogel, polytetran, polytran FS, and sclerogum), with its excellent emulsifying capabilities, could be very suitable as a foam stabilizer. Moreover, its compatibility, without synergism, with various other thickening agents of hydrocolloid nature is also notable. Furthermore, scleroglucan has also shown compatibility with some of the most abundantly used surfactants, for example, sulfates, sulfonates, and quaternary ammonium salts. Also, scleroglucan has been recorded to be soluble even in mixtures consisting of almost 50% of polyols and glycols. With its exceptional rheological properties, as well as stability over a broad range of pH, salinity, and temperature conditions, scleroglucan is suitable for many applications (Wüstenberg 2015).

Both scleroglucan and schizophyllan (SPG), over the years, have grown in significance owing to their superior functionality for various applications. For instance, scleroglucan found its initial utility in the oil industry, displaying greater stability (compared to xanthan that is currently in use) to shear stress, and over a broad range of temperature (20–90 °C), salinity, and pH conditions, imparting enhanced viscosity characteristics, and therefore, improving the hydraulic pressure of the seawater or brine used for oil recovery procedures. Moreover, scleroglucan has also proven to be useful as an oil mud thickener and stabilizer, as well as a biopolymer with low sensitivity to field contaminants (Survase et al. 2007; Gao 2016; Liang et al. 2019). More recently, schizophyllan (trade name sizofiran, or sonifilan) has also been employed for enhanced oil recovery (EOR) or tertiary oil recovery processes in the oilfields, proved to be stable under the high temperature and high salinity conditions, and could substitute partially hydrolyzed polyacrylamide (HPAM) and xanthan gums for commercial EOR projects (Gao 2016; Liang et al. 2019).

A recent application involved the use of scleroglucan as an emulsion stabilizer in bitumen formulations for road surfacing (El Asjadi et al. 2018). Refined scleroglucan has also been used in cosmetics manufacturing, in particular, hair control conditioning products and various skin care preparations such as creams, protective lotions. Similarly, schizophyllan has also been used in skin care products such as skin antiaging and healing preparations (cosmeceuticals), as well as an active ingredient in dermatological formulations aimed at reduction in skin irritation and recovery from sunburns (Gao 2016). Furthermore, scleroglucan has also found applications in the manufacturing of products such as adhesives, paints, watercolors, ceramic glazes, printing ink, various pesticides, and as a stabilizer in fire drencher foams. The utilization of these multipurpose and versatile biopolymers has also been documented for both food, biomedical, and pharmaceutical industries, and the proceeding paragraphs involve a discussion regarding their role in these industries in greater detail.

5.1 Applications in the Food Industry

Worldwide, the food industry uses several thousand tons of polysaccharides every year, and as food product innovation requirements continually demand a host of new,

versatile, and potentially low-cost additives, microbial exopolysaccharides are becoming ubiquitous for various applications in the food industry. Polysaccharide gums such as modified starches, cellulose, curdlan, pectin, guar gum, pullulan, alginates, carrageenans, chitosan, gellan, and xanthan are a few examples being extensively used. In particular, xanthan has become an ingredient of great significance over the years and is being used as a thickening, gelling, and stabilizing agent in a variety of food products including jams, marmalades, soups, confectionery products, aqueous gels, frozen foods, yogurt, ice creams, and low calorie/nonfat products (Survase et al. 2007b; Goff and Guo 2019). However, the exploration for better alternatives continues with research on various polysaccharide-producing strains and novel microbial biopolymers.

5.1.1 As Stabilizers, Viscosifiers/Thickeners, and Gelling Agents

In recent years, scleroglucan has been proposed as a possible substitute for xanthan, especially suitable for food manufacturing operations involving processing at elevated temperatures, owing to its greater thermal stability and process efficiency. Scleroglucan could, therefore, be ideally suited as a hydrocolloid, for stabilizing and viscosifying food products such as salad dressings, sauces, ice creams, and other desserts, as well as prevention of water loss (preservation of retrogradation) (Selvi et al. 2020). The stabilizing properties of scleroglucan were investigated in cooked starch pastes and the results indicated that scleroglucan can retain water in significant amounts, thus reducing syneresis during refrigeration. Moreover, there was evidence that the retention could be improved further by blending scleroglucan with corn starch before adding to the pastes (Selvi et al. 2020).

Although, not inherently a surfactant, scleroglucan has the potential to stabilize oil-in-water (O/W) emulsions as well, by preventing coalescence (Giavasis 2013), to date, scleroglucan has not been approved either by the European Food Safety Authority (EFSA) or the US Food and Drug Administration (FDA), a major impediment in the way of its application on a commercial scale. Many Japanese patents, however, indicate growing acceptance in the form of application of scleroglucan for quality enhancements in a range of frozen/heat-treated and aerated food products, such as Japanese cakes, steamed edibles, rice crackers, and some bakery products (Brigand 2012; Jindal and Khattar 2018; Selvi et al. 2020), and there is increasing hope that scleroglucan could witness wider application in the food industry manufacturing processes worldwide. Since both scleroglucan and schizophyllan are structurally similar, as well as in terms of chemical properties, it is assumed that both can be used alternatively for applications in the food industry.

The tendency of schizophyllan with regards to the formation of co-gels with gelatin has been studied, and it was observed that the concentration of schizophyllan directly influenced the elasticity or rigidness of the gels (higher elasticity was recorded at low gelatin/schizophyllan ratio). Schizophyllan, therefore, can potentially be used for food applications where gelatin is already being utilized as a thickener. Scleroglucan has shown good compatibility with various electrolytes. However, gel formation or flocculation of the solution may take place at higher electrolyte concentrations. Scleroglucan, as already mentioned, is also compatible

with most of the commercially available thickeners, such as locust bean gum, alginates, xanthan, and carrageenans, as well as cellulose derivatives. Pure scleroglucan (∼90–98% EPS) solutions in the water at concentrations of 2 g/L, or lower, yielded highly viscous solutions with non-Newtonian, nonthixotropic, and pseudoplastic behavior (Survase et al. 2007b).

5.1.2 Preparation of Edible Films

Owing to its chemical stability, biocompatibility, and biodegradability, scleroglucan could be used in the preparation of edible films and tablets for nutraceuticals (Giavasis 2013). Similarly, schizophyllan, in combination with lentinan, a polysaccharide obtained from the edible Japanese shiitake mushroom, *Lentinus edodes*, has been studied for possible applications in novel food products and nutraceuticals (Giavasis 2013). The use of schizophyllan for the formation of films with low oxygen permeability, aimed at food preservation, has also been investigated and involved the study of the physicochemical characteristics of films formed from schizophyllan and a chemically modified polymer derived from it. Owing to high water solubility and exceptionally low oxygen permeability, the utility of such films as oxygen barriers (easily removable by water), is a promising prospect (Sutivisedsak et al. 2013).

5.1.3 Production of Functional Foods

A group of Japanese researchers, in a novel approach, added *Schizophyllum commune* (the source for schizophyllan) as a live starter culture for producing a functional cheese-like food. The fungal starter culture induced fermentation and coagulation of milk, owing to its lactate dehydrogenase and proteolytic and milk-clotting enzymes. These proteolytic and clotting enzymes demonstrated higher activity of proteases, albeit a significantly lower clotting activity compared to rennin. The final product consisted of approximately 0.58% β-glucans, which had a significant antithrombotic function (Giavasis 2014). *Schizophyllum commune* has also been significant in terms of the discovery of a special family of small secreted, and moderately hydrophobic proteins, with a structurally characteristic spacing of eight cysteine residues. These proteins have been named hydrophobins and are capable of self-assembly into amphipathic membranes, resulting in the transformation of the attributes of contact surfaces. A highly promising application of hydrophobins involves their use as stabilizers for edible foams and emulsions. The emulsions based on these proteins resemble lipids (fats) in terms of their taste and mouthfeel and, therefore, have the potential for applications not only as natural highly efficient stabilizers but also as fat replacements from emulated products, presenting the opportunities for the creation of novel dietetic foods (Shamtsyan 2016). Scleroglucan, likewise, due to its nondigestibility, acts as a prebiotic (fiber) and has been linked with the formulation of low-calorie foods (Giavasis 2014).

5.2 Biomedical/Pharmaceutical Applications

It has been known for hundreds of years that the consumption of many species of fungi can impart favorable benefits to human health, with the practice widely

prevalent in many of the Far East countries, such as China, Japan, Korea, and some parts of Russia, where they have been used either as dietary or medicinal supplements (Lemieszek and Rzeski 2012). More recently, though, the focus of the researchers has shifted towards the bioactive compounds present in these fungi and their potential impact on the functioning of the human body. This has led to these "mycopharmaceuticals," or "fungal supplements" being introduced to the markets worldwide (Lemieszek and Rzeski 2012). Moreover, the significance of such fungi has warranted their inclusion towards many dietary regimens as "functional foods." The bioactive compounds with the greatest potential in terms of biomedical applications are the β-glucan [(1→3), (1→6)] polysaccharides, including both scleroglucan and schizophyllan, among others.

5.2.1 As Immunostimulants in Anticancer Activities

The anticancer potential of schizophyllan and other similar β-glucan polysaccharides, including scleroglucan, can be attributed to its origin, composition (in terms of types of sugar present) and chemical structure (tertiary), molecular weight, solubility in water, glycosidic linkages, branching rate and form, presence of ligands (such as proteins), and the methods used for isolation. Moreover, the activity and clinical attributes of schizophyllan can be significantly enhanced through chemical modifications, most importantly the Smith degradation (oxido-reducto-hydrolysis), activation by formolysis, and carboxymethylation. Polysaccharides that are water-soluble, have high molecular weights, consist of more β-(1→3) linkages in the main glucan chain, with β-(1→6) at the branch points, and where the value of the degree of branching (DB) with regards to the molecular weight lies between 0.20 and 0.33 have demonstrated the highest biological activity (Lemieszek and Rzeski 2012).

The expression of the anticancer effects of β-glucan polysaccharides involves both direct (cell proliferation inhibition and/or induction of apoptosis) and indirect (immunostimulation) stimulation mechanisms. Indirect action manifests in the stimulation of host defense mechanisms, in particular, the activation of both T and B lymphocytes (tumor-infiltrating), macrophages, Langerhans cells (LCs), polymorphonuclear leukocytes (PMLs), lymphokine-activated killer (LAK) cells, and the natural killer (NK) cells [(also known as the large granular lymphocytes (LGLs)]. Moreover, these polysaccharides are also known to trigger the production of interferons (IFNs), interleukins (ILs), and other cytokines, often considered as the first line of defense in the host immune systems, as well as colony-stimulating factors (CSFs) by specialized receptor-mediated [Toll-like receptor (TLR), dectin-1, and complement receptor 3 (CR3)] induction of gene expression. A host of in vitro and in vivo studies also indicates a direct action of polysaccharides on cancer cells, whereby they have been found to either inhibit the proliferation of tumor cells or cause their death by apoptosis [characterized by the activity of caspase (casp)-3, and the ensuing cleavage of poly (ADP-ribose) polymerase (PARP)] (Lemieszek and Rzeski 2012).

The mechanism for this direct action involves primarily the modulation of the nuclear factor kappa B (NF-**κB**) pathway. Disproportionate activation of NF-**κB**, a proinflammatory inducible transcription factor, has been observed in many types of

cancer, and its activity has direct consequences in the form of promotion of tumor growth owing to increased transcription of genes, therefore, inducing proliferation of cancer cells, inhibition of apoptosis, as well as stimulation of angiogenesis and metastasis. The polysaccharides tend to inhibit the phosphorylation, ubiquitinylation, and degradation of the NF-**κB** inhibitor, **IκBα**, thereby preventing the activation of NF-**κB**, and ultimately, the expression of its subordinate genes (Lemieszek and Rzeski 2012; Giridharan and Srinivasan 2018). Another pathway for the direct action of polysaccharides involves polysaccharopeptide (PSP), a protein complex isolated from the common Turkey Tail fungus, *Trametes versicolor*, and has been shown to induce cell cycle arrest at restrictive points G1/S and G2/M in human myeloid leukemia cells U-937 and human breast cancer cells MDA-MB-231, thereby, also inhibiting the antiapoptotic proteins, resulting in suppression of cell division processes and a marked increase in apoptosis (Lemieszek and Rzeski 2012).

Schizophyllan in conjunction with other anticancer drugs has proven effective for the treatment of cancers of the stomach, lungs, breast, cervix, etc., as well as the prevention of metastasis and side effects associated with chemotherapy treatments (Rakhra et al. 2020). However, the mode of action of schizophyllan specifically does not directly involve the cytotoxicity against cancer cells. Instead, the anticancer activity of schizophyllan manifests by the way of stimulating the secretion of the proinflammatory cytokine, TNF-α, by cells of the human immune system, such as monocytes, macrophages, and platelets (Nie et al. 2013). The bioactivity against various cancers in the case of schizophyllan can be attributed to its high molecular weight, triple-helix conformation, and according to some other studies, its random coil configuration. Moreover, the route of administration could be another significant factor to elucidate the high levels of bioactivity attributed to schizophyllan and other β-glucan polysaccharides (Rakhra et al. 2020).

Scleroglucan, in its immunopharmacological form, Sinophilan, has also found applications for the clinical treatment of various cancers. The antitumor activity of scleroglucan is thought to be mediated by an increase in the number of macrophages in the presence of soluble glucan. Scleroglucan reportedly has a high affinity for human monocytic cells and has two main biological attributes, that is, the stimulation of phagocytic cells and the hemopoietic activity associated with monocytes, neutrophils, and platelets. The polysaccharide exhibited immunomodulating activities against cancerous tumor formation when it was administered either parenterally or orally. The oral administration characteristics of scleroglucan are unique among other glucans and cater favorably in terms of fewer side effects, as well as a significant reduction in pain. Moreover, the oral administration pathway presents the opportunities for incorporation of this biopolymer into foods designed for cancer patients (nutraceuticals), or the individuals at risk of developing the malignancies (Giavasis 2013).

5.2.2 As Immunomodulators in Antitumor Activities

The antitumor action of schizophyllan is dependent on its capability to modulate immune responses (taking place only in the presence of T cells), and according to many reports, by the way of its immune-stimulating properties, it tends to enhance

the ability of immune cells for identification and recognition of tumor cells as foreign entities, thereby increasing and consolidating the effectiveness of the host defense mechanisms. Schizophyllan induces the production of acute-phase proteins (APPs) and colony-stimulating factors, thereby triggering the proliferation of macrophages, peripheral blood mononuclear cells (PBMCs), as well as lymphocytes, and stimulation of the complement system (also termed as complement cascade). Also, this manifests in an increase in the production of the T Helper (Th) lymphocytes and macrophages, indicated by the strong activation of phagocytes, and this, in turn, contributes to increased production of reactive oxygen species (ROS), nitric oxide (NO), proinflammatory cytokines IL-6, IL-8, IL-12, and tumor necrosis factor (TNF-α), as well as increased expression of CD11b and CD69L markers on the surface of leukocytes (Lemieszek and Rzeski 2012).

Schizophyllan was reported to demonstrate antitumor activity against both the solid and ascites forms of Sarcoma 180, as well as sarcoma 37 (the solid form only), Ehrlich-Lettre ascites sarcoma (EAC), Yoshida sarcoma, and Lewis lung carcinoma (LLC). The antitumor activity of schizophyllan was not detected in the thymectomized (thymus gland removed) individuals, indicating that the activity is T-cell mediated. Formyl methylated and aminoethylated derivatives of schizophyllan exhibited enhanced levels of antitumor activities and increased production for both the factors associated with tumor regression and the soluble cytotoxic factors when compared with nonderivatized schizophyllan (Nie et al. 2013). Pretreatment with schizophyllan did not exhibit a significant effect on the survival rates of sarcoma 180 xenografts. However, pre- and post-treatment with the polysaccharide revealed to have increased the chances of survival. The underlying antitumor mechanism involved in these studies can be linked to the improved cellular immunity, resulting from increased production of T lymphocytes, as well as that of the cytokines, interleukin-2, and interferon-γ, generated by the PMBCs, with the ensuing tumor inhibition estimated to be around 95% (Banerjee et al. 2015).

Schizophyllan has been widely approved for clinical applications as a biological response modifier (BRM), as well as a nonspecific stimulator of the immune system in Japan, where multiple pharmaceutical companies are involved in the commercial manufacture of this polysaccharide as immunopotentiators for clinical treatment of patients undergoing various antitumor therapies. The use of schizophyllan, as an immunoadjuvant, in combination with conventional radiotherapy and chemotherapy (antineoplastic drugs) treatments [tegafur or mitomycin C, and 5-fluorouracil (5-FU)], in patients with recurrent and inoperable gastric cancer, has shown a significant improvement in median survival rates, without, however, any significant influence on the size of tumors (Rakhra et al. 2020). Schizophyllan appears to function in terms of controlling or eliminating the microscopic metastatic foci and/or small-sized macroscopic foci observed in stage III patients (Banerjee et al. 2015).

Schizophyllan has also been shown to increase the life span for patients with cancers of the head and neck, aiding in the accelerated recovery of cells that are immunocompromised as a result of radiation, chemotherapy treatments, and surgical procedures. Schizophyllan enhanced the immune-associated function of the regional lymph nodes in these patients, as evidenced by the elevated production of IL-2,

increased cytotoxic activity of effector cells (NK, LAK), as well as the increased numbers of Th lymphocytes (CD4+) in the tumor-uninvolved lymph nodes. However, a subsequent phase II, two-arm, randomized controlled trial (RCT) revealed that the clinical efficacy and tolerability of schizophyllan coupled with standard chemotherapy treatment, in a patient cohort with locally advanced head and neck squamous cell carcinoma, exhibited no significant difference in terms of overall response rate. Although, a comparatively greater number of complete responses (CR) were recorded in the case of Arm A (schizophyllan combined with chemotherapy), against Arm B (chemotherapy treatment regimen alone) (Banerjee et al. 2015).

Schizophyllan is a potent BRM in terms of augmenting the immune function of regional lymph nodes in cervical cancer patients. When combined with radiotherapy treatments, schizophyllan significantly improved the survival time, as well as the time to recurrence (TTR) in stage II cervical cancer patients. However, the effect was not very well pronounced in the patients at Stage III. Another investigation reported that in comparison to the control group, a much higher complete response rate was recorded among patients for both stage II and III. Moreover, the lymphocyte counts that had suffered due to radiotherapy treatments showed rapid and significant improvement. An RCT involving 312 patients being treated with surgery, radiotherapy, chemotherapy (fluorouracil), and schizophyllan in various combinations, the patients who received schizophyllan survived longer. Another prospective RCT is recruiting participants (cervical cancer patients), aged between 20 and 75 years, in Seoul, the Republic of Korea, for a study aimed at evaluation of the impact of schizophyllan (sonifilan) on the quality of life (QOL) during radiation, and concurrent chemoradiotherapy, as well as the complications associated with the treatment involving schizophyllan (Banerjee et al. 2015). The study can be accessed at https://clinicaltrials.gov/ (identifier: NCT01926821).

In the case of chemically induced breast carcinomas, schizophyllan inhibited the incidence of tumors, also leading to reduced cell proliferation in tumor tissue. Schizophyllan and tamoxifen (a drug used for the prevention of breast cancer in women and treatment of breast cancer in both women and men) significantly reduced the incidence of mammary tumors induced by dimethylbenz(α)anthracene, in many cases up to 85% and 75%, respectively, as well as contributed to the suppression of the proliferating cell nuclear antigen (PCNA) labeling index relative to dimethylbenz (α)anthracene. After being exposed to the ultrasound procedures, the immunopotentiating activity of schizophyllan in the context of splenic lymphocytes and RAW264.7 macrophages as well as its antiproliferative action concerning human breast carcinoma T-47D cells increased noticeably (Wong et al. 2020).

In ovarian cancer patients undergoing chemotherapy, schizophyllan has been found to be beneficial against myelosuppression by chemotherapy (Banerjee et al. 2015). Schizophyllan has also been attributed with possessing antiradiation properties, as it has demonstrated the ability to restore mitosis in bone marrow cells that were previously suppressed owing to treatment regimens involving antitumor drugs. Early administration of schizophyllan, either before the initiation of chemotherapy or irradiation, or concurrently for the duration of treatments, has been shown to improve their overall effectiveness (Lemieszek and Rzeski 2012). The radioprotective effects in

bone marrow cell cultures can be attributed to the ability of schizophyllan to induce the expression of macrophage colony-stimulating factor (M-CSF) (Schepetkin and Quinn 2011). Also known as CSF-1 (encoded by the CSF1 gene), this hematopoietic growth factor plays a vital role in promoting the proliferation, differentiation, functional activation, and survival of the cells of the monocyte/macrophage family, reorganization of the cytoskeletal system, and ultimately, the cell spreading and migration of mature osteoclasts (Plotkin and Bivi 2014).

5.2.3 As Antiviral and Antimicrobial Agents

Scleroglucan and schizophyllan have also demonstrated activity against various infectious maladies. In this regard, scleroglucan is significant for its antiviral effect, in particular against rubella (German measles) virus and the herpes simplex virus type 1 (HSV-1) (Survase et al. 2007). Scleroglucan was crucial not only in inhibiting the initial phases of rubella virus infection but in blocking HSV-1 infection as well, in the course of the very early phases of the viral replication (Freitas et al. 2015). The mode of action seems to involve the binding of scleroglucan to the host cell membrane. This binding of the polysaccharide to the glycoproteins of the host cell membrane may reduce or even prevent the viral attachment and the subsequent penetration into the cell. This should be noted, however, that the inhibitory action of scleroglucan has been found to occur only at the early stages of these infections. The key mechanism appears to be that the binding of scleroglucan manifests in disruption of the complex interplay between the virus and the host cell plasma. A further plausible explanation for the antiviral activity of scleroglucan can be that, following the entry of the virus into the cell, the polysaccharide is also internalized in the cell, consequently encapsulating the virus and thus inhibiting its activity. However, this course of action is not deemed possible for polysaccharides with high molecular masses, such as scleroglucan (Survase et al. 2007).

The oral mode of administration of scleroglucan is significant in terms of its antimicrobial action against, for instance, fungal pathogens. The protective action following the ingestion involves the uptake of minute particles of the polysaccharide by the pinocytic microfold cells (or M cells) located in the gut-associated lymphoid tissue (GALT) of the Peyer's patches in the small intestine. Scleroglucan directly binds to the intestinal epithelial cells and the GALT cells, in turn getting internalized by the two cohorts of cells. Once these cells have been activated, they can migrate to the lymph nodes and trigger the activation of other macrophages, NK cells, and T lymphocytes via the release of cytokines. An example of such a phenomenon has been observed in the case of low molecular weight scleroglucan hydrolysates (<5 kDa) stimulating the activation and maturation of dendritic cells derived from monocytes in pigs, primarily through upregulation of CD40, CD80, and CD86 costimulatory proteins (Schepetkin and Quinn 2011).

The stimulation of the dendritic cells with β-glucan polysaccharides such as scleroglucan and schizophyllan induces translocation of dectin-1 to lipid microdomains (also known as lipid "rafts") of the plasma membrane. Molecular patterns of β-glucans bind categorically to two specific classes of pattern recognition receptors in the phagocyte membranes, namely, the Toll-like receptors (TLRs), the C-

lectin-like receptors (CLRs) such as dectin-1 (also known as C-type lectin domain family 7 member A, or CLEC7A). This results in the initiation of the signaling responses, eventually culminating in the release of pro- and anti-inflammatory cytokines, linking up of the innate immune response (IIR) and the adaptive immune response (AIR), and the initiation of processes, such as phagocytosis and intracellular killing. Lipid raft microdomains are considered to be the facilitators of the dectin-1 signaling mechanism, as the activation of dectin-1 results in the recruitment of two key signaling molecules, spleen tyrosine kinase (Syk), and phospholipase Cγ2, to lipid rafts. Downstream signaling pathways promote the activation of NF-**κB** by the way of a Syk-mediated pathway involving the signaling via the nuclear factor of activated T cells (NFAT). This activation of NF-**κB** induces a cascade of immune defenses for the protection of the organisms against various viral, bacterial, and fungal challenges (Bermejo-Jambrina et al. 2018).

Schizophyllan has also been found to be effective as an immunostimulator against tuberculosis (caused by *Mycobacterium tuberculosis*), infections perpetrated by *Staphylococcus sp.*, and *Listeria monocytogenes*, as well as various other microbial infections. Similar to scleroglucan, the antimicrobial activity of schizophyllan is regulated by the augmentation of phagocytosis of invading microorganisms by neutrophils and macrophages. Schizophyllan has also shown significant antiviral activity against the Sendai virus (SeV), now known as murine respirovirus, when administered both orally and intraperitoneally (IP). Moreover, sizofiran, the commercial type of pharmacological schizophyllan, has exhibited the ability to stimulate the immune responses of patients with hepatitis B virus, by enhancing interferon-γ levels, as well as the proliferative response of PBMCs (Survase et al. 2007; Giavasis 2014). Also, there is evidence that sulfated schizophyllans can inhibit the growth of human immunodeficiency virus (HIV), owing to their sulfur content. In the case of the use of schizophyllan for HIV treatment, this sulfur constituent, when compared to the molecular weight or the chemical nature of the polysaccharide component, has been found to be the decisive factor (Survase et al. 2007).

5.2.4 Hypocholesterolemic and Hypoglycemic Effects

Both scleroglucan and schizophyllan, along with many other β-glucans, are also notable for their potential in terms of blood serum cholesterol-lowering action, as well as for their hypolipidemic/hypocholesterolemic and hypoglycemic effects, often acting synergistically. The regulation of these hypolipidemic/hypocholesterolemic effects occurs by the way of the interruption of the enterohepatic circulation of bile acids, increased viscosity in the small intestine (promoted by biopolymers), and hence, decreased absorption of cholesterol and triacylglycerols (TAGs), ultimately resulting in increased removal in the feces. The hypoglycemic effects and therefore, the regulation of blood glucose levels, however, are propagated by the attachment of the indigestible β-glucan biopolymers to the intestinal surface which slows down glucose absorption (Giavasis 2014). The hypocholesterolemic and hypoglycemic activity of these polysaccharides holds the key to designing specialized therapeutic foods and nutritional supplements for obese individuals, as well as diabetics.

5.2.5 Excipients

Excipients, or pharmaceutical inactive ingredients, are integral constituents/components utilized by the pharmaceutical industry for the formulation of active ingredients into finished dosage forms. In a nutshell, excipients are additives that provide a matrix for the handling of the active pharmaceutical ingredients (APIs), or pharmacologically active substances, consequently ensuring an efficient drug delivery. Excipients range between 15% and 99% of the total weight of a given formulation and are highly significant for the drug production processes. They not only increase the bulk of the formulation, but also impart and influence various desired attributes, such as processability, aesthetics, performance, and/or aspects of patient compliance associated with the dosage form (Freitas et al. 2015; Dave 2019). The conventional procedures for synthesis or chemical modification of excipients make use of natural molecules as starting points, for example, cellulose or starch derivatives, as well as synthetic polymers. However, the utilization of entirely natural excipients remains elusive. These natural excipients are superior to their other less natural counterparts, in terms of safety, nontoxicity, biocompatibility, and biodegradability. Keeping this in view, polysaccharides derived from various sources, including yeast and fungi, have been proposed for the development of versatile excipients with improved properties (Freitas et al. 2015).

Native or natural scleroglucan has been extensively used in the pharmaceutical industry, for applications including tablet coatings, ophthalmic/ocular solutions, injectable antibiotic suspensions, and calamine lotion. Scleroglucan has been used in tablets and capsules, for its suitability of use in the formulation of sustained-release, oral dosage forms. The major advantages of using scleroglucan, apart from its controlled release properties, are compatibility, biodegradability, bioadhesiveness, and a broad range of thermal and chemical stability characteristics. Furthermore, the carboxylated derivative of scleroglucan, sclerox (S-1.0), is being used as a matrix for drug delivery in the form of tablets or films. Co-crosslinked scleroglucan/gellan can also be applied for drug delivery (Freitas et al. 2015).

5.2.6 As Carriers for Targeted Delivery of Drugs and Bioactive Compounds

Over the years, natural, nontoxic, and biodegradable polysaccharides have been explored for the development of targeted drug delivery (TDD) agents, chiefly in the form of hydrogels. These hydrogels (and associated nanoparticles) have garnered considerable interest, for their various potential applications in the domains of medicine and pharmacy, for example, as drug carriers, and in the development of biomimetic materials, biosensors, artificial muscles, and environmentally responsive or smart materials, and chemical separations (Freitas et al. 2015). Hydrogels, essentially, are three-dimensional (3D) polymeric crosslinked networks with high water-absorption capacities and controlled drug release (from the matrix) characteristics, therefore improving the overall therapeutic effects (Freitas et al. 2015).

Given that scleroglucan possesses all the desirable prerequisites, such as biocompatibility, biodegradability, and bioadhesion, as well as thermal and chemical stability characteristics, it has been used in hydrogels for drug delivery, especially, as a

slow-release matrix for controlled drug release. It was discovered that scleroglucan can be utilized to formulate a chemical/physical gel in the presence of borax. Consequently, a novel hydrogel, combining scleroglucan and borate ions, has been developed and is being used for controlled drug delivery. This particular hydrogel was loaded with three different model molecules, and after freeze-drying procedures, it has been formulated to be used as a swellable matrix for tablets (Freitas et al. 2015). This has been further corroborated that scleroglucan can certainly play a role in drug delivery owing to its swelling properties, as upon coming in contact with an aqueous medium, it enables the release of drugs through diffusion (Kour et al. 2020).

Scleroglucan has been further explored as a biocompatible material in studies associated with drug-loading effects on the release mechanisms. The investigation involved the incorporation of an aqueous ferrofluid in polyvinyl alcohol (PVA) and scleroglucan hydrogels and loaded with theophylline as a model drug. The results of the analysis for rheological properties indicated that the incorporation of the ferrofluid into the hydrogels yielded a higher storage modulus and a more compact structure (Chen et al. 2016). Carboxymethylated derivative (Scl-CM) of scleroglucan, obtained by reactions with chloroacetic acid in a basic medium, has also been studied for the preparation of physical hydrogels designed for topical drug delivery applications. Physical hydrogels derived from a high-carboxymethylated derivative of scleroglucan ($Scl\text{-}CM_{300}$) as potential drug delivery systems (DDSs) for topical formulations using various therapeutic molecules (fluconazole, diclofenac, and betamethasone) were investigated. Rheological tests on drug-loaded hydrogels, as well as the in vitro release studies, concluded that the hydrogels have a negligible primary skin irritation index (PII) and are highly suitable for both fast and sustained topical release of the drugs (Paolicelli et al. 2017).

In another application of Scl-CM, four nonsteroidal anti-inflammatory drugs (NSAIDs) were loaded into the novel pH-sensitive physical hydrogels obtained from 2.0% (w/v) solutions of the biopolymer. The results obtained from the release studies and physicochemical and biological characterization revealed that new hydrogels have high suitability as colon-specific drug delivery systems. Moreover, the mucoadhesive nature of the hydrogels ensures that the drug release at the site of action is sustained for an extended time. Also, in vivo studies not only substantiated the claims associated with the biocompatibility of this scleroglucan derivative but also indicated that there was no gastric damage postadministration. The enhanced biocompatibility also makes these hydrogels suitable for the administration of biomacromolecules with greater susceptibility to degradation, such as proteins, enzymes (Corrente et al. 2012).

Antisense technologies for targeted inhibition of gene expression are one of the foremost and highly effective measures for the suppression of inflammation. The utilization of antisense oligonucleotides (ODNs), however, has been fraught with problems, severely limiting their effectiveness. Poor uptake of ODNs by the cells, and therefore, their failure to reach the target site, as well as the antisense instability, brought about by hydrolysis, mediated by the enzyme deoxyribonuclease (DNase), are among the major impediments to the effective utilization of the antisense ODNs. Therefore, a delivery system for antisense ODNs aimed at enhancing their antisense

stability while also maintaining the specificity of antisense for its target RNA or DNA was required (Takedatsu et al. 2012).

Schizophyllan has been promoted as a new potential candidate for an antisense ODNs carrier, whereby a derivative of the biopolymer has been used as a binding agent for the modified antisense ODNs. Modification of schizophyllan was mediated by galactose and polyethylene glycol (PEG) units for enhanced uptake by the cells. The resultant antisense effect was maximized due to the use of schizophyllan derivative when the complex was administered to hepatoblastoma HepG2 and melanoma A375 cell lines. These β-glucan-based drug delivery devices can, therefore, serve as versatile biocompatible carriers for a variety of nucleotides (Verma and Gu 2012). The antisense system involving schizophyllan has found applications for various health conditions, most notably, the human inflammatory bowel disease (IBD). Among various advantages of this system, the most significant are: the system enables the effective suppression of targeted RNA or DNA, the schizophyllan complex possesses excellent stability characteristics in vivo, also being resistant to dissolution by deoxyribonuclease, and lastly, the schizophyllan complex is effectively taken up into the macrophages by phagocytosis regulated by dectin-1 (Takedatsu et al. 2012; Vetvicka et al. 2020).

6 Conclusion/Perspectives

Microbial exopolysaccharides offer a vast range of applications in food and biomedical/pharmaceutical industries due to their unique structural and functional properties. In this perspective, scleroglucan and schizophyllan are two important exopolysaccharides of fungal origin are scleroglucan and schizophyllan. Their distinctive chemical, rheological, and physiological attributes make these polysaccharides potential candidates for useful biomedical and functional applications. Both of these exopolysaccharides can be successfully used as alternatives to the other natural polymers for their therapeutic potential due to their controlled release systems and cost-effectiveness. As carriers of drugs and bioactive compounds, scleroglucan and schizophyllan possess more biocompatibility, effective absorption, metabolism and elimination, and less toxicity. However, these compounds should be subjected to pharmaco-toxicological studies for safety analysis as some structural modifications can be hazardous. In this regard, advancements in the exopolysaccharide nanoparticle medical imaging systems can be employed to increase the chances of their clinical approval and marketing. Moreover, development of exopolysaccharide-based nanopharmaceuticals has shown encouraging results through in vivo trials for their safe use in the food and pharmaceutical industries. Hence, nanoparticulate systems based on scleroglucan and schizophyllan must be introduced improved functionality and stability. Additionally, the future research efforts are focused on exopolysaccharide-containing nanopharmaceuticals for brain-targeting in various neurological disorders. Regarding bioactive EPS as potential therapeutics, their challenge is to prove, necessarily in vivo, therapeutic advantages over the drugs available on the market and to observe the abovementioned

well-established protocols for new drugs. Furthermore, the research is directed towards the use of these exopolysaccharide-based nanoparticulate systems for the development of associative formulations with other therapeutic agents for exploring their synergetic effects. Nevertheless, intense world-wide research is being expected for the involvement of these exopolysaccharides in improving human health.

References

Ates O. Systems biology of microbial exopolysaccharides production. Front Bioeng Biotechnol. 2015;3:200.

Banerjee S, Parasramka M, Paruthy SB. Polysaccharides in cancer prevention: from bench to bedside. In: Ramawat KG, Mérillon JM, editors. Polysaccharides: bioactivity and biotechnology. Cham: Springer International Publishing; 2015. p. 2179–214.

Bermejo-Jambrina M, Eder J, Helgers LC, et al. C-type lectin receptors in antiviral immunity and viral escape. Front Immunol. 2018;9:590. https://doi.org/10.3389/fimmu.2018.00590.

Brigand G. Scleroglucan. In: Whistler RL, Bemiller JN, editors. Industrial gums: polysaccharides and their derivatives. San Diego: Academic; 2012. p. 461–74.

Chen L, Liu X, Wong KH. Novel nanoparticle materials for drug/food delivery-polysaccharides. Phys Sci Rev. 2016;2016(8):201653. https://doi.org/10.1515/psr-2016-0053.

Corrente F, Paolicelli P, Matricardi P, et al. Novel pH-sensitive physical hydrogels of carboxymethyl scleroglucan. J Pharm Sci. 2012;101(1):256–67. https://doi.org/10.1002/jps.22766.

Dave VS. QbD considerations for excipient manufacturing. In: Beg S, Hasnain MS, editors. Pharmaceutical quality by design: principles and applications. New Delhi: Academic; 2019. p. 65–76.

El Asjadi S, Nederpel QA, Cotiuga IM, et al. Biopolymer scleroglucan as an emulsion stabilizer. Colloids Surf A Physicochem Eng Asp. 2018;546:326–33. https://doi.org/10.1016/j.colsurfa.2018.02.035.

Farina JI, Sineriz F, Molina OE, et al. Determination of radial growth rate of colonies of *Sclerotium rolfsii* F-6656 for the evaluation of culture medium, optimum incubation temperature, osmo- and halotolerance. Rev Arg Microbiol. 1996;28:190–6.

Farina JI, Sineriz F, Molina OE, et al. Isolation and physicochemical characterization of soluble scleroglucan from S*clerotium rolfsii* – rheological properties, molecular weight and conformational characteristics. Carbohydr Polym. 2001;44:41–50.

Freitas F, Roca C, Reis MA. Fungi as sources of polysaccharides for pharmaceutical and biomedical applications. In: Thakur VK, Thakur MK, editors. Handbook of polymers for pharmaceutical technologies. Volume 3, Biodegradable polymers. Hoboken/Beverly: Scrivener Publishing LLC/Wiley; 2015. p. 61–103.

Gao C. Application of a novel biopolymer to enhance oil recovery. J Petrol Explor Prod Technol. 2016;6:749–53. https://doi.org/10.1007/s13202-015-0213-7.

Giavasis I. Production of microbial polysaccharides for use in food. In: McNeil B, Archer D, Giavasis I, et al., editors. Microbial production of food ingredients, enzymes, and nutraceuticals. Cambridge: Woodhead Publishing; 2013. p. 413–68.

Giavasis I. Bioactive fungal polysaccharides as potential functional ingredients in food and nutraceuticals. Curr Opin Biotechnol. 2014;26:162–73. https://doi.org/10.1016/j.copbio.2014.01.010.

Giridharan S, Srinivasan M. Mechanisms of NF-κB p65 and strategies for therapeutic manipulation. J Inflamm Res. 2018;11:407–19.

Goff HD, Guo Q. The role of hydrocolloids in the development of food structure. In: Spyropoulos F, Lamidis A, Norton IT, editors. Handbook of food structure development. Wales: The Royal Society of Chemistry; 2019. p. 1–28.

Jindal N, Khattar JS. Microbial polysaccharides in food industry. In: Grumezescu AM, Holban AM, editors. Handbook of food bioengineering. Volume 20, Biopolymers for food design. London: Academic; 2018. p. 67–85.
Kour D, Rana KL, Kaur T, et al. Extremophiles for hydrolytic enzymes productions: biodiversity and potential biotechnological applications. In: Molina G, Gupta VK, Singh BN, et al., editors. Bioprocessing for biomolecules production. Hoboken: Wiley; 2020. p. 321–56.
Ladrat CD, Sinquin C, Lebellenger L, et al. Exopolysaccharides produced by marine bacteria and their applications as glycosaminoglycan-like molecules. Front Chem. 2014;2:85.
Lembre P, Lorentz C, Martino PD. Exopolysaccharides of the biofilm matrix: a complex biophysical world. In: Karunaratne DN, editor. The complex world of polysaccharides. Rijeka: Intech; 2012. p. 371–92.
Lemieszek M, Rzeski W. Anticancer properties of polysaccharides isolated from fungi of the Basidiomycetes class. Contemp Oncol. 2012;16(4):285–9. https://doi.org/10.5114/wo.2012.30055.
Li P, Harding SE, Liu Z. Cyanobacterial exopolysaccharides: their nature and potential biotechnological applications. Biotechnol Genet Eng Rev. 2001;18:375–404.
Liang K, Han P, Chen Q, et al. Comparative study on enhancing oil recovery under high temperature and high salinity: polysaccharides versus synthetic polymer. ACS Omega. 2019;4(6):10620–8. https://doi.org/10.1021/acsomega.9b00717.
Madhuri KV, Prabhakar KV. Microbial exopolysaccharides: biosynthesis and potential applications. Orient J Chem. 2014;30:1401–10.
Milani J, Maleki G. Hydrocolloids in food industry. In: Valdez B, editor. Food industrial processes – methods and equipment. Rijeka: Intech; 2012. p. 17–38.
Mishra A, Jha B. Microbial exopolysaccharides. In: Rosenberg E, DeLong EF, Lory S, et al., editors. The prokaryotes. Berlin/Heidelberg: Springer; 2013.
Moscovici M. Present and future medical applications of microbial exopolysaccharides. Frontiers in Microbiology, 2015;6(1012).
Nie H, Zheng Y, Li R, et al. Phosphorylation of FOXP3 controls regulatory T cell function and is inhibited by TNF-α in rheumatoid arthritis. Nat Med. 2013;19(3):322–8.
Paolicelli P, Varani G, Pacelli S, et al. Design and characterization of a biocompatible physical hydrogel based on scleroglucan for topical drug delivery. Carbohydr Polyme. 2017;174:960–9.
Patel AK, Michaud P, Singhania RR, et al. Polysaccharides from probiotics: new developments as food additives. Food Technol Biotechnol. 2010;48:451–63.
Plotkin LI, Bivi N. Local regulation of bone cell function. In: Burr DB, Allen MR, editors. Basic and applied bone biology. San Diego: Academic; 2014. p. 47–73.
Rakhra G, Jaiswal SM, Rakhra G. Bioactive peptides and carbohydrates from natural products: a source of functional foods and nutraceuticals. In: Singh J, Meshram V, Gupta M, editors. Bioactive natural products in drug delivery. Singapore: Springer Nature; 2020. p. 335–54.
Rau U. Production of schizophyllan. In: Bucke C, editor. Carbohydrate biotechnology protocols. Totowa: Humana Press; 2008.
Schepetkin, Igor A., and Mark T. Quinn. "Immunomodulatory effects of botanical polysaccharides." Polysaccharides in medicinal and pharmaceutical applications 35(2011):988–94.
Selvi SS, Eminagic E, Kandur MY, et al. Research and production of microbial polymers for food industry. In: Molina G, Gupta VK, Singh BN, et al., editors. Bioprocessing for biomolecules production. Hoboken: Wiley; 2020. p. 225–6.
Shamtsyan M. Potential to develop functional food products from mushroom bioactive compounds. J Hyg Eng Des. 2016;15:51–9.
Shit SC, Shah PM. Edible polymers: challenges and opportunities. J Polym. 2014;2014:1–13.
Smelcerovic A, Knezevic-Jugovic Z, Petronijevic Z. Microbial polysaccharides and their derivatives as current and prospective pharmaceuticals. Curr Pharm Des. 2008;14:3168–95.
Survase SA, Saudagar PS, Bajaj IB, et al. Scleroglucan: fermentative production, downstream processing and applications. Food Technol Biotechnol. 2007a;45:107–18.
Survase SA, Saudagar PS, Singhal RS. Enhanced production of scleroglucan by *Sclerotium rolfsii* MTCC 2156 by use of metabolic precursors. Bioresour Technol. 2007b;98:410–5.

Sutivisedsak N, Leathers TD, Price NP. Production of schizophyllan from distiller's dried grains with solubles by diverse strains of *Schizophyllum commune*. Springerplus. 2013;2:476. https://doi.org/10.1186/2193-1801-2-476.

Takedatsu H, Mitsuyama K, Mochizuki S, et al. A new therapeutic approach using a schizophyllan-based drug delivery system for inflammatory bowel disease. Mol Ther: J Am Soc Gene Therapy. 2012;20(6):1234–41.

Taurhesia S, McNeil B. Physicochemical factors affecting formation of biological response modifier scleroglucan. J Chem Technol Biotechnol. 1994;59:157–63.

Venugopal V. Polysaccharides: their characteristics and marine sources. Marine polysaccharides food applications. Boca Raton: CRC Press; 2011. p. 3–27.

Verma MS, Gu FX. 1,3-β-glucans: drug delivery and pharmacology. In: Karunaratne DN, editor. The complex world of polysaccharides. IntechOpen; 2012.

Vetvicka V, Vannucci L, Sima P. β-glucan as a new tool in vaccine development. Scandinavian. J Immunol. 2020;91(2):e12833. https://doi.org/10.1111/sji.12833.

Wang Y, McNeil B. Effect of temperature on scleroglucan synthesis and organic acid production by *Sclerotium glucanicum*. Enzym Microb Technol. 1995a;17:893–9.

Wang Y, McNeil B. pH effects on exopolysaccharide and oxalic acid production in cultures of *Sclerotium glucanicum*. Enzym Microb Technol. 1995b;17:124–30.

Wong JH, Ng TB, Chan HHL, et al. Mushroom extracts and compounds with suppressive action on breast cancer: evidence from studies using cultured cancer cells, tumor-bearing animals, and clinical trials. Appl Microbiol Biotechnol. 2020;104:4675–703.

Wüstenberg T. General overview of food hydrocolloids. In: Wüstenberg T, editor. Cellulose and cellulose derivatives in the food industry – fundamentals and applications. Weinheim: Wiley-VCH; 2015. p. 1–68.

Zhang Z, Zhang F, Liu Y. Recent advances in enhancing the sensitivity and resolution of capillary electrophoresis. J Chromatogr Sci. 2013a;51:666–83.

Zhang Y, Kong H, Fang Y, et al. Schizophyllan: a review on its structure, properties, bioactivities and recent developments. Bioact Carbohydr Diet Fiber I. 2013b;2013:53–71.

Sulfated Seaweed Polysaccharides

15

Ana Rita Inácio, Ana C. Carvalho, Catarina Oliveira, Lara Reys, Simone S. Silva, Nuno M. Neves, Albino Martins, Rui L. Reis, and Tiago H. Silva

Contents

Abstract

Mankind has used natural resources for many different applications, from food and clothes to drugs, but only recently is giving the due attention to the need for a responsible management of these resources toward sustainability. One of the approaches is to take the most out of its resources, addressing the several

A. R. Inácio · A. C. Carvalho · C. Oliveira · L. Reys · S. S. Silva · N. M. Neves · A. Martins · R. L. Reis · T. H. Silva (✉)
3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Zona Industrial da Gandra, Barco, Guimarães, Portugal

ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães, Portugal
e-mail: tiago.silva@i3bs.uminho.pt

J. M. Oliveira et al. (eds.), *Polysaccharides of Microbial Origin*,
https://doi.org/10.1007/978-3-030-42215-8_17

components of natural resources, which can be used in many industrial fields. In this regard, many natural compounds have been studied for the evaluation of biological activities with relevance for human health and well-being. Among the explored resources, the survey of marine organisms has been growing, particularly regarding microbiota and seaweeds or macroalgae. Macroalgae are macroscopic algae that are usually found on rocky shores, exhibiting a great diversity of colors, shapes and sizes. They are divided in three large groups, essentially based on their color: green macroalgae, brown macroalgae and red macroalgae. In recent decades, seaweeds have been widely used as a sustainable source of sulfated polysaccharides, which exhibiting diverse chemical and biological properties aim to address the demands of Tissue Engineering and Regenerative Medicine (TERM), as well as of other areas, such as cosmetics and food. This manuscript provides an overview of the paradigmatic examples of this type of biopolymer that can be obtained from the different groups of algae, detailing the chemical structure, general biological characteristics and most revealing properties.

Keywords

Seaweeds · Polysaccharides · Marine biopolymers · Biomedical · Engineered tissues

1 Introduction

Life started in water, home to surprisingly diverse and interesting forms of life. The marine environment embraces 90% of the organisms that inhabit the Earth, being a huge source of biological and chemical diversity. Marine species are increasingly seen as an important source of food and health ingredients. Seaweeds (macroalgae) stand out, as they are perfectly adapted to a range of ecological aquatic niches, exhibiting interesting bioactive compounds and having the potential to provide biomass flows for food and pharmaceutical products, among others (Andrade et al. 2013; Ibañez and Cifuentes 2013; Pangestuti and Kurnianto 2017).

Seaweeds are macroscopic and multicellular aquatic plants that do not have the specialized structures of higher plants, such as true leaves, stems, and roots. Fast-growing and highly efficient, they have a simple life cycle. The seaweeds are divided into three main groups, based on their photosynthetic pigments: Rhodophyceae (red algae), Chlorophyceae (green algae), and Phaeophyceae (brown algae) (Kadam et al. 2015; Venkatesan et al. 2017; Carvalho et al. 2020). Algae, traditionally, has been used as a source of polysaccharides, minerals, vitamins, polyphenols, peptides, amino acids, proteins, lipids, and pigments (Carvalho et al. 2020; Pangestuti and Kurnianto 2017). Among the bioactive ingredients in algae, polysaccharides have been identified as predominant, with uses that include pharmaceutical and cosmeceutical products (Kadam et al. 2015; Patel 2018).

In the last decade, increasing attention has been paid to the exploration of naturally occurring polymers due to their inherent biocompatibility and

biodegradability, whose properties are strictly required by a biomaterial for a safe interaction with biological systems. Among natural polymers, polysaccharides represent the most promising candidates due to their abundance in nature, low cost, chemical versatility and simplicity of extraction and purification processes. In the last decade, algae have been subjected to intensive investigations, as they are known to represent an abundant biomass not being fully used and to contain unequal bioactive compounds and functional polymers (Liu et al. 2015).

Bioactive polysaccharides containing sulfate groups are a group of very interesting and attractive components in algae, as they combine the chemical versatility and biocompatibility of polysaccharides and an incomparable bioactivity, not easily found in any other class of chemical compounds. Sulfated polysaccharides generally operate as structural components of algal cell walls. Different classes of sulfated polysaccharides were identified and classified according to the origin of the algae, being the main representative: ulvan of green algae, fucoidan of brown algae, and agar and carrageenan of red algae. Most of the biological properties of sulfated polysaccharides are attributed to their structural characteristics, such as the presence of sulfate groups (total contents, position and pattern), molar mass, and stereochemistry (Cunha and Grenha 2016). There are no examples of sulfated polysaccharides in higher plants, but there is a resemblance of a particular class of polysaccharides found in animals – glycosaminoglycans – suggesting the exploration of those biopolymers for biotechnological ends, namely, in biomedicine.

Among the biological origin materials, sulfated polysaccharides of algal origin attract attention from the biomedical community due to the generally reported low immunogenicity, non-mutagenic, non-cancerous, hemocompatible, biodegradable, and edible nature, together with the ability to adsorb bioactive molecules, as cell growth factors, not easily found in any other class of chemical compounds (Jacob et al. 2018; Kumar et al. 2019). A wide spectrum of biological properties is described for conferring a multitude of health benefits, such as antioxidants, anti-inflammatory, antiviral, antitumor, anti-angiogenic, antithrombotic, hypocholesterolemic, immunomodulatory, antilipemic, anticoagulant, antibacterial, antiallergic, antiaging, antiprotozoal, osteoprotective, anti-venom, regenerative, among others (Kadam et al. 2015; Patel 2018).

This chapter briefly develops of sulfated polysaccharides of algae origin, namely, regarding their properties and some applications. Several aspects are considered, from the origin of biopolymers to their physic-chemical features and a wide range of biological activities. Special attention is paid to biomedical applications, particularly assessing tissue engineering (TE) and drug delivery.

2 Sulfated Polysaccharides: Their Properties

Sulfated polysaccharides of algal origin are of great interest as a building block or bioactive component for several applications. It was found that the chemical structures of these biopolymers vary according to the group of algae, brown, red and green, enabling the identification of paradigmatic biopolymer families, which

fine chemical features depend on many factors within each of these families (Morelli et al. 2017).

2.1 Fucoidan

Fucoidan is an anionic polysaccharide present in cell walls of brown algae and some marine invertebrate tissues (Anastyuk et al. 2012; Kim 2014; Wang et al. 2010b, 2019). In industry, various extraction methods have been studied and used to produce and preserve high-quality fucoidan. Fucoidan can be extracted using hot acid or ethanol/water solutions (Balboa et al. 2013; Pomin et al. 2005; Rodriguez-Jasso et al. 2011), eventually further assisted by ultrasound and microwave radiation (Ale and Meyer 2013), as well as exploring enzyme-assisted (Ale and Meyer 2013) processes that are at the beginning (Foley et al. 2011; Hahn et al. 2012). Figure 1a shows a scheme of the process for the production of fucoidan that can be divided in three steps: milling seaweeds, extraction/purification (may involve multiple steps, as extended aqueous extractions and acidic solutions and may include calcium to promote the alginate precipitation), and drying/careful storage. Depending on the type of extraction, yields ranging from 0.26% to 20% of algal biomass dry weight can be obtained (Foley et al. 2011; Silva et al. 2012). The physical-chemical characteristics of the extracted fucoidan depend on the severity of the extraction conditions such as

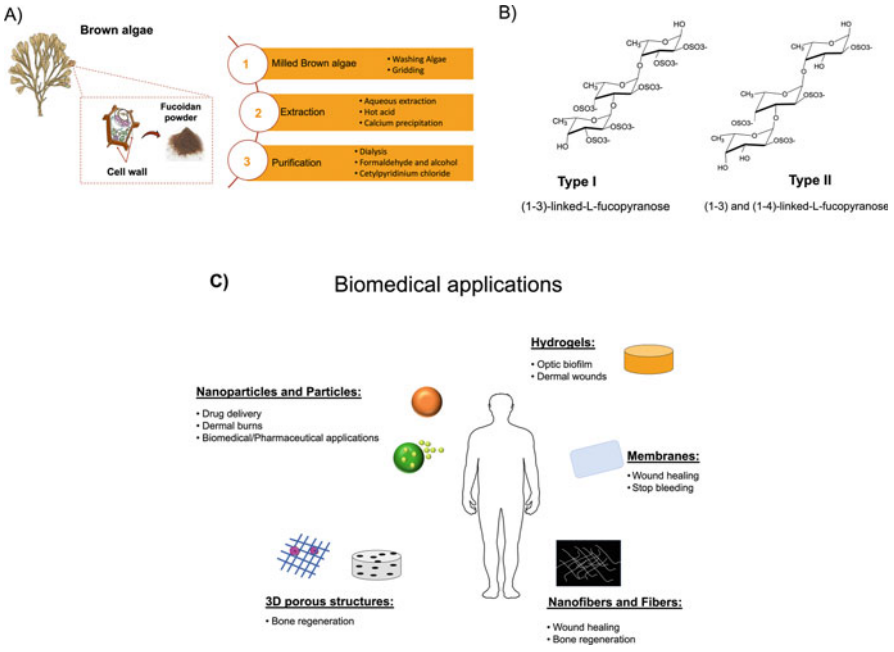


Fig. 1 (**a**) Extraction of fucoidan from brown algae, (**b**) schematic representation of fucoidan type I and II, and (**c**) schematic illustration of fucoidan-based biomaterials and their applications

temperature, reaction time and concentration of the chemicals, and on inherent algae factors, such as algae species and size, local climate, and other environmental factors (Fitton et al. 2007; Holtkamp 2009; Li et al. 2008). Since its discovery by Kylin in 1913, the chemical and structural composition of fucoidan has been studied extensively but has not yet been definitely identified (Mak 2012). Structurally, fucoidan is essentially formed by different amounts of L-fucose and sulfate groups, with other monosaccharides also being present, like mannose (Duarte et al. 2001), galactose (Nagaoka et al. 1999), glucose (Rocha et al. 2005), xylose (Nagaoka et al. 1999), and uronic acids (Holtkamp 2009; Ponce et al. 2003). The chemical structure of fucoidan can be divided into two groups depending on their source (Fig. 1b): one group originates from seaweeds including *Laminaria* species and have their central chains composed by (1-3) α-L-fucopyranose residues; a second group includes fucoidan isolated from *Ascophyllum* and *Fucus* species that have their central chains composed of repeating (1-3) and (1-4) linked α-L- fucopyranose residues. The molecular weight of fucoidan ranges from 13 to 950 kDa and, such as chemical structure, it also depends on several factors such as the source, season, and method of extraction. Furthermore, information regarding the solubility and rheological properties of fucoidan is essential for various applications (Rioux et al. 2007a; Silva et al. 2012). Recent studies have stated in this perspective that rheological characteristics and viscosity of fucoidan depend on the algae species, but regardless of molecular weight and proportion of sulfates and uronic acids. One of those researches, developed by Rioux and colleagues, reports that fucoidan might not be capable of forming a gel, but rather a viscous solution (Rioux et al. 2007b; Silva et al. 2012).

2.2 Carrageenan

Carrageenan is the generic name for a family of high molecular weight sulfated polysaccharides obtained by extraction from various species of red seaweeds (Rhodophyceae), mainly of the genera *Kappaphycus*, *Eucheuma*, *Gigartina*, *Chondrus*, and *Hypnea* (Rhein-Knudsen et al. 2015; Torres et al. 2019). Carrageenans contain between 15% and 40% of sulfate ester groups and a backbone structure based on linear chains of repeating disaccharide units joined by alternating α-(1-3) and β-(1-4) glycosidic linkages, i.e., alternating units of 3-linked β-D-galactopyranose (G-unit) and 4-linked α-D-galactopyranose (D-unit) or 4-linked 3,6-anhydro α-D-galactopyranose (DA-unit) (Jiao et al. 2011). In addition to galactose and sulfate, other carbohydrate residues (e.g., xylose, glucose, and uronic acids), and substituents (e.g., methyl ethers and pyruvate groups) might be present in carrageenans (van de Velde et al. 2002b).

Carrageenans are classified according to their structural characteristics, including the presence of 3,6-anhydro ring in the 4-linked units and the number and position of sulfate ester groups. Among 42 theoretically possible different disaccharide structures, only 15 have been identified and assigned Greek letters (Jiao et al. 2011; Lahaye 2001). The most relevant and commercially exploited carrageenans are kappa (κ)-, iota (ι)-, and lambda (λ)-carrageenans, distinguished

by the presence of one, two, and three sulfate ester groups per repeating disaccharide unit, respectively (Jiao et al. 2011). Sulfate content is one of the features most influencing the properties of carrageenan and it is known that higher sulfate content results in lower solubility temperature and gel strength (Necas and Bartosikova 2013). From the three types of commercial carrageenans, only κ- and ι-carrageenans possess gelling properties, with κ-carrageenan gels being more firm and rigid while ι-carrageenan gels are soft and elastic (Campo et al. 2009). They can be provided separately or as a well-defined mixture, since most seaweeds produce carrageenans as a combination of different sulfated polysaccharides, hybrid structures, and biological precursors, instead of only producing a pure form of one carrageenan type (Torres et al. 2019; van de Velde 2008). The relative presence of different carrageenans is greatly influenced by several factors such as algal source, physiological (nutrient uptake, growth rate, tissue age, stress tolerances, and defenses), environmental (salinity, pH, temperatures, light intensity, and water movement), and extraction conditions (Al-Nahdi et al. 2019). Even though different carrageenans are soluble in hot water, commercial extraction generally takes place in an alkaline solution at elevated temperatures for a specific time. Alkaline treatment decreases sulfate content and increases 3,6-anhydrogalactose content, improving the gelling properties of the final polysaccharide. However, inadequate alkaline treatment may compromise the transformation of the biological precursors into commercial carrageenans, i.e., mu (μ)- and nu (ν)-carrageenans into κ- and ι-carrageenans, respectively. Furthermore, the presence of substantial amounts of precursor units in commercial carrageenan preparations has a significant negative effect on their functional (e.g., gelling) properties (van de Velde et al. 2002a).

2.3 Agar

Agars, also known as agar-agar, are 1,3-α-1,4-β-galactans that can be extracted from the cell walls of red seaweeds (Rhodophyceae). The genera *Gelidium* and *Gracilaria* are the most common sources of agar and the best quality agar is extracted from *Pterocladia*, *Pterocladiella*, and *Gellidium* (Kumar et al. 2008; Torres et al. 2019). Agar contains between 2% and 5% of sulfate residues and is composed of two different polysaccharides, agarose and agaropectin, which may influence its gelling ability (Yarnpakdee et al. 2015). Agarose, a neutral polysaccharide, is the main component of agar with a linear structure of repeating units of the disaccharide agarobiose, a dimmer of D-galactose, and 3,6-anhydro-L-galactose. Agaropectin is an acid polysaccharide containing sulfate groups, pyruvic acid, and D-glucuronic acid conjugated to agarobiose (Arvizu-Higuera et al. 2007). While only one of the components is sulfated, its isolation is not easily achieved, unless chemical modifications and fractionations are explored, with deleterious impact on polymer properties, from which the original mixture – agar – is the considered biopolymer entity when biotechnological uses are foreseen.

Agar is soluble in hot water (above 85 °C), being insoluble in cold water. It forms a gel when cooled down to a temperature between 30 and 40 °C. This parameter depends on agar's purity, concentration and pH (Schiavi et al. 2016). Its extraction is performed in boiling water, after which several filtrations are performed and a gel is obtained after cooling the solution. This freezing and thawing cycle is repeated a few times (Torres et al. 2019; Reis et al. 2008; Rodríguez et al. 2009). However, in some species, like *Gracilaria*, there is the need of a pre-treatment with alkali (NaOH) for the desulfation of the native agar, causing the formation of a 3,6-anhydrogalactose bridge and an increase in the gel strength. The quality and yield of agar may be influenced by the type of treatment before the extraction and the reaction time, since longer alkali treatments may compromise the yield and gel strength (Arvizu-Higuera et al. 2007; Marinho-Soriano and Bourret 2005). Structural variations in the final formulation, namely, the degree of methoxylation and sulfation, also influence gel strength. These characteristics, along with the pattern and degree of substitution, the molecular weight and chemical composition determine the physical properties and consequently the quality of agar. Besides, like most natural compounds, agar properties may also be influenced by the species and culture environment (Fuse and Suzuki 1975; Lee et al. 2017; Vergara-Rodarte et al. 2010; Armisen and Galatas 1987). Although the general aspects of the extraction process are known, this is a time-consuming process that results in considerable amounts of waste. As so, studies are still needed to increase the yield and quality of the product (Arvizu-Higuera et al. 2007).

2.4 Ulvan

Ulvan represents a family of polyanionic sulfated heteropolysaccharides extracted from green algae, mainly belonging to the *Ulva* species. The extraction is conventionally carried out using hot water (80–90 °C) containing ammonium oxalate and the recovery of ulvan is generally achieved by precipitation in ethanol (Chiellini and Morelli 2011). It presents a structure of great complexity and variability, according to the species of algae, time of harvest, geographic distribution, extraction, and purification method of the polysaccharide (Cunha and Grenha 2016; Morelli et al. 2017; Kidgell et al. 2019; Tziveleka et al. 2019). The composition of the ulvans is not yet well established, as research in green algae components and ulvans in particular is quite recent when compared with polysaccharides from other sources. It has been observed a great variation of sugars, such as rhamnose, glucuronic acid, iduronic acid, xylose, as well as its quantity, besides sulfate groups. Other monosaccharides are reported in their composition, such as glucose, galactose, arabinose, and mannose, being unclear whether their presence indicates contamination or a biopolymer component (Kidgell et al. 2019; Morelli et al. 2017; Tziveleka et al. 2019; Popa et al. 2015b).

Monosaccharides are commonly linked to α- and β-(1,4) glycosidic bonds with repeated units of disaccharides. The two main repeating units of disaccharides are aldobiuronic acids, called ulvanobiuronic acids: ulvanobiuronic acid 3-sulfate type A (A_{3s}) and B (B_{3s}). Both units are associated with a rhamnose 3-sulfate residue linked to an uronic residue via a (1-4) glycosidic bond. The A_{3s} disaccharide is

composed of glucuronic acid and sulfated rhamnose, while type B_{3s} is formed by iduronic acid and sulfated rhamnose (Carvalho et al. 2020; Cunha and Grenha 2016; Kidgell et al. 2019; Morelli et al. 2017; Tziveleka et al. 2019).

Many characteristics and properties of this biopolymer remain unknown when extraction conditions vary, that is, the rheological and textural properties. The currently known physic-chemical properties of ulvan may be due to its content in the hydrophobic (methyl) and hydrophilic (hydroxyl, carboxyl, sulphate) groups (Cunha and Grenha 2016; Morelli et al. 2017). It is considered as a family of molecules chemically related with a wide distribution of charge density and molecular weight. Its solubility in water is determined by the high charge density, being common that its dispersion does not form a transparent solution due to the formation of microaggregates. It has a certain hydrophobic character, possibly determined by the presence of a large amount of methyl groups in the repeat rhamnose unit. Ulvan's versatility is hampered by the formation of aggregates in aqueous solutions and its insolubility in almost all organic solvents has been observed by several authors (Bhatnagar and Bhatnagar 2015; Chiellini and Morelli 2011; Cunha and Grenha 2016; Morelli et al. 2017).

Ulvan solutions have been reported to exhibit viscosity that can be compared to gum arabic. The gel formation of this polysaccharide without further functionalization is complex and is not completely clear, being proposed that a concentration of 1.6% (w/v) forms a weak gel in deionized water. The gelling properties are of high interest in gel-forming applications with precise control, for example, toward the development of devices for the sustained or controlled release of particles or molecules trapped under specific conditions. The properties are affected by pH, divalent cations, and boric acid (Bhatnagar and Bhatnagar 2015; Thuy et al. 2015; Usman et al. 2017).

3 Bioactive/Biological Properties of Sulfated Polysaccharides

Sulfated polysaccharides of algal origin have been the focus of research and intense studies, describing a wide spectrum of biological properties, which are related to their structural characteristics, including chemical composition, quantity and position of sulfate groups, conformation of chains, and molecular weight (Morelli et al. 2017; Tziveleka et al. 2019; Alves et al. 2013). The most illustrative examples will be addressed in the following paragraphs, according to the representative sulfated polysaccharides.

3.1 Fucoidan

In recent years, fucoidan form brown algae biomass have been the focus of numerous scientific studies to determine their possible biological activities. These biological activities include, among others, anti-tumoral (Oliveira et al. 2020a; Silva et al.

2012), anti-coagulant and antithrombotic (Ale et al. 2011b; Dobashi et al. 1989; Farias et al. 2000; Silva et al. 2012; Zhu et al. 2010), anti-viral (Baba et al. 1988; Lapshina et al. 2006; Lee et al. 2004b; Silva et al. 2012; Witvrouw and De Clercq 1997), antioxidant (Ale et al. 2011b; Wang et al. 2010a), reduction of blood glucose, and antihyperglycemic effects (Jiang et al. 2015; Kim et al. 2014a; Kumar et al. 2015; Shan et al. 2016). In Table 1, possible application and biological properties of fucoidan are summarized. These biological activities may be influenced by molecular weight, molecular geometry, polymer branching, sugar content, sulfation degree, and pattern (Ale et al. 2011a; Silva et al. 2012), but the structure-activity relationship is not yet well established.

Anti-tumoral fucoidan activity has gain tremendous interest in last years and has been studied over different types of cancer. Studies in vitro and in vivo have shown that fucoidan protects the organism against various forms of cancer, such as leukemia, colon, breast and lung (Boo et al. 2011; Oliveira et al. 2020a). Fucoidan's mechanism of action in anti-tumor therapies is still not well understood

Table 1 Biological properties associated with sulfated polysaccharide fucoidan from brown algae

Biological activity	Application	References
Anticoagulant	Anti-thrombin effect Prolong partial thromboplastin Inhibits the endogenous coagulation cascade Thrombin activity	Kim et al. (2014a, b), Wang et al. (2010a)
Antidiabetic	Prevents hyperglycemia Reduces blood glucose Inhibition of α-amylase and α-glucosidase	Jiang et al. (2015), Kumar et al. (2015), Shan et al. (2016)
Anti-inflammatory	Regulates the inflammatory response Inhibits the release of nitric oxide and reduces inflammation Inhibits the migration of leukocytes	Wang et al. (2019), Irhimeh et al. (2009)
Antioxidant	Avoiding or delaying free radical-mediated illness Radical scavenging ability	Huang et al. (2016), Kyung et al. (2012)
Antitumor	Induces apoptosis in tumor cells Inhibits tumor angiogenesis Enhances immune function Active against several cancers: Lung, breast, cervical, gastric, liver	Wang et al. (2019), Hsu et al. (2014), Marudhupandi et al. (2014), Zhang et al. (2013)
Antiviral	Inhibits human immune deficiency virus Inhibits viral absorption onto cells Impeding virus entry Active against other viruses, as herpes simplex virus, human cytomegalovirus, influenza virus, and corona virus, including SARS-CoV-2	Wang et al. (2019), Lee et al. (2004a), Mandal et al. (2007), Thuy et al. (2015), Wozniak et al. (2015), Kwon et al. (2020)

but may be linked to different factors such as molecular weight (Mw), sulfation degree and pattern, sugar compounds and polymer branching (Oliveira et al. 2017, 2020a), with the first two chemical features being way more investigated than the latter ones. Nevertheless, some studies reveal that fucoidan may regulate and mediate its anti-tumoral potential through different mechanism and cancer related pathways, namely, cell cycle arrest, apoptosis, and immune system activation (Kwak 2014; Lee et al. 2012a; Senthilkumar et al. 2013). It also prevents the development of metastases and enhances the effects of other chemical therapies and compounds (Atashrazm et al. 2015). In fact, the fundamental aspect of a cancer therapy is to eliminate cancer cells without affecting the healthy tissues surrounding them. On this purpose, it is important that precise and targeting treatments be taken into account in order to affect only the tumors, in order to envisage a more successful treatment.

The effect of fucoidan in diabetes mellitus have been carried out on extensive preclinical studies, on in vitro models (on separate cell systems) and in vivo (on different laboratory animals) (Mukhamejanov et al. 2019). The mechanism of action in the treatment of diabetes disease is also poorly known. Some studies reported that sulfate groups and molecular weight (Mw) can have an impact on reducing blood glucose, namely, by inhibition of enzymes α-amylase and α-glycosidase during the digestion of carbohydrates (Kim et al. 2014a; Li et al. 2008), but antioxidant and anti-obesity effects could also be responsible for the use of fucoidan as anti-diabetic property (Li et al. 2015; Mukhamejanov et al. 2019; Wang et al. 2019). The action of fucoidan based on the bioactive properties previously mentioned, when fully understood, can play a major role in the treatment of diabetes mellitus type I and II.

3.2 Carrageenan

Carrageenans are capable of forming thermoreversible gels or viscous solutions and therefore they are frequently used as gelling, thickening, and stabilizing agents in a wide variety of applications in food, pharmaceutical, and cosmetic industries (Necas and Bartosikova 2013; Williams and Phillips 2003).

Commercial κ-, ι-, and λ-carrageenans were approved for the food industry by several regulatory agencies around the world, including the Food and Drugs Administration (FDA), the European Food Safety Agency (EFSA), and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (Younes et al. 2018). Food-grade carrageenans, both refined and semi-refined, have been used for decades as food additives in the production of numerous processed foods, including dairy products, water-based foods, meat products, beverages, condiments, and pet food (McHugh 2003). In the pharmaceutical industry, carrageenans have been increasingly used as a tablet excipient in sustained-release formulations due to their good compatibility, high robustness, and persistent viscoelasticity during compression. Moreover, carrageenan monograph is included into several pharmacopoeias, including the United States Pharmacopeia and the European

Pharmacopeia, suggesting that it may have a promising future as a pharmaceutical excipient (Campo et al. 2009; Li et al. 2015). However, questions have been raised about whether or not it is safe for human consumption. Some scientists argue that carrageenan is highly inflammatory and toxic to the gastrointestinal tract (Feferman et al. 2020; Tobacman 2001), while others defend that only degraded carrageenan is harmful and that the carrageenan employed as food additive and pharmaceutical excipient is not degraded or absorbed in the gastrointestinal tract and, therefore, cannot induce the mentioned inflammatory responses (McKim 2014, 2019; Weiner 2014). Although regulatory agencies reiterated that carrageenan is safe for use as an ingredient in food, carrageenan safety continues to be the subject of much controversy, with arguments being presented by both sides of the discussion (David et al. 2018, 2019; Weiner and McKim 2019). In addition to their applications in the food and pharmaceutical industries, carrageenans have also been used in the formulation of various cosmetics and personal care products, such as toothpastes, skin and hair care products, deodorants, and makeup, as well as in other types of products, such as shoe polish, air fresheners, and firefighting foam (Necas and Bartosikova 2013; Pereira 2018).

In experimental medicine, carrageenan-induced paw edema model has been used to evaluate the anti-inflammatory activity of compounds and to study the mechanisms involved in inflammation, while carrageenan-induced tail thrombosis has been used to evaluate antithrombotic and thrombolytic activity and to study the mechanisms involved in thrombolysis (Arslan et al. 2011; Morris 2003). These deleterious effects of carrageenan have been raising concerns regarding their biomedical use, as discussed later on, but it is important to point out at this moment that these reactions depend on the administration mode, concentration, and carrageenan-type (λ-carrageenan is the used one, characterized by a higher sulfation degree).

Carrageenan polysaccharides and their oligosaccharide derivatives are promising candidates for production of new therapeutic agents for several diseases due to their wide spectrum of biological activities, including antitumor, immunomodulatory (Liu et al. 2013; Luo et al. 2015; Yuan et al. 2006a; Zhou et al. 2004, 2006; Jin et al. 2013), antiangiogenic (Chen et al. 2007; Groult et al. 2019; Poupard et al. 2017; Yao et al. 2014), antihyperlipidemic (Panlasigui et al. 2003; Sokolova et al. 2014a; Valado et al. 2020), anticoagulant (Groult et al. 2019; Silva et al. 2010; Sokolova et al. 2014b), antioxidant (de Souza et al. 2007; Sokolova et al. 2011; Yuan et al. 2005, 2006b), antiviral (Grassauer et al. 2008; Buck et al. 2006; Carlucci et al. 1999; Chiu et al. 2012b; Leibbrandt et al. 2010; Talarico and Damonte 2007), antibacterial (Souza et al. 2018; Zhu et al. 2017), and antifungal (Soares et al. 2016; Souza et al. 2018) (Fig. 2). The beneficial effects of carrageenans on plant growth and defence responses against biotic and abiotic stresses have been confirmed, which open new avenues for their use in the agriculture industry (Bi et al. 2011; Nagorskaya et al. 2010; Sangha et al. 2015; Zou et al. 2018). Besides, its antiviral potential has been explored for the development of nasal spray against common cold (Koenighofer et al. 2014), with the activity against other virus being currently under study, which is particularly relevant in the present context of COVID-19 pandemic (Jang et al. 2021).

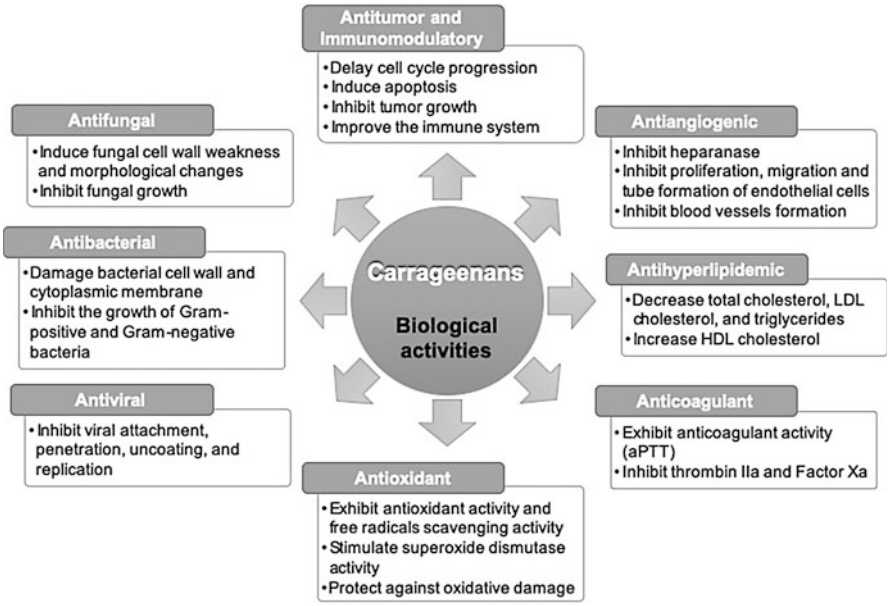


Fig. 2 Biological activities displayed by carrageenan polysaccharides and their oligosaccharide derivatives

3.3 Agar

Commercially available agar is white, shiny, semi-transparent, tasteless, and odorless (Gates 2012). Agar is approved as a food additive in Europe, being generally recognized as safe (Grassauer et al.) in the United States (Pinto 2020). As previously stated the gelling ability is the most important property of agar, which has been applied in different fields. The human food industry accounts for the destination of 80% of agar production, being bacteriological and biotechnological applications the target of the remaining 20% (Torres et al. 2019; Kumar and Fotedar 2009; Venugopal 2019). Illustrative applications are collected in Table 2, mostly associated with its gelling, thickening and stabilizing properties, associated to differences in gelling and melting temperatures. Besides the processing and texturizing role, agar has been also used as a source of water-soluble dietary fiber, being metabolized by intestinal bacteria. Agar is commonly used as a laxative and for the treatment of constipation (Patil et al. 2018; Sanaka et al. 2007). Being a dietary fiber, it has been observed to influence lipid metabolism, reason why its effects on cholesterol have been studied (Patil et al. 2018).

Regarding applications in biotechnology, probably the most recognized use of agar is in the preparation of culture media and other bacteriological applications, with agar diffusion test, also known as disk diffusion test, being a widely used assay to screen the in vitro antimicrobial activity of a compound, or of natural extracts and respective fractions in bioprospecting (Balouiri et al. 2016).

Table 2 General and specific applications of agar

General applications	Specific applications	References
Food	Jams; gelatin; bakery fillings; ice cream; candies, canned meat	Scieszka and Klewicka (2019)
Pharmaceutical	Suspending agent for barium sulfate radiology Intestine regulation Preparation of emulsions, suspensions, capsules	Sanaka et al. (2007), Shahruzzaman et al. (2019)
Bacteriological	Culture medium for microorganism (namely, bacteria growth) Antibacterial activity (agar diffusion assay)	Balouiri et al. (2016)
Dental	Full mouth dental impression Cast duplication	Dilip et al. (2020)
Cosmetics	Deodorants, shampoos, creams and lotions	Priyan Shanura Fernando et al. (2019)

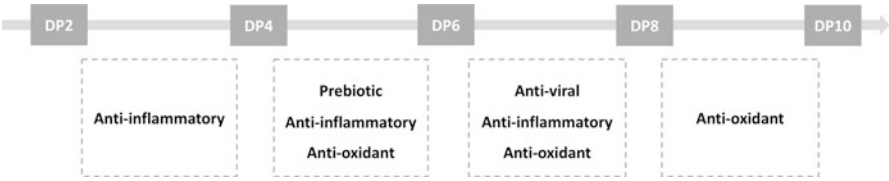


Fig. 3 Relation between biological activity and depolymerization degree (DP) of agar. (Adapted from Torres et al. 2019)

Despite being lately replaced by other materials, mainly related with the expensive equipment required, agar was one of the first materials used for dental impression molds due to reversible properties and hydrophilic nature (Dilip et al. 2020). Due to its stability and inert properties, agar is also used as a gelling agent known to soften and moisturize skin and hair and as an emulsifier and thickening agent in a variety of cosmetics products. Agar presents hygroscopic properties, making it suitable for use as a moisturizing agent (Priyan Shanura Fernando et al. 2019).

Nevertheless, the biological activities are mainly observed in agar oligosaccharides, which can be obtained with enzymatic hydrolysis, being already reported antioxidant, anti-inflammatory, prebiotic, and anti-viral effects (Torres et al. 2019). Agar oligosaccharides can be classified into two different categories: agaro-oligosaccharides (AOS) and neoagaro-oligosaccharides (NAOS) (Chi et al. 2012). Structure and bioactivity relationship studies reported the influence of the degree of depolymerization (DP) in the final biological activities of agar-oligosaccharides (Fig. 3) (Chen et al. 2005).

The anti-inflammatory activity of AOS was studied over mouse macrophages and human monocytes in vitro, demonstrating that AOS blocked elevated levels of nitric oxide and different pro-inflammatory cytokines (Enoki et al. 2010). In a different study, AOS induced heme oxygenase-1 expression, which is associated with significant suppression of neutrophil accumulation and TNF-α expression, being a possibility for inflammatory bowel disease therapeutics (Higashimura et al. 2013). Regarding the anti-oxidant activity, AOS presented DPPH and $ABTS^{+}$ radical scavenging and ferric reduction (Kang et al. 2014). NAOS exhibited increased radical-scavenging activity, which was influenced by the polymer concentration and DP (Xu et al. 2018). Agar extracted from *Gracilaria corticate* did not present cytotoxic effects, showing an antiviral activity against herpes simplex virus types 1 and 2, which may be related with inhibition of the initial virus attachment to the host cells (Mazumder et al. 2002). Different studies have been performed reporting the prebiotic activity of agar-oligosaccharides (Bhattarai and Kashyap 2016; Li et al. 2015), with NAOS revealing an increased beneficial bacteria growth when compared with fructo-oligosaccharides both in vitro and in vivo (Hu et al. 2006).

3.4 Ulvan

Ulvans are used as a source of sugars to synthesize fine chemicals. As an example, rhamnose is an important and rare component, being used as a precursor to synthesize aromatic compounds, as well as in pharmaceutical industry forming combinatorial libraries of glycopeptide mimetics. A rare uronic acid is iduronic acid, which is used for antithrombotic activity through analogues that synthesize heparin fragments (Usman et al. 2017). In this regard, ulvan is considered a heparinoid agent, which means that its chemical structure as well as biological activity is similar to that of heparin as an anticoagulant agent. The presence of sulfate groups was interpreted as determining to provide ulvan with antithrombotic activity, promoting the specific and nonspecific binding of the polysaccharide to the proteins involved in the intrinsic coagulation pathway. Collectively, ulvan is a promising candidate for replacing heparin as an anticoagulant drug, whose use in medicine has been increasingly hampered by biological concerns, namely, related with immunogenicity (Morelli et al. 2017; Pangestuti and Kurnianto 2017). Furthermore, the capacity to eliminate superoxide and hydroxyl radicals, the chelating activity of metals and the reducing power of ulvan have been described as demonstrators of antioxidant activity. These and other biological activities, illustrated in Table 3, are influenced by the molecular weight of the polysaccharide and its oligosaccharides, as well as the content of ulvan sulfate and its derivatives.

In recent years, ulvan has been the focus of numerous scientific studies to determine its possible biological activities. It has shown itself to be a promising candidate for the production of new therapeutic agents for various diseases due to its broad spectrum of activities, including anticoagulant (Faggio et al. 2016; Qi et al.

Table 3 Biological properties associated with sulfated polysaccharide from green algae

Biological activity	Application	References
Anticoagulant	Surrogate of heparin Thrombin inhibition Prolongation of activated partial thromboplastin time (APTT) Prolongation of thrombin time Prolongation of prothrombin time	Qi et al. (2012c, 2013), Adrien et al. (2017), Guerra-Rivas et al. (2011), Shanmugam et al. (2001), Faggio et al. (2016)
Antioxidant	Prevention of oxidative stress and used as a protective drug for several pathologies	Abd-Ellatef et al. (2017), Jose and Kurup (2016), Rahimi et al. (2016), Yuan et al. (2018)
Antitumor	Inhibition of cancer cells proliferation Apoptosis induction and suppression of cell division Cytotoxic activity Chemoprevention	Abd-Ellatef et al. (2017), Ahmed and Ahmed (2014), Thanh et al. (2016)
Antiviral	Treatment of viral infections	Aguilar-Briseño et al. (2015), Chiu et al. (2012a), Jiao et al. (2012), Morán-Santibañez et al. (2016), Song et al. (2016)
Immunomodulating	Therapy for diseases where the immune system is impaired Antinociceptive and anti-inflammatory responses Stronger immunomodulatory activity	Castro et al. (2004), Leiro et al. (2007), Peasura et al. (2016), Rahimi et al. (2016), Tabarsa et al. (2012)
Antihyperlipidemic	Lowering cholesterol levels Regulation of lipid metabolism	Borai et al. (2015), Qi et al. (2012a, b, Rizk et al. (2016a, b), Teng et al. (2013)

2012c), antioxidant (Abd-Ellatef et al. 2017; Jose and Kurup 2016; Rahimi et al. 2016; Yuan et al. 2018), antitumor (Abd-Ellatef et al. 2017; Ahmed and Ahmed 2014; Thanh et al. 2016), antiviral (Aguilar-Briseño et al. 2015; Chiu et al. 2012a; Jiao et al. 2012; Song et al. 2016; Morán-Santibañez et al. 2016), immunomodulating (Castro et al. 2004; Leiro et al. 2007; Peasura et al. 2016; Rahimi et al. 2016; Tabarsa et al. 2012), and antihyperlipidemic (Borai et al. 2015; Qi et al. 2012a, b; Rizk et al. 2016a, b; Teng et al. 2013). As is the case of the other referred sulfated polysaccharides, although several different biological activities have been described, there is yet no definite understanding of the structure-activity relationship, with a certain degree of variability on the exhibition of biological activity depending on the extract, i.e., a non-negligible batch-to-batch variability, thus suggesting that more studies are needed, namely, regarding systematic assessments and specificity of those activities (in comparison to other sulfated polysaccharides), toward the definition of ranges of action.

4 Biomedical Application

4.1 Fucoidan

Due to its unique properties, such as biocompatibility, biodegradable nature, and biological activities, fucoidanis being increasingly used on research for tissue engineering, drug delivery, and application of biosensors (Venkatesan et al. 2019b). Nevertheless, fucoidan has high water solubility, hampering the development of pure fucoidan structures stable in aqueous media, with literature being populated with reports on fucoidan combined with natural/synthetic polymers and materials such as chitosan (Murakami et al. 2010; Sezer et al. 2007), hydroxyapatite (Jeong et al. 2013), silk (Jeong et al. 2013), gelatin (Ko et al. 2012), poly(ε-caprolactone) – PCL (Jin and Kim 2011; Ko et al. 2012), polycarbonates and polyethylene terephthalate (Younes et al. 2018; Lee et al. 2012b). Fucoidan can be processed into hydrogels, nanofibers, microspheres, and porous structures, among other, using different technologies such as electrospinning (Jin and Kim 2011), rapid prototyping (Ko et al. 2012), freeze-drying (Sezer et al. 2007), solvent evaporation, and polyelectrolyte complexation (Indest et al. 2009).

In our group, a recent study demonstrated that it is possible to obtain structures of fucoidan alone using methacrylation reaction, modifying the fucoidan backbone and further obtaining structures by photocrosslinking with visible light (Reys et al. 2016). This methodology has enabled the production of microparticles, which may be find application as drug delivery or polymeric matrices for cell encapsulation (Lee et al. 2009). Other type of microparticles have been produced by combination of fucoidan and chitosan (Fucosphere) for drug delivery (Sezer and Akbuğa 2006), which is a combination enabling also the development of nanoparticles for sustained release of drugs (Huang and Lam 2011; Oliveira et al. 2018). Besides natural polymers, fucoidan has been also blended with synthetic polymers, namely, PCL, following a general melt-plotted process with possibility to be used in tissue engineering (Jin and Kim 2011; Ko et al. 2012). Low molecular weight fucoidan was blended with PCL using rapid-prototyping methodology in order to provide a system with an appropriate pore structure for bone tissue regeneration. Fucoidan was used to induce not only cell proliferation but additionally influence osteoconductive properties including alkaline phosphatase activity, collagen type I expression, and mineral deposition. Besides, micro/nanofibrous membranes and scaffolds of PCL and fucoidan have been produced by electrospinning and airbrushing, aimed for application in bone regeneration (Jin and Kim 2011; Silva et al. 2012), vascularization or anti-tumor devices (Oliveira et al. 2020b). The use of fucoidan on tissue engineering applications have gained particular attention also due to its enhancement of FGF-2 activity (Nakamura et al. 2006), promotion of the formation of fibrillar collagen matrix, support of fibroblastic proliferation (Ko et al. 2012), and stimulation of angiogenesis in vitro and in vivo (Ko et al. 2012; Luyt et al. 2003).

In summary, different approaches have been followed to use fucoidan on biomedical context besides its application as bioactive material, as the proposal of new functional biomaterials is gaining traction, with clear conclusions regarding positive performances being now required, namely, from studies with animal models, to enable scientist giving the next step toward clinical application.

4.2 Carrageenan

Carrageenans have been recognized as promising candidates for diverse biomedical applications, including tissue engineering, drug delivery, and wound healing, due to properties like biocompatibility, biodegradability, gel-forming, and water-retention abilities (Venkatesan et al. 2019a; Zia et al. 2017). Either alone or in combination with other polymers, carrageenans have been processed into several forms (Fig. 4), including pellets, beads, nanoparticles, microparticles, hydrogels, films, and porous matrices (Cunha and Grenha 2016). The interest in carrageenans comes also from their sulfated backbone, resembling naturally occurring glycosaminoglycans in cartilage extracellular matrix (ECM), motivating their study under the scope of cartilage tissue engineering applications (Popa et al. 2015b).

Recently, Popa et al. showed that κ-carrageenan hydrogels supported the viability, proliferation, and chondrogenic differentiation of human adipose-derived stem cells (hASCs) (Popa et al. 2012, 2015a), with mechanical properties being similar to that of natural cartilage tissue. Other authors used carrageenan to improve the quality of gels composed of fibrin and hyaluronic acid (Pereira et al. 2009), with encapsulated human articular chondrocytes maintaining the expression of the typical chondrogenic markers and depositing a cartilage specific ECM. In vivo experiments showed that this hydrogel seeded with human articular chondrocytes was able to regenerate and repair an experimentally made lesion in bovine articular cartilage. In another study, chemical modification of κ-carrageenan hydrogels with methacrylate groups formed covalently

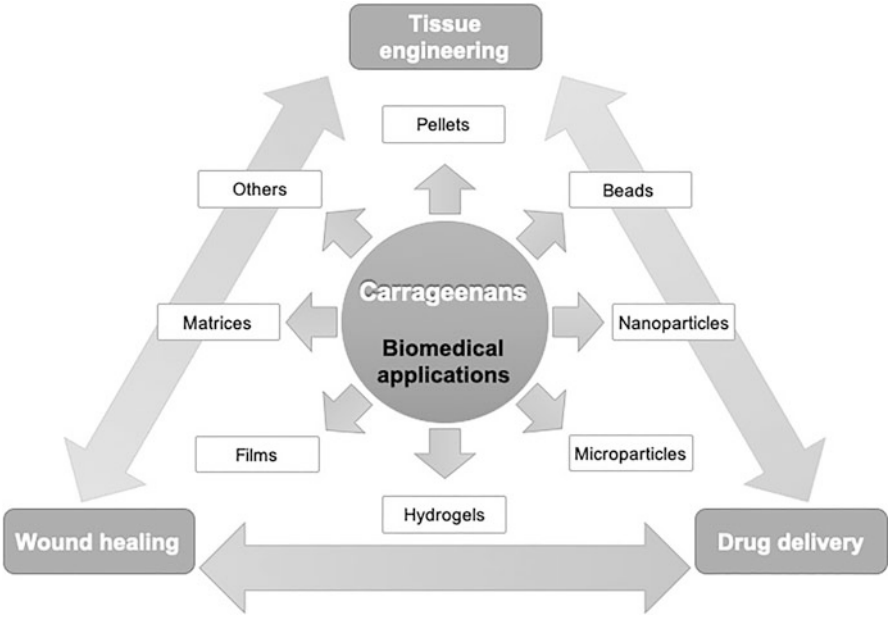


Fig. 4 Schematic representation of the possibilities of processing carrageenan into different forms and their major biomedical applications

crosslinked networks, with physiological stability and supporting the encapsulation of human mesenchymal stem cells (Thakur et al. 2016). More recently, the same research group reported the fabrication of a gradient hydrogel with methacrylated κ-carrageenan (MκCA) and gelatin methacryloyl (GelMA) reinforced with 2D nanosilicates (Cross et al. 2018), with seeded human mesenchymal stem cells displaying round or spread morphology (a characteristic appearance of chondrocyte and osteoblast cells, respectively) depending on the materials composition, suggesting application for the regeneration of bone-cartilage interfacial tissues.

Bone tissue engineering has been also addressed with κ-carrageenan, due to its ability to allow apatite layer formation when incorporated with functional bioactive cues (Daniel-da-Silva et al. 2007). Mihaila et al. (2014) reported the development of κ-carrageenan microfibers coated with chitosan able to release microvascular-like endothelial cells, without compromising their viability and phenotype (Mihaila et al. 2014). More recently, electrospun fibers were obtained by blending polyhydroxybutyrate or polyhydroxybutyrate-valerate with κ-carrageenan (Goonoo et al. 2017), resulting in enhanced NIH3T3 cell proliferation and improved SaOS-2 differentiation and mineralization. Li et al. (2015) fabricated ι-carrageenan/chitosan/gelatin scaffolds that not only could support the attachment and proliferation of adipose-derived mesenchymal stem cells, but also improve their osteogenic differentiation and neovascularization capacities. All the reported studies using carrageenan as component of scaffolds for tissue engineering make use of the less sulfated macromolecules (κ- and ι-), trying to overcome the well-known bottleneck associated to the pro-inflammatory effect associated to carrageenan. Indeed, this pro-inflammatory activity is so established that λ-carrageenan is injected in rat paw for the development of an animal model to test potential anti-inflammatory drugs (Fehrenbacher et al. 2012; Morris 2003). Thus, tissue engineering scaffolds comprising carrageenan should be always assessed in vivo prior to make definitive conclusions regarding performance, as very different outputs can be observed regarding immune response by the host.

The use of carrageenans for the production of drug delivery vehicles have been also explored, as is the case of the hydrogel beads developed with κ-carrageenan for encapsulation of growth factors, which high encapsulation efficiency and the controlled delivery profile resulted attractive for promoting angiogenesis in a tissue engineering approach (Santo et al. 2009). Besides ion gelation or crosslinking with chemical agents, carrageenans have been also combined with other materials. In this regard, Grenha et al. (2009) produced chitosan/κ-carrageenan nanoparticles that supported the sustained and controlled release of ovalbumin, exhibiting low toxicity in contact with fibroblast-like cells (Grenha et al. 2009). Besides, self-assembled hydrogels formulated by interaction between chitosan and ι-carrageenan were studied as potential carriers for transdermal delivery of tramadol (Kamel and Abbas 2013), showing optimized release and permeation profiles, without inducing skin irritancy, therefore promising an optimized pain management while minimizing risk of abuse. Mahdavinia et al. (2014) prepared magnetic and pH-sensitive hydrogel beads based on κ-carrageenan and sodium alginate for colon-targeted delivery of riboflavin, showing a drug release at pH 1.2 remarkably lower than at pH 7.4. A direct

correlation exists between the drug release rate and swelling rate, as evidenced by the low swelling at pH 1.2 and high swelling at pH 7.4, indicating that pH-dependent drug release was controlled by diffusion and polymer relaxation mechanisms. Other magnetic and pH-sensitive composite hydrogels based on κ-carrageenan were successively synthesized for controlled in vitro release of anti-inflammatory (diclofenac) and anticancer (methotrexate) drugs (Mahdavinia et al. 2015, 2017).

Hydrogels have been also studied as a wound dressing material because of their ability to retain water, act as physical barriers against microbial attack, and enhance re-epithelialization by promoting migration of fibroblasts, keratinocytes, and endothelial cells, accelerating the wound healing process (Boateng et al. 2008; Kamoun et al. 2017). In this context, several carrageenan-based hydrogels (in the form of membranes, films, among others) have been developed for wound healing applications (Yegappan et al. 2018). Nair et al. (2016) demonstrated that cyclic β-(1-3) (1-6) glucan/ι-carrageenan hydrogels have good wound healing properties and can accelerate the healing process considerably. Boateng et al. (2012) developed a polyethylene oxide and κ-carrageenan-based composite dressing containing antibacterial and anti-inflammatory drugs that showed a promising effect on the healing of chronic wounds, tackling bacterial infection, swelling and pain at the same time. Azizi et al. (2017) reported that cross-linked κ-carrageenan/silver nanoparticles hydrogel beads presented good antibacterial activities against several bacteria, including the methicillin-resistant *Staphylococcus aureus* that is responsible for several difficult-to-treat wound infections. Strong antibacterial activity was also observed when κ-carrageenan-based hydrogels were combined with other metallic nanoparticles, like zinc oxide and copper oxide nanoparticles (Oun and Rhim 2017).

Overall, these studies emphasize the potential use of carrageenans for cartilage and bone tissue engineering, drug delivery, and would healing. However, translation to the clinical practice will require more studies to broaden our knowledge about the structural, physicochemical, and toxicological properties of carrageenan. Detailed in vivo studies are also essential to validate the therapeutic efficiency of carrageenan-based materials, while evaluating deleterious effects, as severe immune reactions. Despite the obstacles to the successful clinical application of carrageenan, the near future for this versatile material seems to be to enrich the list of marine polysaccharides-based products commercialized in biomedical arena.

4.3 Agar

Agar is used in TE approaches due to its hydrophilic nature, jellylike behavior, controllable size, and concentration-based properties. However, it may also present some restraints due to low mechanical properties such as tensile strength and percent of elongation at break, which may limit its use as a biomaterial in TE, reason why agar is often used in combination with other materials. Nevertheless, its stiffness can be modified due to its concentration-based properties, tuning the scaffolds' mechanical properties (Nayak and Gupta 2015; Nayar et al. 2012). Agar gels are easy to prepare and present a spongy-like behavior due its porous nature. If an agar gel is

dried, it will swell to its original shape and size upon rehydration. Agar gels also allow diffusion of molecules, which is of great importance to allow nutrients and gases exchange when encapsulating cells (Verma et al. 2007).

A blend of keratin/agar was used to produce a porous scaffold by freeze extraction methods, which besides having antimicrobial activity, supported the attachment and proliferation of myofibroblasts, thus showing potential for wound healing and skin regeneration (Nayak and Gupta 2015). Agar was also combined with gelatin to produce an injectable hydrogel aiming peripherical nerve regeneration, which presented suitable rheological properties and appropriate environment for cells' adhesion, proliferation, and migration (Tonda-Turo et al. 2017). An agar-gelatin hybrid hydrogel was also used as mineralization matrix, supporting the in vitro growth of apatite, which significantly enhanced cells' viability and alkaline phosphatase activity, suggesting this system as a possible approach for bone TE (Deng et al. 2013). Agar and gelatin-based scaffolds were also produced using different ratios of the two materials, and a 2:1 ratio presented increased growth kinetics in a mouse fibroblast cell line, being thus a strategy that may be used in various TE strategies and as preliminary evaluation of the toxicity of drugs (Verma et al. 2007). In a different attempt, nanocomposite hydrogels based in polyvinyl alcohol/Agar/Graphene were produced using a green solution mixing method, with the resulting polymeric network exhibiting high strength, toughness, and autonomous self-healing, which may be important parameters for the development of different biomedical applications (Samadi et al. 2018).

Taken together and despite agar is mainly used in the food industry, this versatile sulfated polysaccharide has shown unique properties that have been reported for different biological applications, from which its use on the development of new and innovative systems in the biomedical field should be further investigated.

4.4 Ulvan

Different structures comprising with ulvan have been explored is a variety of biomedical applications, from drug administration to bone regeneration and wound healing. In this regard, different morphologies were manufactured, including nanofibers, particles, hydrogels, and porous three-dimensional (3D) scaffolds (Krishnan 2016).

Nanofibers were produced and evaluated with an extract rich in ulvan with polyvinyl alcohol (PVA), where they exhibited interesting biological and physicochemical properties that can lead to new biomedical applications, such as drug delivery systems or dressings, and even for tissue engineering if mechanical integrity can be reinforced (Bhatnagar and Bhatnagar 2015; Toskas et al. 2011). Alves et al. (2012a) evaluated the potential of 2D polymeric membranes using ulvan for curative purposes, with the capacity to incorporate and further release dexamethasone (model drug) in a sustained manner, which supports the viability of considering these membranes as promising vehicles for the sustained administration of drugs and other biological factors (Alves et al. 2012b; Nguyen and Camci-Unal 2020; Pangestuti and Kurnianto 2017).

Due to its semi-crystalline nature and high hygroscopic characteristics, ulvan has also addressed tissue engineering applications. In a matrix of poly-D, L-lactic acid (PDLLA), the incorporation of ulvan particles resulted in a new scaffold, with cytocompatible characteristics and opening a potential for bone tissue engineering (Alves et al. 2012a; Bhatnagar and Bhatnagar 2015). Besides, cross-linking of ulvan was also explored for the production of biocompatible hydrogels, through the functionalization with methacryloyl moieties and further irradiated with UV to promote photocrosslinking (Morelli and Chiellini 2010), or through enzymatic crosslinking by using a combination of enzyme horseradish peroxidase and H_2O_2 (Morelli et al. 2016), with the latter giving rise to materials with rheological properties compatible with injectability. Dash et al. (2014) investigated another enzymatically cross-linked ulvan scaffold, biofunctionalized with alkaline phosphatase (Fernando et al.), observing homogeneous mineralization and viability of cultured osteoblasts, suggesting that this scaffold can be advantageous as a resorbable bone graft substitute (Dash et al. 2014; Venkatesan et al. 2017).

The preparation of ulvan-based materials is taking advantage of its strong bioactivity and the presence of hydrophilic and hydrophobic groups, with the resulting functional biomaterials being studied in a wide range of biomedical and wellbeing applications (Lakshmi et al. 2020).

5 Conclusions and Final Remarks

In materials science, the ones with biological origin occupy an essential position considering their availability, biodegradability, and ecological nature. Sulfated polysaccharides of algal origin provide a versatile class of materials that can have a wide range of applications, especially medical applications, such as tissue engineering, medication administration, and wound dressings, among others. In this chapter, the biomedical potential of macroalgae sulfated polysaccharides was explored, building on top of their main characteristics and biological activities, which gives us an insight into the chemical diversity of these natural biopolymers and the associated versatility toward applications. The use of these polysaccharides in health-related fields is only at the beginning, with their innate biocompatibility and their wide availability in nature with cost-effective production processes making them attractive for industrial and clinical exploitation, should the on-going and future preclinical trials confirm top performance, as suggested by the high quality in-vitro results obtained with seaweed-derived sulfated polysaccharide-based biomaterials.

Acknowledgments The authors would like to acknowledge the funding received from European Union through INTERREG España-Portugal 2014-2020 Programme, under the scope of the projects 0302_CVMAR_I_1_P and 0474_BLUEBIOLAB_1_E, as well as through ERDF with Programa Operacional Competitividade e Internacionalização (Portugal), under the scope of the project VALORMAR: Valorização Integral dos Recursos Marinhos: Potencial, Inovação Tecnológica e Novas Aplicações (POCI-024517-FEDER).

Declaration of Interest
Authors report no competing interest affecting the contents of the manuscript.

References

Abd-Ellatef G-EF, Ahmed OM, Abdel-Reheim ES, Abdel-Hamid A-HZ. Ulva lactuca polysaccharides prevent Wistar rat breast carcinogenesis through the augmentation of apoptosis, enhancement of antioxidant defense system, and suppression of inflammation. Breast Cancer (Dove Med Press). 2017;9:67–83. https://doi.org/10.2147/BCTT.S125165.

Adrien A, Dufour D, Baudouin S, Maugard T, Bridiau N. Evaluation of the anticoagulant potential of polysaccharide-rich fractions extracted from macroalgae. Nat Prod Res. 2017;31(18):2126–36. https://doi.org/10.1080/14786419.2017.1278595.

Aguilar-Briseño JA, Cruz-Suarez LE, Sassi J-F, Ricque-Marie D, Zapata-Benavides P, Mendoza-Gamboa E, Rodríguez-Padilla C, Trejo-Avila LM. Sulphated polysaccharides from Ulva clathrata and Cladosiphon okamuranus seaweeds both inhibit viral attachment/entry and cell-cell fusion, in NDV infection. Mar Drugs. 2015;13(2):697–712.

Ahmed O, Ahmed R. Anti-proliferative and apoptotic efficacies of ulvan polysaccharides against different types of carcinoma cells. Vitro in vivo. J Cancer Sci Ther. 2014;6:202–8.

Ale MT, Meyer AS. Fucoidans from brown seaweeds: an update on structures, extraction techniques and use of enzymes as tools for structural elucidation. RSC Adv. 2013;3(22):8131–41. https://doi.org/10.1039/C3RA23373A.

Ale MT, Maruyama H, Tamauchi H, Mikkelsen JD, Meyer AS. Fucoidan from Sargassum sp. and Fucus vesiculosus reduces cell viability of lung carcinoma and melanoma cells in vitro and activates natural killer cells in mice in vivo. Int J Biol Macromol. 2011a;49(3):331–6.

Ale MT, Mikkelsen JD, Meyer AS. Important determinants for fucoidan bioactivity: a critical review of structure-function relations and extraction methods for fucose-containing sulfated polysaccharides from brown seaweeds. Mar Drugs. 2011b;9(10):2106–30.

Al-Nahdi ZM, Al-Alawi A, Al-Marhobi I. The effect of extraction conditions on chemical and thermal characteristics of kappa-carrageenan extracted from Hypnea bryoides. J Mar Biol. 2019;2019(1):1–10.

Alves A, Duarte ARC, Mano JF, Sousa RA, Reis RL. PDLLA enriched with ulvan particles as a novel 3D porous scaffold targeted for bone engineering. J Supercrit Fluids. 2012a;65:32–8. https://doi.org/10.1016/j.supflu.2012.02.023.

Alves A, Pinho ED, Neves NM, Sousa RA, Reis RL. Processing ulvan into 2D structures: cross-linked ulvan membranes as new biomaterials for drug delivery applications. Int J Pharm. 2012b;426(1–2):76–81. https://doi.org/10.1016/j.ijpharm.2012.01.021.

Alves A, Sousa RA, Reis RL. A practical perspective on ulvan extracted from green algae. J Appl Phycol. 2013;25(2):407–24.

Anastyuk SD, Imbs TI, Shevchenko NM, Dmitrenok PS, Zvyagintseva TN. ESIMS analysis of fucoidan preparations from Costaria costata, extracted from alga at different life-stages. Carbohydr Polym. 2012;90(2):993–1002. https://doi.org/10.1016/j.carbpol.2012.06.033.

Andrade PB, Barbosa M, Matos RP, Lopes G, Vinholes J, Mouga T, Valentão P. Valuable compounds in macroalgae extracts. Food Chem. 2013;138(2–3):1819–28. https://doi.org/10.1016/j.foodchem.2012.11.081.

Armisen R, Galatas F. Production, properties and uses of agar. In: McHugh DJ, editor. Production utilization of products from commercial seaweeds, vol. 288; 1987. FAO Fish Tech Pap, pp 1–57.

Arslan R, Bor Z, Bektas N, Meriçli AH, Ozturk Y. Antithrombotic effects of ethanol extract of Crataegus orientalis in the carrageenan-induced mice tail thrombosis model. Thromb Res. 2011;127(3):210–3.

Arvizu-Higuera DL, Rodríguez-Montesinos YE, Murillo-Álvarez JI, Muñoz-Ochoa M, Hernández-Carmona G. Effect of alkali treatment time and extraction time on agar from Gracilaria vermiculophylla. J Appl Phycol. 2007;20:515–9.

Atashrazm F, Lowenthal RM, Woods GM, Holloway AF, Dickinson JL. Fucoidan and cancer: a multifunctional molecule with anti-tumor potential. Mar Drugs. 2015;13(4):2327–46.

Azizi, Susan & Mohamad, Rosfarizan & Rahim, Raha & Mohammadinejad, Reza & Ariff, Arbakariya. Hydrogel Beads Bio-nanocomposite based on Kappa-Carrageenan and Green

Synthesized Silver Nanoparticles for Biomedical Applications. Int J Biol Macromol. 2017;104. https://doi.org/10.1016/j.ijbiomac.2017.06.010.

Baba M, Snoeck R, Pauwels R, De Clercq E. Sulfated polysaccharides are potent and selective inhibitors of various enveloped viruses, including herpes simplex virus, cytomegalovirus, vesicular stomatitis virus, and human immunodeficiency virus. Antimicrob Agents Chemother. 1988;32(11):1742–5.

Balboa EM, Rivas S, Moure A, Domínguez H, Parajó JC. Simultaneous extraction and depolymerization of fucoidan from Sargassum muticum in aqueous media. Mar Drugs. 2013;11(11):4612–27. https://doi.org/10.3390/md11114612.

Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: a review. J Pharm Anal. 2016;6(2):71–9.

Bhatnagar M, Bhatnagar A. Wound dressings from algal polymers. In: Kim S-K, Chojnacka K, editors. Marine algae extracts: processes, products, applications. Weinheim: Wiley-VCH Verlag GmbH & Co; 2015. p. 523–56. https://doi.org/10.1002/9783527679577.ch31.

Bhattarai Y, Kashyap PC. Agaro-oligosaccharides: a new frontier in the fight against colon cancer? Am J Physiol Gastrointest Liver Physiol. 2016;310(6):G335–6.

Bi Y, Hu Y, Zhou Z. Genetic variation of Laminaria japonica (Phaeophyta) populations in China as revealed by RAPD markers. Acta Oceanol Sin. 2011;30(2):103–12.

Boateng JS, Matthews KH, Stevens HN, Eccleston GM. Wound healing dressings and drug delivery systems: a review. J Pharm Sci. 2008;97(8):2892–923.

Boateng, Joshua & Pawar, Harshavardhan. Polyox and carrageenan based composite film dressing containing anti-microbial and anti-inflammatory drugs for effective wound healing. Int J Pharm. 2012;441. https://doi.org/10.1016/j.ijpharm.2012.11.045.

Boo HJ, Hyun JH, Kim SC, Kang JI, Kim MK, Kim SY, Cho H, Yoo ES, Kang HK. Fucoidan from Undaria pinnatifida induces apoptosis in A549 human lung carcinoma cells. Phytother Res. 2011;25(7):1082–6.

Borai IH, Ezz MK, Rizk MZ, Matloub A, Aly H, El A, Farrag R, Fouad GI. Hypolipidemic and antiatherogenic effect of sulphated polysaccharides from the green alga Ulva fasciata. Int J Pharm Sci Rev Res. 2015;31(1):1–12.

Buck CB, Thompson CD, Roberts JN, Müller M, Lowy DR, Schiller JT. Carrageenan is a potent inhibitor of papillomavirus infection. PLoS Pathog. 2006;2(7):e69.

Campo VL, Kawano DF, da Silva Jr DB, Carvalho I. Carrageenans: biological properties, chemical modifications and structural analysis–a review. Carbohydr Polym. 2009;77(2):167–80.

Carlucci M, Ciancia M, Matulewicz M, Cerezo A, Damonte E. Antiherpetic activity and mode of action of natural carrageenans of diverse structural types. Antivir Res. 1999;43(2):93–102.

Carvalho DN, Inácio AR, Sousa RO, Reis RL, Silva TH. Seaweed polysaccharides as sustainable building blocks for biomaterials in tissue engineering. In: Sustainable seaweed technologies. Elsevier; 2020. p. 543–87.

Castro R, Zarra I, Lamas J. Water-soluble seaweed extracts modulate the respiratory burst activity of turbot phagocytes. Aquaculture. 2004;229(1–4):67–78.

Chen H-M, Zheng L, Yan X-J. The preparation and bioactivity research of agaro-oligosaccharides. Food Technol Biotechnol. 2005;43(1):29–36.

Chen H, Yan X, Lin J, Wang F, Xu W. Depolymerized products of λ-carrageenan as a potent angiogenesis inhibitor. J Agric Food Chem. 2007;55(17):6910–7.

Chi W-J, Chang Y-K, Hong S-K. Agar degradation by microorganisms and agar-degrading enzymes. Appl Microbiol Biotechnol. 2012;94(4):917–30.

Chiellini F, Morelli A. Ulvan: a versatile platform of biomaterials from renewable resources. Biomater Phys Chem. 2011; 14:75–98.

Chiu Y-H, Chan Y-L, Li T-L, Wu C-J. Inhibition of Japanese encephalitis virus infection by the sulfated polysaccharide extracts from Ulva lactuca. Mar Biotechnol. 2012a;14(4):468–78.

Chiu Y-H, Chan Y-L, Tsai L-W, Li T-L, Wu C-J. Prevention of human enterovirus 71 infection by kappa carrageenan. Antivir Res. 2012b;95(2):128–34.

Cross LM, Shah K, Palani S, Peak CW, Gaharwar AK. Gradient nanocomposite hydrogels for interface tissue engineering. Nanomed: Nanotechnol Biol Med. 2018;14(7):2465–74.

Cunha L, Grenha A. Sulfated seaweed polysaccharides as multifunctional materials in drug delivery applications. Mar Drugs. 2016;14(3):42. https://doi.org/10.3390/md14030042.
Daniel-da-Silva AL, Lopes AB, Gil AM, Correia RN. Synthesis and characterization of porous κ-carrageenan/calcium phosphate nanocomposite scaffolds. J Mater Sci. 2007;42(20):8581–91.
Dash M, Samal SK, Bartoli C, Morelli A, Smet PF, Dubruel P, Chiellini F. Biofunctionalization of ulvan scaffolds for bone tissue engineering. ACS Appl Mater Inter. 2014;6(5):3211–8. https://doi.org/10.1021/am404912c.
David S, Levi CS, Fahoum L, Ungar Y, Meyron-Holtz EG, Shpigelman A, Lesmes U. Revisiting the carrageenan controversy: do we really understand the digestive fate and safety of carrageenan in our foods? Food Funct. 2018;9(3):1344–52.
David S, Fahoum L, Rozen G, Shaoul R, Shpigelman AM-H, Esther G, Lesmes U. Reply to the comment on "Revisiting the carrageenan controversy: do we really understand the digestive fate and safety of carrageenan in our foods?" by M. Weiner and J. McKim, Food Funct. Food Funct. 2019;10(3):1763–6. https://doi.org/10.1039/C8FO01282B.
de Souza MCR, Marques CT, Dore CMG, da Silva FRF, Rocha HAO, Leite EL. Antioxidant activities of sulfated polysaccharides from brown and red seaweeds. J Appl Phycol. 2007;19(2):153–60.
Deng Y, Zhao X, Zhou Y, Zhu P, Zhang L, Wei S. In vitro growth of bioactive nanostructured apatites via agar-gelatin hybrid hydrogel. J Biomed Nanotechnol. 2013;9(12):1972–83.
Dilip A, Gupta R, Geiger Z. Dental alginate impressions. StatPearls. 2020. https://www.ncbi.nlm.nih.gov/books/NBK470480/
Dobashi K, Nishino T, Fujihara M, Nagumo T. Isolation and preliminary characterization of fucose-containing sulfated polysaccharides with blood-anticoagulant activity from the brown seaweed Hizikia fusiforme. Carbohydr Res. 1989;194:315–20.
Duarte ME, Cardoso MA, Noseda MD, Cerezo AS. Structural studies on fucoidans from the brown seaweed Sargassum stenophyllum. Carbohydr Res. 2001;333(4):281–93. https://doi.org/10.1016/s0008-6215(01)00149-5.
Enoki T, Okuda S, Kudo Y, Takashima F, Sagawa H, Kato I. Oligosaccharides from agar inhibit pro-inflammatory mediator release by inducing heme oxygenase 1. Biosci Biotechnol Biochem. 2010;74(4):766–70.
Faggio C, Pagano M, Dottore A, Genovese G, Morabito M. Evaluation of anticoagulant activity of two algal polysaccharides. Nat Prod Res. 2016;30(17):1934–7.
Farias WR, Valente A-P, Pereira MS, Mourão PA. Structure and anticoagulant activity of sulfated galactans isolation of a unique sulfated galactan from the red algaebotryocladia occidentalis and comparison of its anticoagulant action with that of sulfated galactans from invertebrates. J Biol Chem. 2000;275(38):29299–307.
Feferman L, Bhattacharyya S, Oates E, Haggerty N, Wang T, Varady K, Tobacman JK. Carrageenan-free diet shows improved glucose tolerance and insulin signaling in prediabetes: a randomized, pilot clinical trial. J Diabetes Res. 2020;2020:1–16.
Fehrenbacher JC, Vasko MR, Duarte DB. Models of inflammation: carrageenan-or complete freund's adjuvant (CFA)–induced edema and hypersensitivity in the rat. Curr Protoc Pharmacol. 2012;56(1):5.4.1–5.4.4.
Fitton JH, Irhimeh M, Falk N. Macroalgal fucoidan extracts: a new opportunity for marine cosmetics. Cosmetics Toiletries. 2007;122(8):55.
Foley SA, Szegezdi E, Mulloy B, Samali A, Tuohy MG. An unfractionated fucoidan from Ascophyllum nodosum: extraction, characterization, and apoptotic effects in vitro. J Nat Prod. 2011;74(9):1851–61. https://doi.org/10.1021/np200124m.
Fuse T, Suzuki T. Preparation and properties of agar sulfates. Agr Biol Chem. 1975;39(1):119–26.
Gates KW. Marine polysaccharides – food applications. Vazhiyil Venugopal. J Aquat Food Prod Technol. 2012;21(2):181–6.
Goonoo N, Khanbabaee B, Steuber M, Bhaw-Luximon A, Jonas U, Pietsch U, Jhurry D, Schönherr H. κ-Carrageenan enhances the biomineralization and osteogenic differentiation of electrospun

polyhydroxybutyrate and polyhydroxybutyrate valerate fibers. Biomacromolecules. 2017;18(5):1563–73.
Grassauer A, Weinmuellner R, Meier C, Pretsch A, Prieschl-Grassauer E, Unger H. Iota-carrageenan is a potent inhibitor of rhinovirus infection. Virol J. 2008;5(1):1–13.
Grenha A, Gomes ME, Rodrigues M, Santo VE, Mano JF, Neves NM, Reis RL. Development of new chitosan/carrageenan nanoparticles for drug delivery applications. J Biomed Mater Res A. 2009;92(4):1265–72.
Groult H, Cousin R, Chot-Plassot C, Maura M, Bridiau N, Piot J-M, Maugard T, Fruitier-Arnaudin I. λ-Carrageenan oligosaccharides of distinct anti-heparanase and anticoagulant activities inhibit MDA-MB-231 breast cancer cell migration. Mar Drugs. 2019;17(3):140.
Guerra-Rivas G, Gómez-Gutiérrez CM, Alarcón-Arteaga G, Soria-Mercado IE, Ayala-Sánchez NE. Screening for anticoagulant activity in marine algae from the Northwest Mexican Pacific coast. J Appl Phycol. 2011;23(3):495–503. https://doi.org/10.1007/s10811-010-9618-3.
Hahn T, Lang S, Ulber R, Muffler K. Novel procedures for the extraction of fucoidan from brown algae. Process Biochem. 2012;47(12):1691–8.
Higashimura Y, Naito Y, Takagi T, Mizushima K, Hirai Y, Harusato A, Ohnogi H, Yamaji R, Inui H, Nakano Y. Oligosaccharides from agar inhibit murine intestinal inflammation through the induction of heme oxygenase-1 expression. J Gastroenterol. 2013;48(8):897–909.
Holtkamp A. Isolation, characterisation, modification and application of fucoidan from Fucus vesiculosus. 2009. Technische Universität Braunschweig, Doctoral dissertation.
Hsu H-Y, Lin T-Y, Wu Y-C, Tsao S-M, Hwang P-A, Shih Y-W, Hsu J. Fucoidan inhibition of lung cancer in vivo and in vitro: role of the Smurf2-dependent ubiquitin proteasome pathway in TGFβ receptor degradation. Oncotarget. 2014;5(17):7870.
Hu B, Gong Q, Wang Y, Ma Y, Li J, Yu W. Prebiotic effects of neoagaro-oligosaccharides prepared by enzymatic hydrolysis of agarose. Anaerobe. 2006;12(5–6):260–6.
Huang YC, Lam UI. Chitosan/fucoidan pH sensitive nanoparticles for oral delivery system. J Chin Chem Soc. 2011;58(6):779–85.
Huang Y-C, Li R-Y, Chen J-Y, Chen J-K. Biphasic release of gentamicin from chitosan/fucoidan nanoparticles for pulmonary delivery. Carbohydr Polym. 2016;138:114–22.
Ibañez E, Cifuentes A. Benefits of using algae as natural sources of functional ingredients. J Sci Food Agric. 2013;93(4):703–9.
Indest T, Laine J, Johansson L-S, Stana-Kleinschek K, Strnad S, Dworczak R, Ribitsch V. Adsorption of fucoidan and chitosan sulfate on chitosan modified PET films monitored by QCM-D. Biomacromolecules. 2009;10(3):630–7.
Irhimeh MR, Fitton JH, Lowenthal RM. Pilot clinical study to evaluate the anticoagulant activity of fucoidan. Blood Coagul Fibrinolysis. 2009;20(7):607–10.
Jacob J, Haponiuk JT, Thomas S, Gopi S. Biopolymer based nanomaterials in drug delivery systems: a review. Mater Today Chem. 2018;9:43–55. https://doi.org/10.1016/j.mtchem.2018.05.002.
Jang Y, Shin H, Lee MK, Kwon OS, Shin JS, Kim Y-i, Kim CW, Lee H-R, Kim M. Antiviral activity of lambda-carrageenan against influenza viruses and severe acute respiratory syndrome coronavirus 2. Sci Rep. 2021;11(1):1–12. https://doi.org/10.1038/s41598-020-80896-9.
Jeong H-S, Venkatesan J, Kim S-K. Hydroxyapatite-fucoidan nanocomposites for bone tissue engineering. Int J Biol Macromol. 2013;57:138–41.
Jiang X, Yu J, Ma Z, Zhang H, Xie F. Effects of fucoidan on insulin stimulation and pancreatic protection via the cAMP signaling pathway in vivo and in vitro. Mol Med Rep. 2015;12(3):4501–7.
Jiao G, Yu G, Zhang J, Ewart HS. Chemical structures and bioactivities of sulfated polysaccharides from marine algae. Mar Drugs. 2011;9(2):196–223.
Jiao G, Yu G, Wang W, Zhao X, Zhang J, Ewart SH. Properties of polysaccharides in several seaweeds from Atlantic Canada and their potential anti-influenza viral activities. J Ocean Univ China. 2012;11(2):205–12.
Jin G, Kim GH. Rapid-prototyped PCL/fucoidan composite scaffolds for bone tissue regeneration: design, fabrication, and physical/biological properties. J Mater Chem. 2011;21(44):17710–8.

Jin Z, Han Y-X, Han X-R. Degraded iota-carrageenan can induce apoptosis in human osteosarcoma cells via the Wnt/β-catenin signaling pathway. Nutrition Cancer. 2013;65(1):126–31.
Jose GM, Kurup GM. In vitro antioxidant properties of edible marine algae Sargassum swartzii, Ulva fasciata and Chaetomorpha antennina of Kerala coast. Pharm Bioprocess. 2016;4(6):100–8.
Kadam SU, Álvarez C, Tiwari BK, O'Donnell CP. Extraction of biomolecules from seaweeds. In: Seaweed sustainability. Elsevier; 2015. p. 243–69.
Kamel R, Abbas H. Self-assembled carbohydrate hydrogels for prolonged pain management. Pharm Dev Technol. 2013;18(5):990–1004.
Kamoun EA, Kenawy E-RS, Chen X. A review on polymeric hydrogel membranes for wound dressing applications: PVA-based hydrogel dressings. J Adv Res. 2017;8(3):217–33.
Kang OL, Ghani M, Hassan O, Rahmati S, Ramli N. Novel agaro-oligosaccharide production through enzymatic hydrolysis: physicochemical properties and antioxidant activities. Food Hydrocoll. 2014;42:304–8.
Kidgell JT, Magnusson M, de Nys R, Glasson CR. Ulvan: a systematic review of extraction, composition and function. Algal Res. 2019;39:101422.
Kim S-K. Marine carbohydrates: fundamentals and applications, part B. Academic Press; 2014.
Kim K-T, Rioux L-E, Turgeon SL. Alpha-amylase and alpha-glucosidase inhibition is differentially modulated by fucoidan obtained from Fucus vesiculosus and Ascophyllum nodosum. Phytochemistry. 2014a;98:27–33.
Kim MJ, Jeon J, Lee JS. Fucoidan prevents high-fat diet-induced obesity in animals by suppression of fat accumulation. Phytother Res. 2014b;28(1):137–43.
Ko JA, Oh YS, Park HJ. Preparation and characterization of aminated gelatin-fucoidan microparticles. Korean J Food Sci Technol. 2012;44(2):191–5.
Koenighofer M, Lion T, Bodenteich A, Prieschl-Grassauer E, Grassauer A, Unger H, Mueller CA, Fazekas T. Carrageenan nasal spray in virus confirmed common cold: individual patient data analysis of two randomized controlled trials. Multidiscip Respir Med. 2014;9(1):1–12. https://doi.org/10.1186/2049-6958-9-57.
Krishnan UM. Bioengineered skin: progress and prospects. In: Sethuraman S, Kishnan UM, Subramanian A, editors. Biomaterials and nanotechnology for tissue engineering. CRC Press; 2016. p. 127–70.
Kumar V, Fotedar R. Agar extraction process for Gracilaria cliftonii. Carbohydr Polym. 2009;78(4):813–9.
Kumar CS, Ganesan P, Suresh P, Bhaskar N. Seaweeds as a source of nutritionally beneficial compounds-a review. J Food Sci Technol. 2008;45(1):1.
Kumar TV, Lakshmanasenthil S, Geetharamani D, Marudhupandi T, Suja G, Suganya P. Fucoidan–A α-d-glucosidase inhibitor from Sargassum wightii with relevance to type 2 diabetes mellitus therapy. Int J Biol Macromol. 2015;72:1044–7.
Kumar SSD, Houreld NN, Abrahamse H. Biopolymer-based composites for medical applications. In: Hashmi S, Choudhury I, editors. Reference module in materials science and materials engineering. Oxford: Elsevier; 2019. https://doi.org/10.1016/B978-0-12-803581-8.10557-0.
Kwak J-Y. Fucoidan as a marine anticancer agent in preclinical development. Mar Drugs. 2014;12(2):851–70.
Kwon PS, Oh H, Kwon S-J, Jin W, Zhang F, Fraser K, Hong JJ, Linhardt RJ, Dordick JS. Sulfated polysaccharides effectively inhibit SARS-CoV-2 in vitro. Cell Discovery. 2020;6(1):1–4. https://doi.org/10.1038/s41421-020-00192-8.
Kyung J, Kim D, Park D, Yang Y-H, Choi E-K, Lee S-P, Kim T-S, Lee Y-B, Kim Y-B. Synergistic anti-inflammatory effects of Laminaria japonica fucoidan and Cistanche tubulosa extract. Lab Anim Res. 2012;28(2):91–7.
Lahaye M. Developments on gelling algal galactans, their structure and physico-chemistry. J Appl Phycol. 2001;13(2):173–84.

Lakshmi DS, Sankaranarayanan S, Gajaria TK, Li G, Kujawski W, Kujawa J, Navia R. A short review on the valorization of green seaweeds and Ulvan: FEEDSTOCK for chemicals and biomaterials. Biomol Ther. 2020;10(7):991.

Lapshina L, Reunov A, Nagorskaya V, Zvyagintseva T, Shevchenko N. Inhibitory effect of fucoidan from brown alga Fucus evanescens on the spread of infection induced by tobacco mosaic virus in tobacco leaves of two cultivars. Russ J Plant Physiol. 2006;53(2):246–51.

Lee J-B, Hayashi K, Hashimoto M, Nakano T, Hayashi T. Novel antiviral fucoidan from sporophyll of Undaria pinnatifida (Mekabu). Chem Pharm Bull. 2004a;52(9):1091–4.

Lee J-B, Hayashi K, Maeda M, Hayashi T. Antiherpetic activities of sulfated polysaccharides from green algae. Planta Med. 2004b;70(09):813–7.

Lee EJ, Khan SA, Lim K-H. Chitosan-nanoparticle preparation by polyelectrolyte complexation. World J Eng. 2009:541–2.

Lee H, Kim J-S, Kim E. Fucoidan from seaweed Fucus vesiculosus inhibits migration and invasion of human lung cancer cell via PI3K-Akt-mTOR pathways. PLoS One. 2012a;7(11):e50624.

Lee JS, Jin GH, Yeo MG, Jang CH, Lee H, Kim GH. Fabrication of electrospun biocomposites comprising polycaprolactone/fucoidan for tissue regeneration. Carbohydr Polym. 2012b;90(1):181–8.

Lee W-K, Lim Y-Y, Leow AT-C, Namasivayam P, Abdullah JO, Ho C-L. Factors affecting yield and gelling properties of agar. J Appl Phycol. 2017;29(3):1527–40.

Leibbrandt A, Meier C, König-Schuster M, Weinmüllner R, Kalthoff D, Pflugfelder B, Graf P, Frank-Gehrke B, Beer M, Fazekas T. Iota-carrageenan is a potent inhibitor of influenza a virus infection. PLoS One. 2010;5(12):e14320.

Leiro JM, Castro R, Arranz JA, Lamas J. Immunomodulating activities of acidic sulphated polysaccharides obtained from the seaweed Ulva rigida C. Agardh. Int Immunopharmacol. 2007;7(7):879–88.

Li B, Lu F, Wei X, Zhao R. Fucoidan: structure and bioactivity. Molecules. 2008;13(8):1671–95.

Li J, Yang B, Qian Y, Wang Q, Han R, Hao T, Shu Y, Zhang Y, Yao F, Wang C. Iota-carrageenan/chitosan/gelatin scaffold for the osteogenic differentiation of adipose-derived MSCs in vitro. J Biomed Mater Res B Appl Biomater. 2015;103(7):1498–510. https://doi.org/10.1002/jbm.b.33339. Epub 2014 Nov 29. PMID: 25449538.

Liu J, Hafting J, Critchley AT, Banskota AH, Prithiviraj B. Components of the cultivated red seaweed Chondrus crispus enhance the immune response of Caenorhabditis elegans to Pseudomonas aeruginosa through the pmk-1, daf-2/daf-16, and skn-1 pathways. Appl Environ Microbiol. 2013;79(23):7343–50.

Liu J, Willför S, Xu C. A review of bioactive plant polysaccharides: biological activities, functionalization, and biomedical applications. Bioact Carbohydr Diet Fibre. 2015;5(1):31–61.

Luo M, Shao B, Nie W, Wei X-W, Li Y-L, Wang B-L, He Z-Y, Liang X, Ye T-H, Wei Y-Q. Antitumor and adjuvant activity of λ-carrageenan by stimulating immune response in cancer immunotherapy. Sci Rep. 2015;5:11062.

Luyt C-E, Meddahi-Pellé A, Ho-Tin-Noe B, Colliec-Jouault S, Guezennec J, Louedec L, Prats H, Jacob M-P, Osborne-Pellegrin M, Letourneur D. Low-molecular-weight fucoidan promotes therapeutic revascularization in a rat model of critical hindlimb ischemia. J Pharmacol Exp Ther. 2003;305(1):24–30.

Mahdavinia GR, Rahmani Z, Karami S, Pourjavadi A. Magnetic/pH-sensitive κ-carrageenan/sodium alginate hydrogel nanocomposite beads: preparation, swelling behavior, and drug delivery. J Biomater Sci Polym Ed. 2014;25(17):1891–906. https://doi.org/10.1080/09205063.2014.956166.

Mahdavinia GR, Etemadi H, Soleymani F. Magnetic/pH-responsive beads based on caboxymethyl chitosan and κ-carrageenan and controlled drug release. Carbohydr Polym. 2015;128:112–21.

Mahdavinia GR, Mosallanezhad A, Soleymani M, Sabzi M. Magnetic-and pH-responsive κ-carrageenan/chitosan complexes for controlled release of methotrexate anticancer drug. Int J Biol Macromol. 2017;97:209–17.

Mak W. Extraction, characterization and antioxidant activity of Fucoidan from New Zealand Undaria pinnatifida (Harvey) Suringar. Auckland University of Technology; 2012.
Mandal P, Mateu CG, Chattopadhyay K, Pujol CA, Damonte EB, Ray B. Structural features and antiviral activity of sulphated fucans from the brown seaweed Cystoseira indica. Antivir Chem Chemother. 2007;18(3):153–62.
Marinho-Soriano E, Bourret E. Polysaccharides from the red seaweed Gracilaria dura (Gracilariales, Rhodophyta). Bioresour Technol. 2005;96(3):379–82.
Marudhupandi T, Kumar TA, Senthil SL, Devi KN. In vitro antioxidant properties of fucoidan fractions from Sargassum tenerrimum. Pak J Biol Sci. 2014;17(3):402.
Mazumder S, Ghosal PK, Pujol CA, Carlucci MJ, Damonte EB, Ray B. Isolation, chemical investigation and antiviral activity of polysaccharides from Gracilaria corticata (Gracilariaceae, Rhodophyta). Int J Biol Macromol. 2002;31(1–3):87–95.
McHugh DJ. A guide to the seaweed industry, FAO fisheries, technical paper 441. Rome; 2003.
McKim JM. Food additive carrageenan: part I: a critical review of carrageenan in vitro studies, potential pitfalls, and implications for human health and safety. Crit Rev Toxicol. 2014;44(3):211–43.
McKim JM, Willoughby JA Sr, Blakemore WR, Weiner ML. Clarifying the confusion between poligeenan, degraded carrageenan, and carrageenan: a review of the chemistry, nomenclature, and in vivo toxicology by the oral route. Crit Rev Food Sci Nutr. 2019;59(19):3054–73.
Mihaila SM, Popa EG, Reis RL, Marques AP, Gomes ME. Fabrication of endothelial cell-laden carrageenan microfibers for microvascularized bone tissue engineering applications. Biomacromolecules. 2014;15(8):2849–60.
Morán-Santibañez K, Cruz-Suárez LE, Ricque-Marie D, Robledo D, Freile-Pelegrín Y, Peña-Hernández MA, Rodríguez-Padilla C, Trejo-Avila LM. Synergistic effects of sulfated polysaccharides from Mexican seaweeds against measles virus. Hindawi Publishing Corporation, Biomed Res Int. 2016; 2016:11. Article ID 8502123. https://doi.org/10.1155/2016/8502123
Morelli A, Chiellini F. Ulvan as a new type of biomaterial from renewable resources: functionalization and hydrogel preparation. Macromol Chem Phys. 2010;211(7):821–32. https://doi.org/10.1002/macp.200900562.
Morelli A, Betti M, Puppi D, Bartoli C, Gazzarri M, Chiellini F. Enzymatically crosslinked ulvan hydrogels as injectable systems for cell delivery. Macromol Chem Phys. 2016;217(4):581–90. https://doi.org/10.1002/macp.201500353.
Morelli A, Puppi D, Chiellini F. Perspectives on biomedical applications of ulvan. In: Seaweed polysaccharides. Elsevier; 2017. p. 305–30.
Morris CJ. Carrageenan-induced paw edema in the rat and mouse. Methods Mol Biol. 2003;225:115–21.
Mukhamejanov E, Kon G, Erjanova S, Kirgizbaeva A, Muhamadieva E. Fucoidan – new principle prevention and treatment of diabetes. J Pharm Pharmacol. 2019;7:316–22.
Murakami K, Aoki H, Nakamura S, Nakamura S-i, Takikawa M, Hanzawa M, Kishimoto S, Hattori H, Tanaka Y, Kiyosawa T. Hydrogel blends of chitin/chitosan, fucoidan and alginate as healing-impaired wound dressings. Biomaterials. 2010;31(1):83–90.
Nagaoka M, Shibata H, Kimura-Takagi I, Hashimoto S, Kimura K, Makino T, Aiyama R, Ueyama S, Yokokura T. Structural study of fucoidan from Cladosiphon okamuranus Tokida. Glycoconj J. 1999;16(1):19–26.
Nagorskaya V, Reunov A, Lapshina L, Ermak I, Barabanova A. Inhibitory effect of κ/β-carrageenan from red alga Tichocarpus crinitus on the development of a potato virus X infection in leaves of Datura stramonium L. Biol Bull. 2010;37(6):653–8.
Nair, Anju & Raman, Maya & Doble, Mukesh. Cyclic β-(1→3) (1→6) glucan/carrageenan hydrogels for wound healing applications. RSC Adv. 2016;6:98545–98553. https://doi.org/10.1039/C6RA23386D.
Nakamura S, Ishihara M, Obara K, Masuoka K, Ishizuka T, Kanatani Y, Takase B, Matsui T, Hattori H, Sato T. Controlled release of fibroblast growth factor-2 from an injectable 6-O-desulfated heparin hydrogel and subsequent effect on in vivo vascularization. J Biomed Mater Res A. 2006;78(2):364–71.

Nayak KK, Gupta P. In vitro biocompatibility study of keratin/agar scaffold for tissue engineering. Int J Biol Macromol. 2015;81:1–10.
Nayar V, Weiland J, Nelson C, Hodge A. Elastic and viscoelastic characterization of agar. J Mech Behav Biomed Mater. 2012;7:60–8.
Necas J, Bartosikova L. Carrageenan: a review. Veterinarni Medicina. 2013;58(4).
Nguyen MA, Camci-Unal G. Unconventional tissue engineering materials in disguise. Trends Biotechnol. 2020;38(2):178–90.
Oliveira C, Ferreira AS, Novoa-Carballal R, Nunes C, Pashkuleva I, Neves NM, Coimbra MA, Reis RL, Martins A, Silva TH. The key role of sulfation and branching on fucoidan antitumor activity. Macromol Biosci. 2017;17(5):1600340.
Oliveira C, Neves NM, Reis RL, Martins A, Silva TH. Gemcitabine delivered by fucoidan/chitosan nanoparticles presents increased toxicity over human breast cancer cells. Nanomedicine. 2018;13(16):2037–50. https://doi.org/10.2217/nnm-2018-0004.
Oliveira C, Neves NM, Reis RL, Martins A, Silva TH. A review on fucoidan antitumor strategies: from a biological active agent to a structural component of fucoidan-based systems. Carbohydr Polym. 2020a; 239:116131.
Oliveira C, Soares AI, Neves NM, Reis RL, Marques AP, Silva TH, Martins A. Fucoidan immobilized at the surface of a fibrous mesh presents toxic effects over melanoma cells, but not over noncancer skin cells. Biomacromolecules. 2020b;21(7):2745–54.
Oun AA, Rhim J-W. Carrageenan-based hydrogels and films: effect of ZnO and CuO nanoparticles on the physical, mechanical, and antimicrobial properties. Food Hydrocoll. 2017;67:45–53.
Pangestuti R, Kurnianto D. Green seaweeds-derived polysaccharides ulvan: occurrence, medicinal value and potential applications. In: Seaweed polysaccharides. Elsevier; 2017. p. 205–21.
Panlasigui LN, Baello OQ, Dimatangal JM, Dumelod BD. Blood cholesterol and lipid-lowering effects of carrageenan on human volunteers. Asia Pac J Clin Nutr. 2003;12(2).
Patel S. Seaweed-derived sulfated polysaccharides: scopes and challenges in implication in health care. In: Bioactive seaweeds for food applications. Elsevier; 2018. p. 71–93.
Patil NP, Le V, Sligar AD, Mei L, Chavarria D, Yang EY, Baker AB. Algal polysaccharides as therapeutic agents for atherosclerosis. Front Cardiovasc Med. 2018;5:153.
Peasura N, Laohakunjit N, Kerdchoechuen O, Vongsawasdi P, Chao LK. Assessment of biochemical and immunomodulatory activity of sulphated polysaccharides from Ulva intestinalis. Int J Biol Macromol. 2016;91:269–77.
Pereira L. Seaweeds as source of bioactive substances and skin care therapy – cosmeceuticals, algotheraphy, and thalassotherapy. Cosmetics. 2018;5(4):68.
Pereira RC, Scaranari M, Castagnola P, Grandizio M, Azevedo HS, Reis R, Cancedda R, Gentili C. Novel injectable gel (system) as a vehicle for human articular chondrocytes in cartilage tissue regeneration. J Tissue Eng Regen Med. 2009;3(2):97–106.
Pinto DC. Seaweeds secondary metabolites: successes in and/or probable therapeutic applications. MDPI; 2020.
Pomin VH, Valente AP, Pereira MS, Mourão PA. Mild acid hydrolysis of sulfated fucans: a selective 2-desulfation reaction and an alternative approach for preparing tailored sulfated oligosaccharides. Glycobiology. 2005;15(12):1376–85.
Ponce NM, Pujol CA, Damonte EB, Flores ML, Stortz CA. Fucoidans from the brown seaweed Adenocystis utricularis: extraction methods, antiviral activity and structural studies. Carbohydr Res. 2003;338(2):153–65.
Popa E, Reis R, Gomes M. Chondrogenic phenotype of different cells encapsulated in κ-carrageenan hydrogels for cartilage regeneration strategies. Biotechnol Appl Biochem. 2012;59(2):132–41.
Popa EG, Caridade SG, Mano JF, Reis RL, Gomes ME. Chondrogenic potential of injectable κ-carrageenan hydrogel with encapsulated adipose stem cells for cartilage tissue-engineering applications. J Tissue Eng Regen Med. 2015a;9(5):550–63.

Popa EG, Reis RL, Gomes ME. Seaweed polysaccharide-based hydrogels used for the regeneration of articular cartilage. Crit Rev Biotechnol. 2015b;35(3):410–24.
Poupard N, Badarou P, Fasani F, Groult H, Bridiau N, Sannier F, Bordenave-Juchereau S, Kieda C, Piot J-M, Grillon C. Assessment of heparanase-mediated angiogenesis using microvascular endothelial cells: identification of λ-Carrageenan derivative as a potent anti angiogenic agent. Mar Drugs. 2017;15(5):134.
Priyan Shanura Fernando I, Kim K-N, Kim D, Jeon Y-J. Algal polysaccharides: potential bioactive substances for cosmeceutical applications. Crit Rev Biotechnol. 2019;39(1):99–113.
Qi H, Huang L, Liu X, Liu D, Zhang Q, Liu S. Antihyperlipidemic activity of high sulfate content derivative of polysaccharide extracted from Ulva pertusa (Chlorophyta). Carbohydr Polym. 2012a;87(2):1637–40.
Qi H, Liu X, Zhang J, Duan Y, Wang X, Zhang Q. Synthesis and antihyperlipidemic activity of acetylated derivative of ulvan from Ulva pertusa. Int J Biol Macromol. 2012b;50(1):270–2.
Qi X, Mao W, Gao Y, Chen Y, Chen Y, Zhao C, Li N, Wang C, Yan M, Lin C. Chemical characteristic of an anticoagulant-active sulfated polysaccharide from Enteromorpha clathrata. Carbohydr Polym. 2012c;90(4):1804–10.
Qi X, Mao W, Chen Y, Chen Y, Zhao C, Li N, Wang C. Chemical characteristics and anticoagulant activities of two sulfated polysaccharides from Enteromorpha linza (Chlorophyta). J Ocean Univ China. 2013;12(1):175–82. https://doi.org/10.1007/s11802-013-2057-4.
Rahimi F, Tabarsa M, Rezaei M. Ulvan from green algae Ulva intestinalis: optimization of ultrasound-assisted extraction and antioxidant activity. J Appl Phycol. 2016;28(5):2979–90.
Reis RL, Neves NM, Mano JF, Gomes ME, Marques AP, Azevedo HS. Natural-based polymers for biomedical applications. 1st ed. Elsevier; 2008.
Reys LL, Silva SS, Soares da Costa D, Oliveira NM, Mano JF, Reis RL, Silva TH. Fucoidan hydrogels photo-cross-linked with visible radiation as matrices for cell culture. ACS Biomater Sci Eng. 2016;2(7):1151–61.
Rhein-Knudsen N, Ale MT, Meyer AS. Seaweed hydrocolloid production: an update on enzyme assisted extraction and modification technologies. Mar Drugs. 2015;13(6):3340–59.
Rioux L-E, Turgeon SL, Beaulieu M. Characterization of polysaccharides extracted from brown seaweeds. Carbohydr Polym. 2007a;69(3):530–7.
Rioux LE, Turgeon SL, Beaulieu M. Rheological characterisation of polysaccharides extracted from brown seaweeds. J Sci Food Agric. 2007b;87(9):1630–8.
Rizk MZ, Aly HF, Matloub AA, Fouad GI. The anti-hypercholesterolemic effect of ulvan polysaccharide extracted from the green alga Ulva fasciata on aged hypercholesterolemic rats. Asian J Pharm Clin Res. 2016a;9(3):165–76.
Rizk MZ, El-Sherbiny M, Borai IH, Ezz MK, Aly HF, Matloub AA, Farrag A, Ghadha I, Fouad G. Sulphated polysaccharides (SPS) from the green alga ulva fasciata extract modulates liver and kidney function in high fat diet-induced hypercholesterolemic rats. Int J Pharm Pharm Sci. 2016b;8(6):43–55.
Rocha HA, Moraes FA, Trindade ES, Franco CR, Torquato RJ, Veiga SS, Valente AP, Mourão PA, Leite EL, Nader H. Structural and hemostatic activities of a sulfated galactofucan from the brown alga Spatoglossum schroederi AN IDEAL ANTITHROMBOTIC AGENT? J Biol Chem. 2005;280(50):41278–88.
Rodríguez MC, Matulewicz MC, Noseda M, Ducatti D, Leonardi PI. Agar from Gracilaria gracilis (Gracilariales, Rhodophyta) of the Patagonic coast of Argentina–content, structure and physical properties. Bioresour Technol. 2009;100(3):1435–41.
Rodriguez-Jasso RM, Mussatto SI, Pastrana L, Aguilar CN, Teixeira JA. Microwave-assisted extraction of sulfated polysaccharides (fucoidan) from brown seaweed. Carbohydr Polym. 2011;86(3):1137–44.
Samadi N, Sabzi M, Babaahmadi M. Self-healing and tough hydrogels with physically cross-linked triple networks based on Agar/PVA/Graphene. Int J Biol Macromol. 2018;107:2291–7.
Sanaka M, Yamamoto T, Anjiki H, Nagasawa K, Kuyama Y. Effects of agar and pectin on gastric emptying and post-prandial glycaemic profiles in healthy human volunteers. Clin Exp Pharmacol Physiol. 2007;34(11):1151–5.

Sangha JS, Kandasamy S, Khan W, Bahia NS, Singh RP, Critchley AT, Prithiviraj B. λ-carrageenan suppresses tomato chlorotic dwarf viroid (TCDVd) replication and symptom expression in tomatoes. Mar Drugs. 2015;13(5):2875–89.

Santo VE, Frias AM, Carida M, Cancedda R, Gomes ME, Mano JF, Reis RL. Carrageenan-based hydrogels for the controlled delivery of PDGF-BB in bone tissue engineering applications. Biomacromolecules. 2009;10(6):1392–401.

Schiavi A, Cuccaro R, Troia A. Strain-rate and temperature dependent material properties of Agar and Gellan Gum used in biomedical applications. J Mech Behav Biomed Mater. 2016;53:119–30.

Scieszka S, Klewicka E. Algae in food: a general review. Crit Rev Food Sci Nutr. 2019;59(21): 3538–47. https://doi.org/10.1080/10408398.2018.1496319.

Senthilkumar K, Manivasagan P, Venkatesan J, Kim S-K. Brown seaweed fucoidan: biological activity and apoptosis, growth signaling mechanism in cancer. Int J Biol Macromol. 2013;60: 366–74.

Sezer A, Akbuğa J. Fucosphere – new microsphere carriers for peptide and protein delivery: preparation and in vitro characterization. J Microencapsul. 2006;23(5):513–22.

Sezer AD, Hatipoglu F, Cevher E, Oğurtan Z, Bas AL, Akbuğa J. Chitosan film containing fucoidan as a wound dressing for dermal burn healing: preparation and in vitro/in vivo evaluation. AAPS PharmSciTech. 2007;8(2):E94–E101.

Shahruzzaman M, Sakib MN, Biswas S, Rahman MM, Haque P, Mallik A. Pharmaceutical applications of agar-agar. In: Nayak AK, Hasnain MS, Pal D, editors. Natural polymers for pharmaceutical applications, volume 2: marine and microbiologically derived polymers. Apple Academic Press; 2019.

Shan X, Liu X, Hao J, Cai C, Fan F, Dun Y, Zhao X, Liu X, Li C, Yu G. In vitro and in vivo hypoglycemic effects of brown algal fucoidans. Int J Biol Macromol. 2016;82:249–55.

Shanmugam M, Ramavat B, Mody K, Oza R, Tewari A. Distribution of heparinoid-active sulphated polysaccharides in some Indian marine green algae. Indian J Mar Sci. 2001;30(4):222–7.

Silva F, Dore C, Marques C, Nascimento M, Benevides N, Rocha H, Chavante S, Leite E. Anticoagulant activity, paw edema and pleurisy induced carrageenan: action of major types of commercial carrageenans. Carbohydr Polym. 2010;79(1):26–33.

Silva TH, Alves A, Popa EG, Reys LL, Gomes ME, Sousa RA, Silva SS, Mano JF, Reis RL. Marine algae sulfated polysaccharides for tissue engineering and drug delivery approaches. Biomatter. 2012;2(4):278–89.

Soares F, Fernandes C, Silva P, Pereira L, Gonçalves T. Antifungal activity of carrageenan extracts from the red alga Chondracanthus teedei var. lusitanicus. J Appl Phycol. 2016;28(5):2991–8.

Sokolova E, Barabanova A, Homenko V, Solov'eva T, Bogdanovich R, Yermak I. In vitro and ex vivo studies of antioxidant activity of carrageenans, sulfated polysaccharides from red algae. Bull Exp Bio Med. 2011;150(4):426.

Sokolova E, Bogdanovich L, Ivanova T, Byankina A, Kryzhanovskiy S, Yermak I. Effect of carrageenan food supplement on patients with cardiovascular disease results in normalization of lipid profile and moderate modulation of immunity system markers. PharmaNutrition. 2014a;2(2):33–7.

Sokolova EV, Byankina AO, Kalitnik AA, Kim YH, Bogdanovich LN, Solov'eva TF, Yermak IM. Influence of red algal sulfated polysaccharides on blood coagulation and platelets activation in vitro. J Biomed Mater Res A. 2014b;102(5):1431–8.

Song L, Chen X, Liu X, Zhang F, Hu L, Yue Y, Li K, Li P. Characterization and comparison of the structural features, immune-modulatory and anti-avian influenza virus activities conferred by three algal sulfated polysaccharides. Mar Drugs. 2016;14(1):4.

Souza RB, Frota AF, Silva J, Alves C, Neugebauer AZ, Pinteus S, Rodrigues JAG, Cordeiro EMS, de Almeida RR, Pedrosa R. In vitro activities of kappa-carrageenan isolated from red marine alga Hypnea musciformis: antimicrobial, anticancer and neuroprotective potential. Int J Biol Macromol. 2018;112:1248–56.

Tabarsa M, Han JH, Kim CY, You SG. Molecular characteristics and immunomodulatory activities of water-soluble sulfated polysaccharides from Ulva pertusa. J Med Food. 2012;15(2):135–44.

Talarico LB, Damonte EB. Interference in dengue virus adsorption and uncoating by carrageenans. Virology. 2007;363(2):473–85.
Teng Z, Qian L, Zhou Y. Hypolipidemic activity of the polysaccharides from Enteromorpha prolifera. Int J Biol Macromol. 2013;62:254–6.
Thakur A, Jaiswal MK, Peak CW, Carrow JK, Gentry J, Dolatshahi-Pirouz A, Gaharwar AK. Injectable shear-thinning nanoengineered hydrogels for stem cell delivery. Nanoscale. 2016;8(24):12362–72.
Thanh TTT, Quach TMT, Nguyen TN, Luong DV, Bui ML, Van Tran TT. Structure and cytotoxic activity of ulvan extracted from green seaweed Ulva lactuca. Int J Biol Macromol. 2016;93: 695–702.
Thuy TTT, Ly BM, Van TTT, Van Quang N, Tu HC, Zheng Y, Seguin-Devaux C, Mi B, Ai U. Anti-HIV activity of fucoidans from three brown seaweed species. Carbohydr Polym. 2015;115:122–8.
Tobacman JK. Review of harmful gastrointestinal effects of carrageenan in animal experiments. Environ Health Perspect. 2001;109(10):983–94.
Tonda-Turo C, Gnavi S, Ruini F, Gambarotta G, Gioffredi E, Chiono V, Perroteau I, Ciardelli G. Development and characterization of novel agar and gelatin injectable hydrogel as filler for peripheral nerve guidance channels. J Tissue Eng Regen Med. 2017;11(1):197–208.
Torres MD, Flórez-Fernández N, Domínguez H. Integral utilization of red seaweed for bioactive production. Mar Drugs. 2019;17(6):314.
Toskas G, Hund R-D, Laourine E, Cherif C, Smyrniotopoulos V, Roussis V. Nanofibers based on polysaccharides from the green seaweed Ulva rigida. Carbohydr Polym. 2011;84(3):1093–102.
Tziveleka L-A, Ioannou E, Roussis V. Ulvan, a bioactive marine sulphated polysaccharide as a key constituent of hybrid biomaterials: a review. Carbohydr Polym. 2019;218:355–70.
Usman A, Khalid S, Usman A, Hussain Z, Wang Y. Algal polysaccharides, novel application, and outlook. In: Algae based polymers, blends, and composites. Elsevier; 2017. p. 115–53.
Valado A, Pereira M, Caseiro A, Figueiredo JP, Loureiro H, Almeida C, Cotas J, Pereira L. Effect of carrageenans on vegetable jelly in humans with hypercholesterolemia. Mar Drugs. 2020;18(1):19.
van de Velde F. Structure and function of hybrid carrageenans. Food Hydrocoll. 2008;22(5):727–34.
van de Velde F, Knutsen S, Usov A, Rollema H, Cerezo A. 1H and 13C high resolution NMR spectroscopy of carrageenans: application in research and industry. Trends Food Sci Technol. 2002a;13(3):73–92.
van de Velde F, Lourenço ND, Pinheiro HM, Bakker M. Carrageenan: a food-grade and biocompatible support for immobilisation techniques. Adv Synth Catal. 2002b;344(8):815–35.
Venkatesan J, Lowe B, Anil S, Ealla KKR, Kim S-K. Marine biopolymers in bone tissue repair and regeneration. In: Sudha PN, editors. Industrial applications of marine biopolymers. 1st ed. 2017. CRC Press. ISBN: 9781315313535
Venkatesan J, Anil S, Rao S, Bhatnagar I, Kim S-K. Sulfated polysaccharides from macroalgae for bone tissue regeneration. Curr Pharm Des. 2019a;25(11):1200–9.
Venkatesan J, Anil S, Rao S, Kim S-K. Macroalgal Fucoidan for biomedical applications. In: Ravishankar GA, Ambati RR, editors. Handbook of algal technologies and phytochemicals. CRC Press; 2019b. p. 13–23.
Venugopal V. Sulfated and non-sulfated polysaccharides from seaweeds and their uses: an overview. EC Nutr. 2019;14:126–41.
Vergara-Rodarte MA, Hernández-Carmona G, Rodríguez-Montesinos YE, Arvizu-Higuera DL, Riosmena-Rodríguez R, Murillo-Álvarez JI. Seasonal variation of agar from Gracilaria vermiculophylla, effect of alkali treatment time, and stability of its Colagar. J Appl Phycol. 2010;22(6):753–9.
Verma V, Verma P, Kar S, Ray P, Ray AR. Fabrication of agar-gelatin hybrid scaffolds using a novel entrapment method for in vitro tissue engineering applications. Biotechnol Bioeng. 2007;96(2): 392–400.

Wang J, Zhang Q, Zhang Z, Song H, Li P. Potential antioxidant and anticoagulant capacity of low molecular weight fucoidan fractions extracted from Laminaria japonica. Int J Biol Macromol. 2010a;46(1):6–12.
Wang J, Zhang Q, Zhang Z, Zhang H, Niu X. Structural studies on a novel fucogalactan sulfate extracted from the brown seaweed Laminaria japonica. Int J Biol Macromol. 2010b;47(2):126–31.
Wang Y, Xing M, Cao Q, Ji A, Liang H, Song S. Biological activities of fucoidan and the factors mediating its therapeutic effects: a review of recent studies. Mar Drugs. 2019;17(3):183.
Weiner ML. Food additive carrageenan: part II: a critical review of carrageenan in vivo safety studies. Crit Rev Toxicol. 2014;44(3):244–69.
Weiner ML, McKim JM. Comment on "revisiting the carrageenan controversy: do we really understand the digestive fate and safety of carrageenan in our foods?" by S. David, CS Levi, L. Fahoum, Y. Ungar, EG Meyron-Holtz, A. Shpigelman and U. Lesmes, Food Funct., 2018, 9, 1344–1352. Food Funct. 2019;10(3):1760–2.
Williams PA, Phillips GO. GUMS | properties of individual GUMS. In: Caballero B, editor. Encyclopedia of food sciences and nutrition. Academic Press; 2003. p. 2992–3001. https://doi.org/10.1016/B0-12-227055-X/00573-3.
Witvrouw M, De Clercq E. Sulfated polysaccharides extracted from sea algae as potential antiviral drugs. Gen Pharmacol Vasc S. 1997;29(4):497–511.
Wozniak M, Bell T, Dénes Á, Falshaw R, Itzhaki R. Anti-HSV1 activity of brown algal polysaccharides and possible relevance to the treatment of Alzheimer's disease. Int J Biol Macromol. 2015;74:530–40.
Xu S-Y, Kan J, Hu Z, Liu Y, Du H, Pang G-C, Cheong K-L. Quantification of neoagaro-oligosaccharide production through enzymatic hydrolysis and its anti-oxidant activities. Molecules. 2018;23(6):1354.
Yao Z, Wu H, Zhang S, Du Y. Enzymatic preparation of κ-carrageenan oligosaccharides and their anti-angiogenic activity. Carbohydr Polym. 2014;101:359–67.
Yarnpakdee S, Benjakul S, Kingwascharapong P. Physico-chemical and gel properties of agar from Gracilaria tenuistipitata from the lake of Songkhla, Thailand. Food Hydrocoll. 2015;51:217–26.
Yegappan R, Selvaprithiviraj V, Amirthalingam S, Jayakumar R. Carrageenan based hydrogels for drug delivery, tissue engineering and wound healing. Carbohydr Polym. 2018;198:385–400.
Younes M, Aggett P, Aguilar F, Crebelli R, Filipič M, Frutos MJ, Galtier P, Gott D, Gundert-Remy U. Re-evaluation of carrageenan (E 407) and processed Eucheuma seaweed (E 407a) as food additives. EFSA J. 2018;16(4):5238.
Yuan H, Zhang W, Li X, Lü X, Li N, Gao X, Song J. Preparation and in vitro antioxidant activity of κ-carrageenan oligosaccharides and their oversulfated, acetylated, and phosphorylated derivatives. Carbohydr Res. 2005;340(4):685–92.
Yuan H, Song J, Li X, Li N, Dai J. Immunomodulation and antitumor activity of κ-carrageenan oligosaccharides. Cancer Lett. 2006a;243(2):228–34.
Yuan H, Song J, Zhang W, Li X, Li N, Gao X. Antioxidant activity and cytoprotective effect of κ-carrageenan oligosaccharides and their different derivatives. Bioorganic Med Chem Lett. 2006b;16(5):1329–34.
Yuan Y, Xu X, Jing C, Zou P, Zhang C, Li Y. Microwave assisted hydrothermal extraction of polysaccharides from Ulva prolifera: functional properties and bioactivities. Carbohydr Polym. 2018;181:902–10.
Zhang Z, Teruya K, Yoshida T, Eto H, Shirahata S. Fucoidan extract enhances the anti-cancer activity of chemotherapeutic agents in MDA-MB-231 and MCF-7 breast cancer cells. Mar Drugs. 2013;11(1):81–98.
Zhou G, Sun Y, Xin H, Zhang Y, Li Z, Xu Z. In vivo antitumor and immunomodulation activities of different molecular weight lambda-carrageenans from Chondrus ocellatus. Pharmacol Res. 2004;50(1):47–53.
Zhou G, Sheng W, Yao W, Wang C. Effect of low molecular λ-carrageenan from Chondrus ocellatus on antitumor H-22 activity of 5-Fu. Pharmacol Res. 2006;53(2):129–34.

Zhu Z, Zhang Q, Chen L, Ren S, Xu P, Tang Y, Luo D. Higher specificity of the activity of low molecular weight fucoidan for thrombin-induced platelet aggregation. Thromb Res. 2010;125 (5):419–26.

Zhu M, Ge L, Lyu Y, Zi Y, Li X, Li D, Mu C. Preparation, characterization and antibacterial activity of oxidized κ-carrageenan. Carbohydr Polym. 2017;174:1051–8.

Zia KM, Tabasum S, Nasif M, Sultan N, Aslam N, Noreen A, Zuber M. A review on synthesis, properties and applications of natural polymer based carrageenan blends and composites. Int J Biol Macromol. 2017;96:282–301.

Zou P, Lu X, Jing C, Yuan Y, Lu Y, Zhang C, Meng L, Zhao H, Li Y. Low-molecular-weightt polysaccharides from Pyropia yezoensis enhance tolerance of wheat seedlings (Triticum aestivum L.) to salt stress. Front Plant Sci. 2018;9:427.

Polysaccharides Produced by Microalgae

16

Antonio Trincone

Contents

Abstract

The class of polysaccharides is recognized to be of paramount importance in modern technology; the possible molecular chemodiversity of these biomaterials that can be found in microalgae can play an important role in industrial sectors such as food, pharmaceutical, cosmetic, nutraceutical, and aquaculture. Some

A. Trincone (✉)
Institute of Biomolecular Chemistry, Consiglio Nazionale delle Ricerche, Pozzuoli (Naples), Italy
e-mail: antonio.trincone@icb.cnr.it

J. M. Oliveira et al. (eds.), *Polysaccharides of Microbial Origin*,
https://doi.org/10.1007/978-3-030-42215-8_18

species of microalgae are already commercially available and studies about their bioculturing are advanced, thus offering high-value products for commercial applications. In this chapter the results of a searching the literature from 2013 to 2019 has been conducted on the structural identification and characterization of polysaccharides from these microorganisms. This report has been restricted to species commercially available, which are well known and prone to possible utilization and will not cover any possible discovery about rare or new species. In addition, prompted by the recent sudden outbreak of COVID-19, some literature reports on antiviral potential of microalgae polysaccharides among the commercial species are underlined. The overall results of this analysis seem not encouraging about flourishing of new detailed knowledge of fine structural features of these polysaccharides. The situation is reflecting the historical one observed for the launch of polysaccharides in biotechnology since 1950s. Intensive studies on possible exploitation using crude or partially purified and hardly standardized biomass preexisted to more demanding applications with standardized preparations with possible applications in more demanding fields. Only an interdisciplinary effort can lead soon to research for new chemical knowledge on fine structural details that are necessary to increase possible extensions of applications of pure material.

Keywords

Polysaccharides · Microalgae · Bioactivity · Antiviral · Carbohydrate

1 Introduction

Algae are photosynthetic organisms thriving in different aquatic environments (lakes, ponds, rivers, oceans, and wastewater). It has been estimated that many species are unknown, in fact 200,000–800,000 different species of these organisms exist, while only 50,000 (Algaebase 2020) have been already described in the literature and probably less than 100 cultivated, at least at lab scale (Richmond 2004). These organisms are resistant to different temperatures, salinities, pH values, and light intensities. They are classified as Rhodophyta (red algae), Phaeophyta (brown algae), and Chlorophyta (green algae) and generally categorized by size, as macroalgae or microalgae. Macroalgae or seaweeds are multicellular, large-size algae while microalgae are microscopic, single cells prokaryotic (cyanobacteria) or eukaryotic (green algae, Chlorophyta) in nature.

In the current decade, the study of microalgae is centered mainly on two challenges: (i) carbon dioxide sequestration and (ii) production of the third generation of biofuel. Microalgae play also a role in the following sectors of biotechnological applications: wastewater bioremediation and dietary supplements for animal and human nutrition. However, interest for microalgae is also focused about sourcing of high-value bioproducts and the attention in literature for this aspect is high with editorial initiatives promoted by important journals (MDPI publishing 2020).

Different species of marine microalgae produce and accumulate biotoxins and other interesting natural products such as antioxidants. Two hundred bioactives are estimated originating from cyanobacteria and thousands more from eukaryotic microalgae (Barkia et al. 2019).

Within the long list of bioproducts above indicated, the class of polysaccharides is recognized to be of paramount importance. These biopolymers are currently obtained from other biomasses as important technological agents or as bioactives; moreover possible molecular chemodiversity of these molecules extracted from microalgae can play an important role in the industry (Phélippé et al. 2019). Also, general advantages of microalgae as source biomass are recognized as (i) ease to grow and manipulation and (ii) climate-related composition (especially for polysaccharides) is not an issue to be faced with for microalgae culturing as happens for macroalgae.

The current situation about technology of microalgae culturing at both pilot-phase and industrial level, and related upgrades, has been recently discussed (Khan et al. 2018). Ultimately, the genetic modification is substantiating these developments for targeting products of interest and making the technology economically feasible. The world market of microalgae and cyanobacteria is considered as very emergent; industrial algaculture, estimated at 10,000–20,000 tons, will lead to a decrease in costs for biomass production and extraction. This will empower exploitation of metabolites such as polysaccharides. Industrial sectors such as food, pharmaceutical, cosmetic, nutraceutical, and aquaculture will take proficiency from these biotechnological advances. Indeed, some species of microalgae are already commercially available and emphasis on some important microalgal species, offering high-value by-products for commercial applications, is present in recent literature (Mobin and Alam 2017). Four microalgae are reported to be of major importance in biotechnological applications: *Spirulina* (*Arthrospira*), *Chlorella vulgaris*, *Dunaliella salina*, and *Haematococcus pluvialis*. Additional insights about the importance of polysaccharides from marine microalgae to act as prebiotics and dietary fibers are also reviewed (De Jesus Raposo et al. 2016; Barkia et al. 2019).

Interest in biotechnology for red microalgae is dated back a long time ago. In 1991 an interesting article (Geresh and Arad 1991) reported results of a research program aimed at the industrial development of sulfated polysaccharides from these organisms. Three species were studied: *Porphyridium* sp., *P. aerugineum*, and *Rhodella reticulata*. Although authors found differences in the composition of single sugar components and sulfate groups as well as glucuronic acid content, a disaccharide isolated was identical in all species and its structure and stereochemistry of galactose moiety were determined to be 3-O-(α-D-glucopyranosyluronic acid)-L-galactopyranose, utilizing enzymatic analysis applying three different enzymes. The authors postulated that the disaccharide unit could be part of a basic building block in the cell-wall polysaccharides of the red microalgae. Other old articles related to polysaccharides from *Chlorella* sp. were also present in the literature, reporting about the presence and abundance of glucuronic acid in these molecules (Yalcin et al. 1994). In more recent scientific reports, some modern reference work can be found addressing the bioactivity and applications of general (De Jesus Raposo et al. 2015) and sulfated

polysaccharides (De Jesus Raposo et al. 2013). These authors pointed out several interesting properties of microalgae polysaccharides for human nutrition and health. However, for the use in biomedicine and bio-applications, these molecules are yet to be explored being some issues still encountered due to the high molecular weight and to the difficulty assessing the precise composition and structural identity that have been found different according to the method used for the extraction and hydrolysis. Consequently, very few commercial products from isolated and purified polysaccharides are present on the market, despite the outlook for such products is highly considered.

In this chapter the results of a searching the literature from 2013 to 2019 has been conducted using the name of the species of microalgae and the word polysaccharide in titles and abstracts, opening up to retrieving as much as articles on the structural identification and characterization of these molecules from these microorganisms. This report has been restricted to species commercially available, which are well known and prone to possible utilization and will not cover any possible discovery about rare or new species. Besides, the recent sudden outbreak of COVID-19 among the general human population caused by the transmission of coronavirus SARS-CoV-2 prompted us to enlighten the literature reports on the antiviral potential of microalgal polysaccharides among the commercial species listed below. Both complete extracts and purified polysaccharide mixtures have been generally evaluated from microalgae as antiviral agents (Dewi et al. 2018). In particular sulfated exopolysaccharides have been hypothesized to interfere with virus penetration whose initial cellular receptors are anionic (sulfated) carbohydrates into the host cells; however other molecular mechanisms could be involved including inhibition of viral replication. Cyanobacterial lectins, also recently reviewed (Singh et al. 2017) for their potential antiviral action, are not considered here.

2 General Structures of Polysaccharides from Commercial Microalgae

In 2007, the structure of a mucous exopolysaccharide from the marine dinoflagellate *Gyrodinium impudicum* was reported (Yim et al. 2007) detailing about interesting bioactivities, particularly the bioflocculant action useful in a variety of industrial processes such as water treatment, food production, and other bioprocesses. High sulfate content in a mainly galactose-based molecule was established without other fine structural detail.

A β-1,3 glucan structure was already known for another polysaccharide from *Chlorella vulgaris* and in 1996 the structure of calcium spirulan was reported from green alga *Spirulina platensis*. Purification and chemical experiments concluded only about general structural elements: a sulfated polysaccharide chelating calcium ion and mainly composed of rhamnose and fructose (Hayashi et al. 1996).

A recent report (De Jesus Raposo et al. 2015) tabulated many examples of polysaccharides from marine unicellular algae, which are heteropolymers in nature, based on Xyl (xylose), Gal (galactose), and Glc (glucose) in different proportions

along with other neutral sugars such as Rha (rhamnose), Man (mannose), or Fuc (fucose), and also some methylated sugars. Moreover, for their glucuronic acid and sulfate group content these polysaccharides have a general acidic characteristic. Their anionic properties influence important physicochemical characteristics and bioactivity. A general glucuronomannan structure is present in *Phaeodactylum tricornutum*, and some hypothetical oligosaccharide fragments with 1,3-linked mannopyranose chain were produced by mild acid hydrolysis of cell wall extracts; however no strict evidence for the stereochemistry of interglycosidic linkages was detailed. This type of carbohydrate chain may be synthesized also in other diatom species. Among soluble polysaccharides, the β-(1,3) glucan, also called chrysolaminarin, was mentioned to be present in many species including *Nitzschia sigmoidea*, *Cymatopleura solea*, *Pinnularia* sp., and *Melosira varians* and other diatom species such as *Skeletonema*, *Phaeodactylum*, *Chaetoceros*, *Thalassiosira*, *Saccharina latissima, P. tricornutum*. According to species, variations could be also present: β-(1,6) branching, presence or absence of mannitol at reducing end, and molecular weight diversity.

Important chemical characteristics of exopolysaccharides of microalgae and cyanobacteria and the factors affecting their synthesis were also reviewed in 2016 (Rossi and De Philippis 2016). Complex heteroglycans are reported among exopolysaccharides synthesized by diatoms with partially similar sugar composition as indicated above: rhamnose, fucose, galactose, glucose, mannose, xylose, and/or uronic acids as well as some arabinose in lower proportion. Although indication of a large diversity of linkages were noted, different species were tabulated only reporting percentage composition of monosaccharides, sulfate content and with rare annotation of interglycosidic linkages: *Amphora* sp. *Asterionella socialis*, *Chaetoceros* sp., *Coscinodiscus nobilis*, *Cylindrotheca* sp., *Cyclotella nana*, *Melosira nummuloides*, *Navicula* sp., *Nitzschia* sp., *Pinnularia viridis*, *Thalassiosira* sp. (Gügi et al. 2015). Aqueous extracts of the thermophilic microalgae *Graeisiella* sp. contain polysaccharides with antiproliferative activity; their partial characterization has been recently reported (Trabelsi et al. 2016) based on eight neutral sugars: glucose, galactose, mannose, fucose, rhamnose, xylose, arabinose, and ribose.

Specific reference works more oriented on structural details of these polysaccharides seem to be absent in the literature and in the following paragraphs, the search for original articles more oriented on this aspect is reported for commercial microalgae.

3 Commercial Microalgae

The first country where the commercial application of microalgae was implemented was Japan since the 1960s with *Chlorella* being the first species (Borowitzka 1999). However four microalgae have been listed during the last 20 years, for which biotechnological applications were envisaged or exploited; this list includes a) *Spirulina* (*Arthrospira*), b) *Chlorella vulgaris*, c) *Dunaliella salina*, and d) *Haematococcus pluvialis* that have already been recognized as safe or authorized as additives for

humans and animals, as recently summarized (Mobin and Alam 2017) with a list of microalgal species according to the products and applications. *Scenedesmus almeriensis* and *Nannochloropsis* sp. resulted not yet marketed (Molino et al. 2018). For carbohydrate extracts, *Chlorella* spp. and *Chlorella vulgaris* are mentioned in addition to *Aphanizomenon flos-aquae* indicated for application in the food industry (Varfolomeeva and Wasserman 2011; Benedetti et al. 2004). Others are tabulated as current microalgae authorized in Europe and by the US FDA in food (nutraceutical) sectors: *Spongiococcum*, *Tetraselmis chuii*, *Odontella aurita*, *Ulkenia* sp., *Schizochytrium* sp. (Molino et al. 2018). Ten different microalgae have recently been investigated as potential functional food ingredients. Although carbohydrate content is not structurally detailed, this work contains interesting conclusions (Bernaerts et al. 2018). The investigated species were *Arthrospira platensis*, *Chlorella vulgaris*, *Diacronema lutheri*, *Tisochrysis lutea*, *Nannochloropsis sp.*, *Odontella aurita*, *Phaeodactylum tricornutum*, *Porphyridium cruentum*, *Schizochytrium* sp., and *Tetraselmis chuii*. The amounts of storage polysaccharides observed were low (2–8%) while extracellular polymeric substances were only present in *P. cruentum*, *O. aurita*, *C. vulgaris*, and *A. platensis*. Polysaccharides of the cell wall contributed approximately 10% of the biomass and were composed of heteropolysaccharides with five different monosaccharides in their composition. Uronic acids and sulfate groups provide anionic characteristics and as a result these polysaccharides show the potential to display interesting functionalities (bioactivity, technological interest, etc.).

With the reported benefits for human consumptions, numerous studies are indicating moderate to severe side effects that raised doubts for use of microalgae in humans (Altaner et al. 2019). In addition, extraction procedures especially for polysaccharides, can induce changes in the composition and chemical structures although new systems of extraction (microwave-, ultrasonic-, and enzyme-assisted extractions) and some purification steps are usually required.

Microalgae can represent a source of beneficial carbohydrates; however, their use in food has been limited and microalgal polysaccharides are gaining more importance in the cosmetic industry as hygroscopic agents and antioxidants for topical applications. An interesting list of major producers of microalgae is reported here (Barkia et al. 2019) with different species for which biochemical composition (i.e., percentages of carbohydrates, proteins, and lipids) are tabulated.

Issues for mass production of microalgae are also already discussed in depth (Pires 2015). The author, after relating about species selection and basic culturing techniques for production (nutrition, media, and growth kinetics), emphasized the technological aspects with the importance of the integration of processes contributing both to enhancing the economic viability of microalgae mass production and to mitigate environmental impact.

3.1 Species

No results were sorted for *Nitzschia*, *Chlamydomonas,* and *Prymnesium* sp. and generally few are the articles with fine structural details about polysaccharides,

indeed articles related to general studies and tentative applications, simple presence, and detection of bioactivities can be found as detailed below for the most important commercial species.

3.2 *Spirulina*

The importance of this cyanobacterium (*Arthrospira*) has been recently reviewed especially in food science (Mathur 2018). The commercial interest it is due to important dietary fibers and polysaccharides contents found in *Spirulina platensis*. Notwithstanding it is worth noting that anionic exopolysaccharides from cyanobacterium *A. platensis* possessed antiviral activities (Radonić et al. 2018) as known since the 1990 attributed to calcium spirulan against different viruses including influenza virus with no cytotoxicity to infected cells.

Polysaccharides have been studied as the agent for increasing the quality attributes of fish burgers (Barkallah et al. 2019) with recognition of many other bioactivities: inhibition of attachment of *Herpes simplex* virus to human keratinocytes (Mader et al. 2016), the cytotoxic effect on human acute leukemia, partly due to polysaccharides present in the extracts (Hernandez et al. 2017), the effect on ameliorating symptoms of constipation in mice, etc.

The structure of a sulfated polysaccharide isolated from blue-green alga *Spirulina platensis* (Lee et al. 2000) is already reported and the anticoagulant and antiviral activities have been related to the presence of sulfate groups in varying amounts and different positions along the macromolecular backbone. A disaccharide unit is suggested to be - > 3)-α-L-Rha-(1- > 2)-α-L-Aco-(1->, while acidic portions were also revealed to be composed of repeating structures of O-hexuronosyl-rhamnose. However, a more systematic study recently appeared investigating also the influence of culture conditions on polysaccharides abundance and composition. After performing a meta-analysis in literature about culture conditions, the authors identified incident light as the most influencing parameter and proceeded to experimental quantification and analysis of the EPS/glycogen ratio (Phélippé et al. 2019). They reported about the accumulation and composition of polysaccharides in *Arthrospira platensis*. Although their meta-bibliographic analysis indicated a great variability, due to different operating conditions or extraction/analysis methods, this study allowed to define clear tendencies resulting that among the operating conditions, the light intensity can strongly modulate the accumulation of these molecules. They also found that uronic acids content decreased at high photon flux density, and the neutral monosaccharides components are modified, suggesting potential modulation of the biological activities modifying culture conditions.

Extraction processes used for *Arthrospira* were studied for obtaining high-value chemicals such as phycocyanin, lipids/total fatty acids (TFA), and polysaccharides (Kurd and Samavati 2015; Chaiklahan et al. 2018). The study was contextualized in the economic analysis showing that producing phycocyanin alone was economically feasible, but producing coproducts (lipid/TFA and polysaccharides) was not. A

microwave-assisted process has been introduced recently to study the efficiency of water extraction following the principles of green chemistry (de Sousa et al. 2018).

During the cultivation process and study of growth (de Jesus et al. 2019), efforts for enhancement of production of extracellular polymeric substances (EPS) in *Spirulina* (*Arthrospira* sp.) were reported (Chentir et al. 2017) with the partial characterization of polysaccharidic moiety; however this study is limited to differential scanning calorimetry and infrared spectroscopy as general technique to assess the structures.

A further study indicated that a spirulan like polysaccharide PUF2 from old culture medium had an effective anticoagulant activity and the culture medium of *A. platensis* may constitute a cheap and abundant source of this biomolecule that has been shown to be more effective than dermatan sulfate of porcine origin. However, its obtention by ultrafiltration may represent an extraction procedure of interest (Majdoub et al. 2009). Authors indicated generally that the sugar components in the structure of the sulfated polysaccharide were rhamnose (49.7%) and uronic acids (32% of total sugar) as glucuronic and galacturonic acids.

3.3 *Chlorella*

Microalgae not only have the potential to replace the use of antibiotics, but also can improve the growth of cultured animals owing to their high nutritional value. The effects of polysaccharides of the unicellular green eukaryotic microalgae *Chlorella vulgaris* when used as a feed or feed additive have been studied. One of the interesting aspects of this study is that in the adopted conditions of growth of *C. vulgaris*, the accumulation of polysaccharides from was up to 32.7%. An enhancement of the disease resistance of *Trachemys scripta elegans* due to functional role of these molecules was also reported (Gui et al. 2019).

Two different fractions of EPS from *Chlorella vulgaris* were studied (Zhao et al. 2018) in the context of their roles in protecting cells against environmental stresses, in particular about cell damage caused by nano-ZnO and capability to adhere directly to the outside of cells. The amount of bound and soluble types of EPS were generally quantified but no structurally analyzed.

The influence of extraction methodology on the bioactivity of polysaccharides is known. Very recently, extracts of polysaccharides were reported from *Chlorella vulgaris* (Yu et al. 2019) in the context of a comprehensive study exploring the antioxidant properties that these molecules can offer. The study is focused on the preparation, and limited to yields, monosaccharide composition and molecular weight distribution relating to different extraction methods (freeze-thawing, microwave-assisted-, ultrasonic wave-, alkali-, hot water-, and cellulase-based). The alkali extraction method had the highest yield of the crude polysaccharides. Anti-oxidant properties were also studied in vitro and in vivo. Ultrasonic wave extraction produces extracts with activities superior with respect to other methods.

Significant production of extracellular polysaccharide (EPS) during the cultivation of *Chlorella vulgaris* has been reported (Barboríková et al. 2019). The report's

preliminary findings at the structural level indicated interesting complexity regarding interglycosidic linkages as determined by methylation analysis. In particular, the dominant galactose has been found involved in six different types of linkages in both pyranose and furanose forms while the second most abundant sugar, arabinose found in six types of different linkages in furanose form. Rhamnose with five different types of linkages was found along with glucose, and mannose residues in minor amounts. Significant effects in the in vivo animal testing were reported: bronchodilatory, anti-inflammatory, and antitussive. These exopolysaccharides thus appears to be interesting agents for the prevention of chronic inflammation in many respiratory diseases. Interestingly in a strain of *Chlorella* isolated from the coldest and driest arctic ecosystems, three polysaccharides fractions were also studied recently (Song et al. 2018). A crude yield of ca. 10% dry weight is reported after optimization of extraction using the response surface methodology. The most homogeneous fraction, based on infrared spectroscopy, high-performance liquid chromatography, and ^{1}H and ^{13}C nuclear magnetic resonance, was shown to possess sulfate group and α- and β-type linkages, and composed mainly of galactose (66%), with variable amounts of rhamnose (10%), arabinose (16.3%), and glucose (6.8%). NMR (nuclear magnetic resonance) details allowed the exclusion of furan rings as the resonance at δ107–109 ppm for their anomeric carbons resulted absent. Indeed, the presence of C-1 signals (δ93.46–103.87 ppm) indicated that the sugar residues were all in the pyranose form: H-1 signal at δ5.19 ppm and the C-1 signal at δ99.03 ppm were likely due to α-galactose residues. The H-1 signal at δ4.06 ppm and C-1 signal at δ101.43 ppm suggested that the polysaccharide contained β-arabinose residues and the H-1 signal at δ4.83 ppm and C-1 signal at δ103.87 ppm indicated a β-anomeric configuration for (1→4)-linked β-D-glucopyranosyl units.

In a recent study, crude biomasses of six different representative microalgae were analyzed for their monosaccharide composition: *Nannochloropsis salina*, *P. tricornutum*, *C. vulgaris* and *D. salina, P. purpureum* and *S. ovalternus* were investigated. Trifluoroacetic acid (TFA) hydrolysis of algal biomasses and application of a high throughput method for the identification of complex carbohydrates were used. The latter is based on the selective derivatization by 1-phenyl-3-methyl-5-pyrazolone, UHPLC separation, and MS analysis of the derivatives (Ortiz-Tena et al. 2016) allowing the identification of uncommon monosaccharides. Usually found neutral sugars, uronic acids, amino sugars, methylated and phosphorylated or sulfated monosaccharides were identified. This methodology is promising both in general utilization and in strain screening to maximize biomass development, allowing the detection of rare sugars.

Polysaccharide-based nanomaterials can be used as drug carriers or tissue-engineered scaffolds, in theranostic approach as the material constituting nanotools for cancer treatments, in wound dressings, as antimicrobial agents and biosensors, etc. The use of polysaccharides for the synthesis of metallic nanoparticles is a growing scientific topic and microalgal polysaccharides can be used as templates for the growth and stabilization of nanoparticles. Since these molecules are rich in reducing groups they can bind and reduce metal ions; chelation and bioaccumulation of heavy metals performed by microalgae are indeed mainly due to them. *Chlorella*

pyrenoidosa was included in a study of production and investigation of exo-polysaccharides in silver nanoparticles preparation (Gallón Navarro et al. 2019). With a novel approach, the authors described the green-based production of these nanoparticles and their activity as antibacterial agents. Additionally, for the topic of removal of ammonium and orthophosphate from wastewater by algae-based techniques, the role of cadmium ions was studied using *Chlorella vulgaris* polysaccharides showing the roles of these carbohydrate molecules in protecting the microalga in high cadmium environment (Chen et al. 2015).

The polysaccharide fraction of *Chlorella vulgaris* was evaluated against herpes simplex virus type 1 although no direct virucidal activity against the virus was noticed. However, water and ethanol extracts were able to inhibit the in vitro virus replication in a significant manner (Santoyo et al. 2010).

3.4 *Nannochloropsis*

Water-soluble polysaccharides from *Nannochloropsis oculata* were structurally analyzed, and tested for their immunostimulatory activity (Pandeirada et al. 2019); complex structural features were discovered in the hot water extracted polysaccharides after defatting the biomass. The polysaccharidic material was fractionated by ethanol precipitation, size exclusion chromatography, and anion exchange chromatography, and the usual chemical technology were used for interglycosidic linkage detection. Carbohydrate microarrays technology was also used in this screening analysis [Liu et al. 2009). Solid matrices coupled with only a few amounts of sample were probed with proteins with well-known carbohydrate binding specificities to elucidate the carbohydrate structures; glucans and mannans of known structures were used as polysaccharide controls. The complete analysis of polysaccharides from *Nannochloropsis oculata* revealed the presence of mixed-linked (β1→3, β1→4)-glucans and (α1→3)-, (α1→4)-mannans, an important asset in considering this organism as a source of dietary fibers. Additionally, a complex structure of anionic sulfated heteropolysaccharide composed of GlcA, Rha, Xyl, Gal, Fuc, and a sulfated heterorhamnan was also found. These authors indicated also potential immunostimulatory activity of these molecules.

3.5 *Tetraselmis*

In the context of an evaluation of nutritional potential and toxicological effects of *Tetraselmis* sp. CTP4 biomass, produced in photobioreactors at an industrial level, the presence of starch-like polysaccharides was declared (Pereira et al. 2019). Interglycosidic linkage analysis of polysaccharides showed the presence of 1,4-linked Glc (57 mol%) and 1,4-linked Gal (22 mol%). A content of 4.4 mol% of 1,4-Glc is substituted at C6 (1,4,6–Glc) confirming the presence of starch-like polysaccharides containing a percentage of branching residues. Similarly, as inferred by the

presence of 1,3,4–Gal (2 mol%), *Tetraselmis* polysaccharides seem to also be constituted by a galactan, with 1,4–Gal linkage in the backbone and substituted at C3.

As for *Tetraselmis suecica* a study of enzymatic hydrolysis for polysaccharide characterization appeared in the literature (Kermanshahi-Pour et al. 2014); the authors used this technique as an analytical procedure for the characterization of carbohydrate composition. This analysis shows that this microalga consists of approximately 42 wt % glucose as starch and 5 wt% 3-deoxy-D-manno-oct-2-ulosonic acid (Kdo) on a dry weight basis of microalgae when grown under specific conditions (i.e., varying nitrate concentrations). Kdo, a crucial part of the structure of lipid A-based lipopolysaccharides, is very expensive than glucose per mole and a complex challenge molecule for chemical synthesis. However, a robust and cost-effective extraction process must be developed for this compound from *Tetraselmis suecica*.

From the marine microalgae *Tetraselmis* sp. KCTC 12432BP and KCTC 12236BP, potential biodiesel producers, water soluble polysaccharides were identified as antioxidant materials for industrial applications (Dogra et al. 2017), their monosaccharide composition was identified by using HPAEC analysis and the authors partially concluded about galactosyl and glucosyl interglycosidic linkages without further details.

3.6 *Isochrysis*

Three polysaccharides were isolated from the marine microalgae *Isochrysis galbana* (Sun et al. 2014a) by ion-exchange chromatography. Previous work found that this species was rich in polysaccharides found at level up to 25% dry cell weight. Structural insights were obtained by infrared spectroscopy, electrospray ionization-mass spectrometry (ESI-MS), and ^{1}H nuclear magnetic resonance. The most effective fraction concerning antioxidant activity possessed a molecular weight of 15.934 kDa and was shown to be composed by variable amounts of glucose, galactose, and rhamnose. Anomeric hydrogen signals in ^{1}H spectroscopy allowed the identification as β-forms for glucose and galactose and α-form for rhamnose. From the same organism, the group of Usov isolated at a very interesting yield (44 mg of total neutral glucan from 500 mg of lyophilized cells of *Isochrysis galbana* of a pure highly branched (1–3,1–6)-β-D-glucan (4 mg) (Sadovskaya et al. 2014). The structure of this pure glucan was analyzed by methylation and Smith degradation, as well as ESI and MALDI TOF (matrix-assisted laser desorption/ionization time-of-flight) mass spectrometry and current proton and carbon 1-D and 2-D NMR spectroscopy. Interesting inhibition of the proliferation of U937 human leukemic monocyte lymphoma cells has been reported with a claimed potential of anti-tumor activity.

3.7 *Thalassiosira*

An investigation on extracellular carbohydrates of *Thalassiosira pseudonana* was reported in a collective study for three species including *Cylindrotheca closterium*

and *Skeletonema costatum*. It was mainly aimed at providing insights into the potential role of these species in mucilage formation of the Adriatic Sea. However, this study hardly reports any detail about structures of substances under analysis (Urbani et al. 2005). The authors have expressed production rates by gas-chromatographic analysis of the dissolved extracellular fraction, indicating glucose as the most abundant monomer in exponentially growing cultures. Of the three species analyzed that the highest abundance of polysaccharides was found in *T. pseudonana* followed by *C. closterium* and *S. costatum*. However, the composition varied according to the phase of growth with increase in other sugars (galactose, mannose, xylose, rhamnose, and fucose content) evaluated as storage glucans and heteropolysaccharides.

For *Thalassiosira pseudonana* the released polysaccharides and proteins, as major components of extracellular polymeric substances (EPS), were also measured to assess the potential effects of quantum dots in the aquatic environments by the interactions between quantum dots and the microalga (Zhang et al. 2013). The hypothesis of this work is that toxicity of quantum dots can be ameliorated by interaction with EPS from microalgae; the authors measured the extent of dissolution/aggregation of quantum dots relating it to environmental factors using *T. pseudonana* in different nutrient conditions. The production of carbohydrates increased with the addition of quantum dots but it did not show any relationship with particle's stability; thus EPS proteins might be more involved in the detoxification.

An interesting study related to the degradation of carbohydrates in *Thalassiosira weissflogii* during various stresses has been also published (Suroy et al. 2015); an increase in the carbon content was essentially reflected in the carbohydrate component of the TW cells. Three monosaccharides ribose, glucose, and galactose exhibited the highest degradation rate constants and the authors correlated this aspect to the differences in their initial macromolecular origin (e.g., storage vs structural carbohydrates).

3.8 *Dunaliella*

Five polysaccharide fractions were isolated from *Duanaliella salina* and studied by means of high-performance chromatography with precolumn derivatizations (Dai et al. 2010), a technique allowing that some minute quantities of polysaccharide samples can be analyzed with high resolution separation and simultaneous determination of many kinds of component sugars. Two fractions were acidic heteropolysaccharides mainly containing glucose and galactose, respectively, one possessing sulfate groups, two others were glucans, and the latter was a complex of polysaccharide linked with nucleic acids by covalent bonds.

A linear (1–4)-α-D-glucan has been identified in the microalgae *Dunaliella tertiolecta* in a work (Goo et al. 2013) framed to evaluate the potential for the production of fermentable monomeric sugars. The structural assignment was based on enzymatic hydrolysis by α-amylase (endo-type) allowing the authors to conclude

that EPS from *D. tertiolecta* are quite different from those obtained from other *Dunaliella* species, the structure was also confirmed by NMR analysis.

In a study (Santoyo et al. 2012) about antiviral compounds sourced from microalgae used as carotenoid producers both *Dunaliella salina* and *Haematococcus pluvialis* were investigated. Although *Dunaliella* extracts were found with less potency, in both cases the antiviral activity was found to be related to polysaccharides since carbohydrate-rich fractions isolated from extracts showed higher activity.

3.9 *Phaeodactylum*

The cell wall of the diatom *Phaeodactylum tricornutum* is poor in silica and mainly composed of organic molecules, notably, a sulfated glucuronomannan identified in a pioneering work (Husemann 1968). Structural insights were recently gained (Costaouëc et al. 2017; Yang et al. 2019) on this polysaccharide using modern technology showing that the backbone is a linear poly-α-(1→3) mannan decorated with sulfate ester groups and β-D-glucuronic residues. This compound could play a role in shaping the architecture of the frustule. These authors, discussing monosaccharide composition of other diatoms, suggested that sulfated glucomannan structure is conserved, although with variations in the amount and modality of distribution of sulfate ester groups and glucuronic residues branched on the α-mannan backbone, depending on the diatom species and their morphotypes.

A microalgal polysaccharide-enriched extract of this microalgae was used in the study of modulation of immune response in fish aquaculture (Carballo et al. 2019). The data reported by authors indicate that orally administrated insoluble yeast β-glucans in the sole aquaculture modulated the immune response and controlled the *Vibrio* abundance in the intestine of fishes; since microalgal polysaccharide caused a transient systemic anti-inflammatory response, they concluded that these polysaccharides are a promising source of prebiotics for the sole aquaculture industry.

3.10 *Porphyridium*

The red microalga *Porphyridium* sp. was shown to be a promising source of new sustainable thickening agents that shows a higher intrinsic viscosity compared to commercially used hydrocolloids. The EPS are composed of galactose, glucose, xylose, and glucuronic acid and their physicochemical properties were related with a different molecular structural organization of monosaccharides and sulfate groups constituting the polymer when compared to cell wall polysaccharides. Purification of carbohydrate fractions to obtain polysaccharide extracts with low protein and salt contents remain the main challenge for commercialization of extracellular polysaccharides of *Porphyridium* sp. (Bernaerts et al. 2018a). The process of purification has been indeed studied by membrane technology (Marcati et al. 2014) and also more recently (Balti et al. 2018) reporting about exopolysaccharide concentration

and purification. In the first study the method used allowed recovering both B-phycoerythrin and soluble polysaccharides from *Porphyridium* biomass. The authors of the more recent study showed that exopolysaccharides purified by a ceramic membrane filtration with high yield of recovery, were concentrated more than six folds.

For this microorganism too the antiviral activity has been recently reported (Radonić et al. 2018) as due to the presence of EPS from *P. purpureum*. The action is due to their anionic nature and the production of these compounds, which is possible in photo-bioreactors, is of great interest being the whole process easily scalable. The influence of sulfate on growth of microalgae was also monitored along with the composition and antibacterial and antiviral properties of the exo-polysaccharide from *Porphyridium cruentum* thus showing that enrichment of the culture medium with sulfate improved sulfate content of exopolysaccharides and they presented a relevant activity against *Vesicular stomatitis* virus (De Jesus Raposo et al. 2014).

3.11 *Botryococcus*

In an investigation recently published, the interest toward the microalga *Botryococcus braunii* has increased despite slow growth rates of the organism; indeed some strains were grown relatively efficiently in continuous cultures in controlled photobioreactors and studies about milking process (extraction and recovery of polysaccharides (García-Cubero et al. 2018) were conducted obtaining good EPS productivity. A previous study (Jin et al. 2016) was focused on the ways to improve the return of hydrocarbons production with co-valorization of the biomass residues, according to a biorefinery approach to producing compounds of industrial interest such as polysaccharides. In another report (Atobe et al. 2015), it was shown that the water-soluble polymers that can be released from the extracellular matrix of *Botryococcus braunii* by thermal pretreatment, before hydrocarbon extraction, have to be reduced to less than 0.5% for optimal hydrocarbon recovery. The water-soluble polymers are high molecular weight polysaccharides, mainly comprised of galactose, arabinose, and uronic acid. The authors suggest that these polymers are desirable as industrial emulsifiers and thickeners. A more in-depth study showed also the presence of a protein to which polysaccharides are linked studied using mass spectroscopy and bioinformatics approach (Tatli et al. 2018).

3.12 *Chaetoceros*

Some reports on polysaccharide material from this diatom of importance in biofuels since long time (McGinnis et al. 1997) can be traced in more modern literature. In a study on issues induced by algal blooms in fresh and saline waters, aimed to minimizing adverse effects in membrane-based water treatment systems, *Chaetoceros affinis* was included among species studied. The algal organic matter

was shown mainly composed by biopolymers (e.g., polysaccharides and proteins) containing fucose and sulfated functional groups (Villacorte et al. 2015) by lectin-binding affinity.

Similar studies on organic matter generated by marine algal species were also present (Chowdhury et al. 2016; Dhakal et al. 2018). A modern study using lectin-binding demonstrated that conspicuous setae of the diatom *Chaetoceros* were preferred habitats for *Polaribacter* and these microorganisms are able to bind and degrade sulfated fucose-containing polysaccharides coating the *Chaetoceros* setae (Bennke et al. 2013), an interesting study for possible insight on structural elucidation of microalgae polysaccharide material.

3.13 *Chlamydomonas*

The genus of green alga *Chlamydomonas* is important in the study of biohydrogen production. A study of polysaccharides present in *Chlamydomonas reinhardtii* is dated back to 1975 showing the presence of a polysaccharide of molecular weight of 41,500 containing molar proportion of monosaccharides: arabinose, mannose, and galactose. The latter in the polymer was shown to consist of a mixture of the D and L enantiomers in a ratio of 84: 16. No more than monosaccharide composition was reported though (Shii and Barber 1975).

3.14 *Pavlova*

The scientific interest for the species *Pavlova viridis* and other Chrysophyta is to be found in fish feed preparation. The two articles found on *Pavlova viridis* focused on the study and potential of antioxidant activity of polysaccharides extracted in water and of their readily absorbable degradation fragments (Sun et al. 2014b) and on their potential about immunomodulation and antitumor activity (Sun et al. 2016). As for molecular structural details reported in these articles, the authors did not go in-depth more than sugar composition and sulfate content although they concluded interestingly that all the polysaccharide fragments evoked a significant dose-dependent scavenging ability and they could be considered as a potential antioxidant and that the polysaccharides of *Pavlova viridis* had potential antitumor activities by improving immune response; the bioactivities, however, depend on their molecular weights.

3.15 Scenedesmus

Efficient biomass harvesting is one of the bottlenecks to develop a cost-effective process in microalgal cultivation technology. Flocculation is one of the processes used in which freely suspended cells are aggregated together to form large particles, and cells can then be easily harvested by sedimentation. The study of flocculant agents is then important as a promising technology for low-cost biomass recovery.

Scenedesmus obliquus, a green microalga that has been widely used in fish feed, is a self-flocculating microalga recently studied (Guo et al. 2013). The bioflocculant polymers were studied for composition and consist of glucose, mannose, galactose, rhamnose, and fructose with the molar ratio of 8:5:3:2:1 but no more precise details on structures were furnished although the author stated that this is the first report on the characterization of flocculation agent from self-flocculating microalga.

3.16 *Synechococcus*

Two interesting articles sorted from literature search for this species for production of two polymers, hyaluronic acid and heparosan using a photo-biorefinery approach. In the first (Zhang et al. 2019), the authors explored *Synechococcus* sp. PCC 7002, considered as safe hosts for biotechnological applications, for photosynthetic hyaluronic acid production system. For bacterial engineered solutions (*Bacillus subtilis* and *Escherichia coli* strains overexpressing different HA synthases) in fact, substantial amounts of carbon feedstocks, such as glucose or sucrose are necessary for fermentative processes on which these systems are based. The work emphasizes cyanobacteria as promising hosts to produce biomedically relevant sugar polymers solely by photosynthesis. In the second article, the authors used *Synechococcus elongatus* PCC 7942 for production of heparosan (Sarnaik et al. 2019); after cultivating the cells were under different environmental conditions they found yielding maximum heparosan production to 2.8 μg/L, which is useful for production of high-value chemicals from low-cost inputs.

3.17 Conclusion

Even before their structures were known, polysaccharides, including starch and cellulose from a historical perspective, have always been exploited before complete structural detail assessment. Only after the 1970s a serious resurgence of the interest for this class of biomolecules, considered as renewable resources, occurred and this new interest reflected and paralleled the advent of the new analytical techniques since the 1950s. Gas chromatography, mass spectrometry, and nuclear magnetic resonance spectroscopy together with permethylation procedure and selective enzymatic digestion induced major progress in the structural definition of polysaccharides. Currently, concern about the quality of polysaccharides preparations is still present; necessary standardization for commercial exploitation (Han 2018) and the poor repeatability of the methods used in sample preparation and lack of information about chemical characterization are still hampering research and product development. Even in a recent overview (Gray et al. 2019) of advanced solutions for the carbohydrate sequencing challenge, the authors stated that "our understanding of endogenous carbohydrate structure and function still lags behind that of other biomolecules, despite carbohydrates representing the most abundant biological class." Whole extract utilization, at least for external or cosmetic applications can be partly

possible but in the field of drug development a more demanding situation is real; solving unavailability of supply and acquiring detailed structural and composition information are necessary.

The main conclusion of this report is based on the two aspects depicted above. Many review reports were generally found on natural products from microalgae with few ones specialized for polysaccharide material and widely indicated in the general parts above. Microalgae culturing seems to be a very active field in development due to successes of the genetic modification and this could have a positive impact avoiding issues for seasonality of macroalgae production. However, extraction procedures for polysaccharides, which can induce changes in the composition and chemical structures, have to be assessed and standardized and articles on these aspects are also present in literature about new systems of extraction. It is worth mentioning the microwave-assisted and the ultrasonic based processes to increase the efficiency of water extraction following the principles of green chemistry.

Even if this report has been based on a literature search since 2013, articles reporting fine structural details of this biomaterial are hardly present. In fact very rare exceptions are the articles with plenty NMR details. If present they are obtained on whole material (*Chlorella* and *Isochrysis* polysaccharides) or after enzymatic degradation (*Dunaliella*). Structural analysis of polysaccharides of *Nannochloropsis oculata* by a very modern approach using proteins with well-known carbohydrate-binding specificities to elucidate the carbohydrate structures is worth mentioning. A linear poly-α-(1→3) mannan decorated with sulfate ester groups has been reported for the diatom *Phaeodactylum tricornutum*. Biomedically relevant and structurally known sugar polymers can be obtained by photosynthesis in *Synechococcus*.

4 Conclusions

From this search in literature and previously published review reports, it seems that the situation for polysaccharide from microalgae is reflecting the historical one, with focusing first on collection of data on possible exploitation of potential in applications (rheological features, bioactivities, etc.) where the use of crude or partially purified and hardly standardized biomass is relatively possible in different fields. Hope is that, in future, with interdisciplinary efforts, new knowledge on structural details will increase with possible extension of applications in more demanding fields.

References

Algaebase. Ryan Institute, National University of Ireland, University Road, Galway, Ireland. 2020. https://www.algaebase.org/content/. Accessed May 2020.

Altaner S, Jaeger S, Fotler R, Zemskov I, Wittmann V, Schreiber F, Dietrich D. Machine learning prediction of cyanobacterial toxin (microcystin) toxicodynamics in humans. ALTEX. 2019;37(1):24–36. https://doi.org/10.14573/altex.1904031.

Atobe S, Saga K, Hasegawa F, Furuhashi K, Tashiro Y, Suzuki T, Okada S, Imou K. Effect of amphiphilic polysaccharides released from *Botryococcus braunii* Showa on hydrocarbon recovery. Algal Res. 2015;10:172–6.

Balti R, Balc'h RL, Brodu N, Gilbert M, Gouic BL, Gall SL, Sinquin C, Massé A. Concentration and purification of *Porphyridium cruentum* exopolysaccharides by membrane filtration at various cross-flow velocities. Process Biochem. 2018;74:175–84.

Barboríková J, Šutovská M, Kazimierová I, Jošková M, Fraňová S, Kopecký J, Capek P. Extracellular polysaccharide produced by *Chlorella vulgaris* – chemical characterization and anti-asthmatic profile. Int J Biol Macromol. 2019;135:1–11. https://doi.org/10.1016/j.ijbiomac.2019.05.104.

Barkallah M, Atitallah B, Hentati F, Dammak M, Hadrich B, Fendri I, Ayadi MA, Michaud P, Abdelkafi S. Effect of *Spirulina platensis* biomass with high polysaccharides content on quality attributes of common carp (*Cyprinus carpio*) and common barbel (*Barbus barbus*) fish burgers. Appl Sci. 2019;9:2197.

Barkia I, Saari N, Manning SR. Microalgae for high-value products towards human health and nutrition. Mar Drugs. 2019;17:304.

Benedetti S, Benvenuti F, Pagliarani S, Francogli S, Sconglio S, Canestrari F. Antioxidant properties of a novel phycocyanin extract from the blue-green alga *Aphanizomenon flos-aquae*. Life Sci. 2004;75:2353–62.

Bennke CM, Neu TR, Fuchs BM, Amann R. Mapping glycoconjugate-mediated interactions of marine Bacteroidetes with diatoms. Syst Appl Microbiol. 2013;36(6):417–25.

Bernaerts TMM, Gheysen L, Kyomugasho C, Kermani ZJ, Vandionant S, Foubert I, Hendrickx ME, Van Loey AM. Comparison of microalgal biomasses as functional food ingredients: focus on the composition of cell wall related polysaccharides. Algal Res. 2018;32:150–61.

Bernaerts TMM, Kyomugasho C, Looveren NV, Gheysen L, Foubert I, Hendrickx ME, Loey AMV. Molecular and rheological characterization of different cell wall fractions of *Porphyridium cruentum*. Carbohydr Polym. 2018a;195:542–50.

Borowitzka MA. Commercial production of microalgae: ponds, tanks, and fermenters. Prog Ind Microbiol. 1999;35:313–21.

Carballo C, Pinto PIS, Mateus AP, Berbel C, Guerreiro CC, Martinez-Blanch JF, Codoñer FM, Mantecon L, Power DM, Manchado M. Yeast β-glucans and microalgal extracts modulate the immune response and gut microbiome in Senegalese sole (*Solea senegalensis*). Fish Shellfish Immunol. 2019;92:31–9.

Chaiklahan R, Chirasuwan N, Loha V, Tia S, Bunnag B. Stepwise extraction of high-value chemicals from *Arthrospira* (*Spirulina*) and an economic feasibility study. Biotechnol Rep. 2018;20:e00280. https://doi.org/10.1016/j.btre.2018.e00280.

Chen B, Li F, Liu N, Ge F, Xiao H, Yang Y. Role of extracellular polymeric substances from *Chlorella vulgaris* in the removal of ammonium and orthophosphate under the stress of cadmium. Bioresour Technol. 2015;190:299–306.

Chentir I, Hamdi M, Doumandji A, Abelkader H, Ouada HB, Nasri M, Mourad J. Enhancement of extracellular polymeric substances (EPS) production in *Spirulina* (*Arthrospira* sp.) by two-step cultivation process and partial characterization of their polysaccharidic moiety. Int J Biol Macromol. 2017;105:1412–20.

Chowdhury C, Majumder N, Jana TK. Seasonal distribution and correlates of transparent exopolymer particles (TEP) in the waters surrounding mangroves in the Sundarbans. J Sea Res. 2016;112:65–74.

Costaouëc TL, Unamunzaga C, Mantecon L, Helbert W. New structural insights into the cell-wall polysaccharide of the diatom *Phaeodactylum tricornutum*. Algal Res. 2017;26:172–9.

Dai J, Wu Y, Shang-wei C, Song Z, Hong-ping Y, Min W, Jian T. Sugar compositional determination of polysaccharides from *Dunaliella salina* by modified RP-HPLC method of precolumn derivatization with 1-phenyl-3-methyl-5-pyrazolone. Carbohydr Polym. 2010;82(3):629–35.

De Jesus Raposo FM, De Morais MR, de Morais MA. Bioactivity and applications of sulphated polysaccharides from marine microalgae. Mar Drugs. 2013;11:233–52.

De Jesus Raposo MF, de Morais AM, de Morais RM. Influence of sulphate on the composition and antibacterial and antiviral properties of the exopolysaccharide from *Porphyridium cruentum*. Life Sci. 2014;101(1–2):56–63.

De Jesus Raposo MF, Alcina MF, De Morais MM, De Morais SC. Bioactivity and applications of polysaccharides from marine microalgae. In: Ramawat KG, Merillon GM, editors. Polysaccharides. Springer; 2015. p. 1683–727.

De Jesus Raposo FM, De Morais MA, De Morais MR. Emergent sources of prebiotics: seaweeds and microalgae. Mar Drugs. 2016;14:27.

de Jesus CS, de Jesus Assis D, Rodriguez MB, Menezes Filho JA, Vieira Costa JA, de Souza Ferreira E, Druzian JB. Pilot-scale isolation and characterization of extracellular polymeric substances (EPS) from cell-free medium of *spirulina* sp. LEB-18 cultures under outdoor conditions. Int J Biol Macromol. 2019;124:1106–14.

de Sousa SA, de Magalhães WT, Teixeira M, Marques LM, Valderez Ponte RM, Karine Pessoa BA. Microwave-assisted extraction of polysaccharides from *Arthrospira* (*Spirulina*) platensis using the concept of green chemistry. Algal Res. 2018;35:178–84. https://doi.org/10.1016/j.algal.2018.08.015.

Dewi IC, Falaise C, Hellio C, Bourgougnon N, Mouget JL. Anticancer, antiviral, antibacterial, and antifungal properties in microalgae. In: Levine IA, Fleurence J, editors. Microalgae in health and disease prevention. Academic Press; 2018. p. 235–61. https://doi.org/10.1016/B978-0-12-811405-6.00012-8.

Dhakal N, Salinas-Rodriguez SG, Ouda A, Schippers JC, Kennedy MD. Fouling of ultrafiltration membranes by organic matter generated by marine algal species. J Membr Sci. 2018;555:418–28.

Dogra B, Amna S, Park YI, Park JK. Biochemical properties of water soluble polysaccharides from photosynthetic marine microalgae Tetraselmis species. Macromol Res. 2017;25:172–9. https://doi.org/10.1007/s13233-017-5016-x.

Gallón Navarro SM, Alpaslan E, Wang M, Larese-Casanova P, Londoño ME, Atehortúa L, Pavón JJ, Webster TJ. Characterization and study of the antibacterial mechanisms of silver nanoparticles prepared with microalgal exopolysaccharides. Mater Sci Eng C. 2019;99:685–95.

García-Cubero R, Wang W, Martín J, Bermejo E, Sijtsma L, Togtema A, Barbosa MJ, Kleinegris DMM. Milking exopolysaccharides from *Botryococcus braunii* CCALA778 by membrane filtration. Algal Res. 2018;34:175–81.

Geresh S, Arad S. The extracellular polysaccharides of the red microalgae: chemistry and rheology. Bioresour Technol. 1991;38:195–201.

Goo BG, Baek G, Choi DJ, Park Y, Synytsya A, Bleha R, Seong DH, Lee CG, Park JK. Characterization of a renewable extracellular polysaccharide from defatted microalgae *Dunaliella tertiolecta*. Bioresour Technol. 2013;129:343–50. https://doi.org/10.1016/j.biortech.2012.11.077.

Gray CJ, Migas LG, Barran PE, Pagel K, Peter H, Seeberger PH, Eyers CE, Boons GJ, Pohl NLB, Compagnon I, Widmalm G, Flitsch SL. Advancing solutions to the carbohydrate sequencing challenge. J Am Chem Soc. 2019;141(37):14463–79. https://doi.org/10.1021/jacs.9b06406.

Gügi B, Le Costaouec T, Burel C, Lerouge P, Helbert W, Bardor M. Diatom-specific oligosaccharide and polysaccharide structures help to unravel biosynthetic capabilities in diatoms. Mar Drugs. 2015;13:5993–6018.

Gui J, Tong W, Huang S, Liang X, Fang Z, Wang W, Zhang Y. Effects of Chlorella vulgaris polysaccharides accumulation on growth characteristics of *Trachemys scripta elegans*. Int J Biol Macromol. 2019;141:1304–13. https://doi.org/10.1016/j.ijbiomac.2019.08.248.

Guo SL, Zhao XQ, Wan C, Huang ZY, Yang YL, Alam MA, Ho SH, Bai FW, Chang JS. Characterization of flocculating agent from the self-flocculating microalga *Scenedesmus obliquus* AS-6-1 for efficient biomass harvest. Bioresour Technol. 2013;145:285–9.

Han QB. Critical problems stalling progress in natural bioactive polysaccharide research and development. J Agric Food Chem. 2018;66(18):4581–3.

Hayashi T, Hayashi K, Maeda M, Kojima I. Calcium spirulan, an inhibitor of enveloped virus replication, from a blue-green alga *Spirulina platensis*. J Nat Prod. 1996;59:83–7.

Hernandez FYF, Khandual S, Guadalupe I, López R. Cytotoxic effect of *Spirulina platensis* extracts on human acute leukemia Kasumi-1 and chronic myelogenous leukemia K-562 cell lines. Asian Pac J Trop Biomed. 2017;7(1):14–9.

Husemann E. Chemistry and enzymology of marine algal polysaccharides. Von E. Percival und R. H. McDowell. Academic Press, London-New York 1967. 1. Aufl., XII, 219 S., mehrere Abb. u. Tab., geb. 60s. Angew Chem. 1968;80:856. https://doi.org/10.1002/ange.19680802022.

Jin J, Dupré C, Yoneda K, Watanabe MM, Legrand J, Grizeau D. Characteristics of extracellular hydrocarbon-rich microalga *Botryococcus braunii* for biofuels production: recent advances and opportunities. Process Biochem. 2016;51(11):1866–75.

Kermanshahi-Pour A, Sommer TJ, Anastas PT, Zimmerman JB. Enzymatic and acid hydrolysis of Tetraselmis suecica for polysaccharide characterization. Bioresour Technol. 2014;173:415–21. https://doi.org/10.1016/j.biortech.2014.09.048.

Khan MI, Shin JH, Kim JD. The promising future of microalgae: current status, challenges, and optimization of a sustainable and renewable industry for biofuels, feed, and other products. Microb Cell Factories. 2018;17:36.

Kurd F, Samavati V. Water soluble polysaccharides from *Spirulina platensis*: extraction and in vitro anti-cancer activity. Int J Biol Macromol. 2015;74:498–506.

Lee JB, Hayashi T, Hayashi K, Sankawa U. Structural analysis of calcium spirulan (Ca-SP)-derived oligosaccharides using electrospray ionization mass spectrometry. J Nat Prod. 2000;63:136–8.

Liu Y, Palma AS, Feizi T. Carbohydrate microarrays: key developments in glycobiology. Biol Chem. 2009;390(7):647–56.

Mader J, Gallo A, Schommartz T, Handke W, Nagel CH, Günther P, Brune W, Reich K. Calcium spirulan derived from *Spirulina platensis* inhibits herpes simplex virus 1 attachment to human keratinocytes and protects against herpes labialis. J Allergy Clin Immunol. 2016;137(1):197–203.e3.

Majdoub H, Mansour MB, Chaubet F, Roudesli MS, Maaroufi RM. Anticoagulant activity of a sulfated polysaccharide from the green alga Arthrospira platensis. Biochim Biophys Acta Gen Subj. 2009;10:1377–81. https://doi.org/10.1016/j.bbagen.2009.07.013.

Marcati A, Ursu AV, Laroche C, Soanen N, Marchal L, Jubeau S, Djelveh G, Michaud P. Extraction and fractionation of polysaccharides and B-phycoerythrin from the microalga *Porphyridium cruentum* by membrane technology. Algal Res. 2014;5:258–63.

Mathur M. Bioactive molecules of *spirulina*: a food supplement. In: Mérillon JM, Ramawat K, editors. Bioactive molecules in food. Reference series in Phytochemistry. Cham: Springer; 2018. https://doi.org/10.1007/978-3-319-78030-6_97.

McGinnis KM, Dempster TA, Sommerfeld MR. Characterization of the growth and lipid content of the diatom Chaetoceros muelleri. J Appl Phycol. 1997;9:19–24.

MDPI Publishing. 2020. https://www.mdpi.com/journal/marinedrugs/special_issues/bioactive_compounds_from_microalgae. Accessed May 2020.

Mobin S, Alam F. Some promising microalgal species for commercial applications: a review. Energy Procedia. 2017;110:510–7.

Molino A, Iovine A, Casella P, Mahariya S, Chianese S, Cerbone A, Rimauro J, Musmarra D. Microalgae characterization for consolidated and new application in human food, animal feed and nutraceuticals. Int J Environ Res Public Health. 2018;15:2436.

Ortiz-Tena JG, Rühmann BR, Schieder D, Sieber V. Revealing the diversity of algal monosaccharides: fast carbohydrate fingerprinting of microalgae using crude biomass and showcasing sugar distribution in *Chlorella vulgaris* by biomass fractionation. Algal Res. 2016;17:227–35.

Pandeirada CO, Maricato È, Ferreira SS, Correia VG, Pinheiro BA, Evtuguin V, Palma AS, Correia A, Vilanova M, Coimbra MA, Nunes C. Structural analysis and potential immunostimulatory activity of *Nannochloropsis oculata* polysaccharides. Carbohydr Polym. 2019;222:114962.

Pereira H, Silva J, Santos T, Gangadhar KN, Raposo A, Nunes C, Coimbra MA, Gouveia L, Barreira L, Varela J. Nutritional potential and toxicological evaluation of *Tetraselmis* sp. CTP4 microalgal biomass produced in industrial photobioreactors. Molecules. 2019;24(17):3192.

Phélippé M, Gonçalves O, Thouand G, Cogne G, Laroche C. Characterization of the polysaccharides chemical diversity of the cyanobacteria *Arthrospira platensis*. Algal Res. 2019;38:101426.
Pires JCM. Mass production of microalgae. In: Kim S-K J, editor. Handbook of marine microalgae. New York: Academic Press; 2015. p. 55–68.
Radonić A, Thulke S, Achenbach J, Kurth A, Vreemann A, König T, Walter C, Possinger K, Nitsche A. Anionic polysaccharides from phototrophic microorganisms exhibit antiviral activities to vaccinia virus. J Antivir Antiretrovir. 2018;2:051–5. https://doi.org/10.4172/jaa.1000023.
Richmond A. Handbook of microalgae culture: biotechnology and phycology. Oxford: Blackwell Science; 2004.
Rossi F, De Philippis R. Exocellular polysaccharides in microalgae and cyanobacteria: chemical features, role and enzymes and genes involved in their biosynthesis. In: Borowitzka M, Beardall J, Raven J, editors. The physiology of microalgae. Developments in applied phycology, vol. 6. Cham: Springer; 2016.
Sadovskaya I, Souissi A, Souissi S, Grard T, Lencel P, Greene CM, Duin S, Dmitrenok PS, Chizhov AO, Shashkov AS, Usov AI. Chemical structure and biological activity of a highly branched (1→3,1→6)-β-d-glucan from *Isochrysis galbana*. Carbohydr Polym. 2014;111:139–48.
Santoyo S, Plaza M, Jaime L, Ibañez E, Reglero G, Señorans FJ. Pressurized liquid extraction as an alternative process to obtain antiviral agents from the edible microalga *Chlorella vulgaris*. J Agric Food Chem. 2010;58(15):8522–7.
Santoyo S, Jaime L, Plaza M, Herrero M, Rodriguez-Meizoso I, Ibañez E, Reglero G. Antiviral compounds obtained from microalgae commonly used as carotenoid sources. J Appl Phycol. 2012;24:731–41.
Sarnaik A, Abernathy MH, Han X, Ouyang Y, Xia K, Chen Y, Cress B, Zhang F, Lali A, Pandit R, Linhardt RJ, Tang YJ, Koffas MAG. Metabolic engineering of cyanobacteria for photoautotrophic production of heparosan, a pharmaceutical precursor of heparin. Algal Res. 2019;37:57–63.
Shii JK, Barber GA. Polysaccharide from cell walls of *Chlamydomonas reinhardtii*. Phytochemistry. 1975;14(11):2459–61.
Singh RS, Walia AK, Khattar JS, Singh DP, Kennedy JF. Cyanobacterial lectins characteristics and their role as antiviral agents. Int J Biol Macromol. 2017;102:475–96.
Song H, He M, Gu C, Wei D, Liang Y, Yan J, Wang C. Extraction optimization, purification, antioxidant activity, and preliminary structural characterization of crude polysaccharide from an arctic *Chlorella* sp. Polymers. 2018;10(3):292.
Sun L, Wang LLJ, Liu H. Characterization and antioxidant activities of degraded polysaccharides from two marine Chrysophyta. Food Chem. 2014a;160:1–7.
Sun Y, Wang H, Guo G, Pu Y, Yan B. The isolation and antioxidant activity of polysaccharides from the marine microalgae Isochrysis galbana. Carbohydr Polym. 2014b;113:22–31.
Sun L, Chu JSZ, Chen L. Physicochemical properties, immunomodulation and antitumor activities of polysaccharide from *Pavlova viridis*. Life Sci. 2016;144:156–61.
Suroy M, Panagiotopoulos C, Boutorh J, Goutx M, Moriceau B. Degradation of diatom carbohydrates: a case study with N- and Si-stressed *Thalassiosira weissflogii*. J Exp Mar Biol Ecol. 2015;470:1–11.
Tatli M, Ishihara M, Heiss C, Browne DR, Dangott LJ, Vitha S, Azadi P, Devarenne TP. Polysaccharide associated protein (PSAP) from the green microalga *Botryococcus braunii* is a unique extracellular matrix hydroxyproline-rich glycoprotein. Algal Res. 2018;29:92–103.
Trabelsi L, Chaieb O, Mnari A, Abid-Essafi S, Aleya L. Partial characterization and antioxidant and antiproliferative activities of the aqueous extracellular polysaccharides from the thermophilic microalgae *Graesiella* sp. BMC Complement Altern Med. 2016;16:210.
Urbani R, Magaletti E, Sist P, Cicero AM. Extracellular carbohydrates released by the marine diatoms *Cylindrotheca closterium*, *Thalassiosira pseudonana* and Skeletonema costatum: effect of P-depletion and growth status. Sci Total Environ. 2005;353(1):300–6.
Varfolomeeva SD, Wasserman LA. Microalgae as source of biofuel, food, fodder, and medicines. Appl Biochem Microbiol. 2011;47:789–807.

Villacorte LO, Ekowati Y, Neu TR, Kleijn JM, Winters H, Amy G, Schippers JC, Kennedy MD. Characterisation of algal organic matter produced by bloom-forming marine and freshwater algae. Water Res. 2015;73:216–30.

Yalcin I, Hicsasmaz Z, Boz B, Bozoglu F. Characterization of extracellular polysaccharide from freshwater microalgae *Chlorella* sp. LWT Food Sci Technol. 1994;27:158–65.

Yang S, Wan H, Wang R, Hao D. Sulfated polysaccharides from *Phaeodactylum tricornutum*: isolation, structural characteristics, and inhibiting HepG2 growth activity in vitro. PeerJ. 2019;7:e6409.

Yim JH, Kim SJ, Ah SH, Lee HK. Characterization of a novel bioflocculant, p-KG03, from a marine dinoflagellate, *Gyrodinium impudicum* KG03. Bioresour Technol. 2007;98:361–7.

Yu M, Chen M, Gui J, Huang S, Liu Y, Shentu H, He J, Fang Z, Wang W, Zhang Y. Preparation of *Chlorella vulgaris* polysaccharides and their antioxidant activity in vitro and in vivo. Int J Biol Macromol. 2019;137:139–50. https://doi.org/10.1016/j.ijbiomac.2019.06.222.

Zhang S, Jiang Y, Chen CS, Creeley DS, Quigg KA, Chin A, Santschi PH. Ameliorating effects of extracellular polymeric substances excreted by *Thalassiosira pseudonana* on algal toxicity of CdSe quantum dots. Aquat Toxicol. 2013;126:214–23. https://doi.org/10.1016/j.aquatox.2012.11.012.

Zhang L, Selão T, Toscano N, Peter J, Norling B. Photosynthetic conversion of CO_2 to hyaluronic acid by engineered strains of the cyanobacterium *Synechococcus* sp. PCC 7002. Algal Res. 2019;44:101702.

Zhao J, Liu S, Liu N, Zhang H, Zhou Q, Ge F. Accelerated productions and physicochemical characterizations of different extracellular polymeric substances from *Chlorella vulgaris* with nano-ZnO. Sci Total Environ. 2018;658:582–9. https://doi.org/10.1016/j.scitotenv.2018.12.019.

Microalgae Polysaccharides with Potential Biomedical Application

17

Michele Greque Morais, Gabriel Martins Rosa, Luiza Moraes, Ana Gabrielle Pires Alvarenga, Jacinta Lutécia Vitorino da Silva, and Jorge Alberto Vieira Costa

Contents

Abstract

Microalgae and cyanobacteria contain several biomolecules of commercial interest, including pigments, fatty acids, proteins, lipids, and carbohydrates. Among these, polysaccharides (PS) and extracellular polymeric substances (EPS) have been highlighted for their high levels of biological activity. In this context, this chapter aimed to not only highlight the main types, growing conditions, and downstream processes that influence the PS and EPS yield of microalgae but also

M. G. Morais (✉) · A. G. P. Alvarenga
Laboratory of Microbiology and Biochemistry, College of Chemistry and Food Engineering, Federal University of Rio Grande, Rio Grande, RS, Brazil

G. M. Rosa · L. Moraes · J. L. V. da Silva · J. A. V. Costa
Laboratory of Biochemical Engineering, College of Chemistry and Food Engineering, Federal University of Rio Grande, Rio Grande, RS, Brazil

J. M. Oliveira et al. (eds.), *Polysaccharides of Microbial Origin*,
https://doi.org/10.1007/978-3-030-42215-8_20

to report on the promising biomedical effects of their applications. The Increased production of PS or EPS by microalgae can be obtained mostly by providing stress conditions through nutritional limitation and/or adverse extrinsic growth conditions. In addition, the separation and purification stages can influence the acquisition of these biomolecules. Therefore, this chapter demonstrates that PS and EPS of microalgae, especially when a high concentration of sulfate is present, can act efficiently as bioactive molecules in the health field compared to specific reference drugs.

Keywords

Biomass · EPS · PS · Sulfated polysaccharides

1 Introduction

Microalgae are photosynthetic microorganisms with potential for applications in many areas, including nutrition and health, due to their biochemical compositions, with high concentrations of proteins, lipids, carbohydrates, fatty acids, phenolic compounds, and carotenoids. As such, biomass, extracted bioactive compounds, or biomolecules recovered from the culture medium are widely used in the food, pharmaceutical, cosmetics, and nutraceutical industries (Costa et al. 2020).

Polysaccharides (PS), a type of macromolecule produced by microalgae, play an important role in cells as energy and structural reserve components, as well as being produced and excreted into the extracellular environment (Delattre et al. 2016; Rossi and De Philippis 2016; Phélippé et al. 2019). This last group of biopolymers has a complex structure that consists mainly of different carbohydrate monomers linked in several structural arrangements. In addition, these biomolecules contain substituents other than sugars, such as sulfate and pyruvate groups (Delattre et al. 2016; Rossi and De Philippis 2016).

Microalgal extracellular biopolymers, or exopolysaccharides, exist as extracellular polymeric substances (EPS), extracellular polysaccharides (ECPS), released polysaccharides (RPS), capsular polysaccharides (CPS), and sulfated polysaccharides (sPS) (Bhunia et al. 2018). Although the morphology of these molecules is distinct, they can be classified into two groups: (a) those that form capsules, establishing strong molecular bonds with the outer cell surface; (b) viscous biomolecules that are weakly attached to capsules or released into the surrounding environment cellular (Han et al. 2014). Under ideal growing conditions, these biomolecules are produced at different stages of microalgal growth. However, an increase in the production of PS or EPS can be promoted by stress, such as changes in nutritional factors (Bafana 2013; Tiwari et al. 2020), salinity (Chentir et al. 2017), or light intensity (Ge et al. 2014).

The complexity of PS and EPS has made it is difficult to characterize the structures of and bonds between monomers and other non-sugar substituents present in their composition. However, their rheological and biological properties have led

to increased interest in their study, with views to apply them in several industrial sectors, particularly in the biomedical area (Delattre et al. 2016; Phélippé et al. 2019). The following biological activities stand out among those listed in the literature: antioxidant (Chen et al. 2016; Belhaj et al. 2017), antitumor (Sheng et al. 2007; Zhang et al. 2019b), antiviral (Huleihel et al. 2001; Mader et al. 2016), anti-inflammatory (Motoyama et al. 2016), anticoagulant (Majdoub 2009), and antimicrobial (Belhaj et al. 2017).

Therefore, this chapter addresses the main types of PS or EPS, the cultivation conditions that influence their production, and the techniques used for their extraction and purification, as well as evaluates the biological activities of these biomolecules, obtained from freshwater microalgae and cyanobacteria.

2 Polysaccharides and Extracellular Polymeric Substances Founded in Microalgae

Polysaccharides (PS) constitute the largest portion of carbohydrate macromolecules in microalgae and cyanobacteria. They are found in the cell wall, can be excreted by the cell, and serve as reserve components (Rossi and De Philippis 2016). Eukaryotic microalgae are capable of synthesizing starch molecules (composed of amylopectin and amylose) in plastids. They form their reserve compounds in the chloroplast, unlike prokaryotes, which form these compounds in the cytosol. Cyanobacteria are capable of synthesizing glycogen (α-polyglucan), with particle sizes $<0.04\mu m$, containing α-1,6-glucoside bonds between monomers (Lee 2008).

Eukaryotic microalgae are often surrounded by a cell wall composed of PS, partially produced and secreted by the Golgi apparatus. The wall of these microorganisms is composed of cellulose as the main PS, although xylans or mannans are also often used as basal components. Some single-celled microalgae have an amorphous matrix comprised of sulfated PS, and a PS protection film with a high protein concentration can occur outside the cell wall. PS acids and glycoproteins can also be present in the cell wall of some microalgae. Cyanobacteria have a cell wall similar to that of Gram-negative bacteria, containing a layer formed by huge polymers (peptidoglycan) composed of two sugar derivatives (N-acetylglucosamine and N-acetylmuramic acid) and amino acids, such as glutamic acid, alanine, and diaminopimelic acid (Lee 2008).

EPS produced by microalgae and cyanobacteria can be divided into two groups: those associated with the cell surface, which are referred to as capsule polysaccharides (CPS), sheath and slime, and those released into the culture medium (RPS) (De Philippis and Vincenzini 1998). In microalgae, the Golgi apparatus synthesizes vesicles with large amounts of PS, which are then transported to the cell membrane. There, vesicles fuse with the cell membrane and release their content out of the cell by exocytosis (Myklestad 1995). By contrast, in cyanobacteria, synthesis occurs in the cytoplasm. The main stages of EPS synthesis are the production of activated sugars, the assembly of this enzyme by glycosyltransferase enzymes, processing, and the export of polymeric substances in the extracellular medium or as a constituent of the endomembrane (Rossi and De Philippis 2016).

PS or EPS have complex structures, with high numbers and many kinds of monosaccharides in their composition. Studies have reported that EPS in cyanobacteria can consist of six or more monosaccharides (De Philippis and Vincenzini 1998). EPS are normally classified as complex heteropolymers, although homopolymers have been identified in microalgae. An example of a homopolymer, which is formed by repeated units of monomers, is the sulfated EPS produced by the microalgae *Gyrodinium impudicum* KG03, composed of galactose (Yim et al. 2007). Another example is PS β-(1,3)-glucan in *Chlorella vulgaris*, which is composed of glucose (Iwamoto 2004).

Heteropolymers are formed by more than one kind of monosaccharide unit, consisting mainly of neutral sugars, such as glucose, galactose, and xylose; in addition, mannose, ribose, fucose, arabinose, and rhamnose may also be present. Methylated sugars or uronic acids, such as galacturonic acid and glucuronic acid, may also be constituents (De Philippis and Vincenzini 1998; Delattre et al. 2016; Rossi and De Philippis 2016). In addition, non-sugar substituents, such as pyruvate, acetyl, methyl, protein, and inorganic groups, such as sulfate, can be linked to these structures (De Philippis and Vincenzini 1998; Delattre et al. 2016).

Sacran is a heteropolymer found in cyanobacteria. It is formed by glucose, galactose, mannose, xylose, rhamnose, fucose, galacturonic, and glucuronic acid, as well as traces of alanine, galactosamine, and muramic acid (Motoyama et al. 2016). Bafana (2013) characterized EPS produced by *C. reinhardtii*, and found it was composed of galacturonic acid, ribose, arabinose, xylose, glucose, galactose, rhamnose, and pyruvate. In another study (Majdoub 2009), the sulfated PS extracted from the *Arthrospira platensis* culture medium, called PUF2, was found to be composed of rhamnose (49.7%), sulfate (20%), galacturonic acid (16.9%), glucuronic acid (15%), xylose (5.9%), galactose (5.8%), glucose (4.3%), and mannose (0.9%). Figure 1 shows the composition of the main constituents of EPS in *Phormidium versicolor* (Belhaj et al. 2017), *Spirulina platensis* (Rajasekar et al. 2019), *C. vulgaris* (El-Naggar et al. 2020), and PS in *Aphanothece sacrum* (Motoyama et al. 2016) and *S. platensis* (Lee et al. 1998).

3 The Cultivation Conditions that Increase Polysaccharides or Extracellular Polymeric Substances Production in Microalgae

A strategy that is frequently used to increase PS and/or EPS production in microalgae is nutrient starvation, which results in an undesirable reduction of the biomass concentration. To match the high growth and production of these biomolecules, a two-stage batch cultivation strategy can be used: in stage (1), the ideal cultivation conditions for biomass production are used; in stage (2), adverse growth conditions are imposed to increase biomolecule production (Delattre et al. 2016). Microalgae PS and/or EPS increase with biomedical potential is similarly promoted, with an emphasis on the use of isolated strains, extraction methodology, technique, and harvesting period (Raposo et al. 2014).

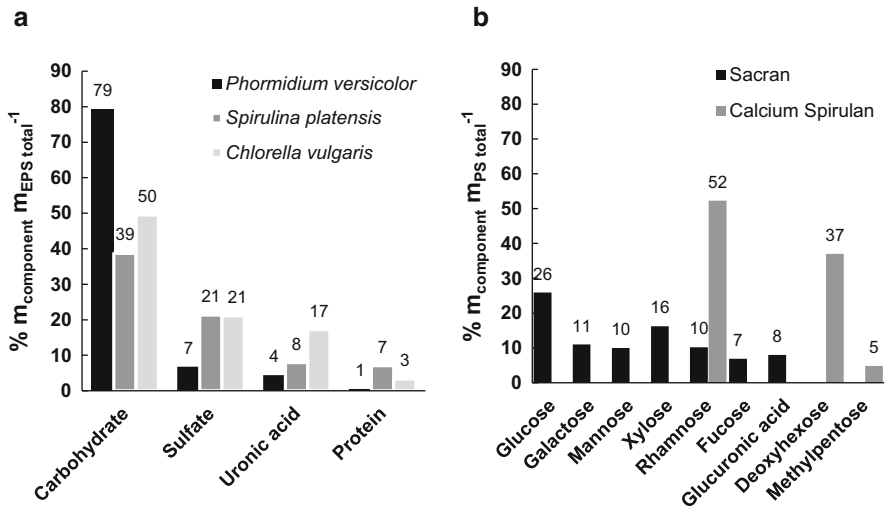


Fig. 1 Main constituents found in (**a**) extracellular polymeric substances in *Phormidium versicolor*, *Spirulina platensis*, and *Chlorella vulgaris* and (**b**) exopolysaccharides of *Aphanothece sacrum* (Sacran) and *S. platensis* (Calcium Spirulan)

From the nutritional optimization of *C. reinhardtii*, cultivation resulted in a significant increase in EPS production (0.63 g L^{-1}) (Bafana 2013). To produce these biomolecules, the reduction and/or deprivation of nitrogen, phosphorus, and sulfur, as well as different concentrations of calcium and magnesium, have been studied (Delattre et al. 2016). EPS production by *Botryococcus braunii* UC58 in 14 d with 2 mmol L^{-1} from different nitrogen sources was found to increase with nitrate (2.4 g L^{-1}) more than with ammonium (2.1 g L^{-1}) and urea (1.8 g L^{-1}), with a biomass concentration that was practically unchanged (1.1–1.2 g L^{-1}) (Lupi et al. 1994) (Table 1). The production of EPS by *Anabaena* sp. CCC-746 was maximized (0.34 g L^{-1}) when grown in BG-11 medium supplemented with 10 mmol L^{-1} of $CaCl_2$. In this strain, the fractions of EPS (CPS and RPS) showed antioxidant activity and were composed of glucose (40% mol mol^{-1}), xylose (40% mol mol^{-1}), and glucuronic acid (17% mol mol^{-1}) (Tiwari et al. 2020).

Saline stress, caused by sodium chloride (NaCl, 0.3–0.7 mol L^{-1}), increased the production of EPS by approximately 63% in the cyanobacterium *Microcoleus vaginatus* compared to cultivation without the presence of salt, acting as a protective cell response (Chen et al. 2006). *Synechocystis* sp. BASO444 cultivated with 0.4 mol L^{-1} of NaCl has secreted ~0.5 g L^{-1} of EPS, composed of glucose (97%) and galacturonic acid (3%) (Ozturk and Aslim 2010). Two-stage cultivation showed that an increase in NaCl (0.7 mol L^{-1}) combined with a reduction in light intensity (10μmol m^{-2} s^{-1}) in the second stage increased by 1.7-fold the production of EPS by *Spirulina* sp. compared to that obtained under the ideal growth conditions (10μmol m^{-2} s^{-1}, 1 g L^{-1} NaCl, 30 °C) (Chentir et al. 2017). Microwave irradiation (100 W, 40% duty cycle, and 2 min treatment time), representing stress conditions

Table 1 Parameters that influence the production of polysaccharides (PS) or extracellular polymeric substances (EPS) by microalgae and cyanobacteria

Microorganism	Cultivation condition for production	Differential factor used	PS or EPS production		Other important results	Reference
			$g\ L^{-1}$	$g\ g_{biomass}^{-1}$		
Anabaena sp. CCC-746	Conical flask (1 L); BG-11 medium; 28 °C; 12 h light (4 klx) and 12 h dark; manual shaking every 24 h; 30 d	Supplementation of 10 mmol L^{-1} $CaCl_2$	0.34	–	CPS and RPS with antioxidant activity	Tiwari et al. (2020)
Arthrospira platensis	Erlenmeyer (5 L); Zarrouk medium; 32 °C; light intensity (study factor); 11 d	Mixotrophic cultivation (100μmol m^{-2} s^{-1} and 1,5 g L^{-1} glucose)	0.30	0.35	Biomass concentration increase	Trabelsi et al. (2013)
Botryococcus braunii UC58	Cylindrical vessel; Chu13 medium; 1% CO_2, 25 °C; 250 $\mu E \cdot m^{-2} \cdot s^{-1}$, 31 d	Inorganic and organic sources of nitrogen	2.4	2.2	Unchanged biomass concentration	Lupi et al. (1994)
Chlamydomonas reinhardtii	Flask (1 L); medium and pH (study factors); room temperature; 12 h light (20μmol m^{-2} s^{-1}) and 12 h dark; 9 d	Optimized medium (mg L^{-1}): $CaCl_2$ (74), $NaNO_3$ (422), K_2HPO_4 (10), MgSO4 (200), pH 7	0.63	–	CPS = 60 mg L^{-1}	Bafana (2013)
Chlorella vulgaris	Conical flask (1 L); BG-11 medium; 28 °C; 12 h (light) and 12 h (dark); 120μmol m^{-2} s^{-1}; 16 d	Static magnetic field (800 G)	0.07	0.05	Increased biomass concentration	Luo et al. (2020)
Chlorella zofingiensis	Flask; BG-11 + glucose; 25 °C; 150 rpm; continuous light (40 $\mu E \cdot m^{-2} \cdot s^{-1}$); 5 d.	Mixotrophic cultivation (BG-11+ 5.0 g L^{-1} glucose)	0.21	0.09	Potential antitumoral agent	Zhang et al. (2019a)

Scenedesmus sp.	BG-11 medium; 27 °C; two-step cultivation: Growth (50μmol m^{-2} s^{-1}, pH 7.5, 12 d) and stress conditions (microwaves radiation)	Microwaves radiation (100 W, 40% duty cycle, 2 min treatment time)	0.12	0.04	Increased of biomass concentration and lipids	Sivaramakrishnan et al. (2020)
Spirulina sp.	Zarrouk medium; 30 °C; two-step cultivation: Growth (5 L flask, 80μmol m^{-2} s^{-1}, 1 g L^{-1} NaCl, 5 d) and stress conditions (0.25 L flask, 10μmol m^{-2} s^{-1}, 40 g L^{-1} NaCl, 3 d)	Increased salinity combined with reduced light	–	1.0	EPS with reduced protein concentration	Chentir et al. (2017)
Nostoc sp.	Flask (0.25 L); axenic cultivation; BG-11 medium; 25 °C; 20 d	Continuous lighting (80 μE m^{-2} s^{-1})	0.17	0.21	Unchanged biomass concentration	Ge et al. (2014)

(second-stage), was found to increase the production of EPS (140%), lipids (87%), and biomass (146%) produce by *Scenedesmus* sp. compared to a control culture (Sivaramakrishnan et al. 2020).

C. vulgaris mixotrophic cultivation increased both the growth and production of EPS. The composition of this aggregate was (m m^{-1}): carbohydrate (49.5%), sulfate (21.1%), uronic acid (17.2%), and protein (3.3%). This cultivation also resulted in a complex arrangement of monosaccharide bonds (% m m^{-1}): glucose (8.1), rhamnose (1.9), arabinose (1.4), maltose (0.8), lactose (0.7), and fructose (0.4) (El-Naggar et al. 2020). The production of EPS and its biomass concentration by *A. platensis* (0.30 g L^{-1}) were found to be 32% and 57% higher, respectively, in a mixotrophic culture (100μmol m^{-2} s^{-1} and 1.5 g L^{-1} of glucose as a carbon source) compared to photoautotrophic cultivation (Trabelsi et al. 2013). Mixotrophic cultivation with glucose or sucrose as carbon sources promoted the production of EPS by *Chlorella* sp. (0.03 g L^{-1}), and the biomass concentration was five-fold higher than that under control conditions (photoautotrophic assay) (Vo et al. 2020).

EPS production is increased by continuous exposure to high-intensity light, which has little influence on the PS composition. The cultivation of *Nostoc* sp. has shown no significant change in the biomass concentration. However, at a higher light intensity (80 μE m^{-2} s^{-1}), the production of RPS and CPS increased by 37.3% and 24.6% m m^{-1}, respectively, compared to cultivation with half the incidence of light (Ge et al. 2014). The blue monochromatic light incidence (460 nm) in the *N. flagelliforme* cultivation was found to increase the biomass concentration by 16% and EPS production by 51% compared to the control condition (white light (0.03 g $g_{biomass}{}^{-1}$)). However, a 43% increase in the CPS fraction by this cyanobacterium was obtained when using a red electromagnetic spectrum (660 nm) compared to the control culture (Han et al. 2014).

The production of EPS by microalgae can also be maximized by other parameters. For example, increasing the intensity of the static magnetic field has been found to increase the bound fraction of EPS (B-EPS) and decrease the formation of colonies by *C. vulgaris*. An intensity of 800 G increased the biomass concentration by 35.7% compared to cultivation without using a static magnetic field (Luo et al. 2020). The nonionic surfactant nonylphenol (8 mg L^{-1}) increased the production of soluble EPS (S-EPS) by *Dictyosphaerium* sp. by 58% after 72 h of cultivation compared to the control culture (Cheng et al. 2020). Meanwhile, the concentration of EPS produced by *C. vulgaris* was found to increase by 27.5% after exposure to a high concentration of zinc oxide nanoparticles (nano-ZnO, 0.04 mmol L^{-1}) compared to the control culture (Zhao et al. 2019).

4 Extraction and Purification of Microalgae Polysaccharides or Extracellular Polymeric Substances

In addition to the cultivation conditions, downstream steps, such as extraction, separation, and purification, also influence the PS and EPS yield (Chaiklahan et al. 2014; Delattre et al. 2016). In this sense, it is necessary to develop extraction/

separation methods that are not only efficient for obtaining a high yield of these compounds but also economical and environmentally friendly (Delattre et al. 2016). Moreover, the effects of the methods on the bioactivity of these compounds must be considered (Yu et al. 2019).

When PS or EPS are released into the culture medium, techniques such as filtration or centrifugation can be used to separate the cells from the permeate and supernatant. Afterward, the EPS or PS present in the soluble fraction (supernatant or permeate) are separated by alcoholic precipitation or with the use of membranes (tangential ultrafiltration) (Delattre et al. 2016). On the other hand, when PS is bound to cells, it is necessary to use methods to release the compounds into the liquid medium. Among these is the use of saline solutions (Bafana 2013), alkalis (Yu et al. 2019), and water (Chaiklahan et al. 2013; Delattre et al. 2016; Yu et al. 2019), which can undergo heating, freezing, and unfreezing cycles (Liu et al. 2010), followed by sonication, complexation, and cation exchange resin application, among others. Thereafter, alcoholic precipitation or membrane separation can be used for PS or EPS purification (Delattre et al. 2016).

Hot water is widely used for extracting PS from microalgal biomass (dry or fresh) (Chaiklahan et al. 2013, 2014; Yu et al. 2019) and after cell disruption (Chen et al. 2016). The binomial temperature/extraction time and the biomass/water ratio influences the PS extraction yield. Chaiklahan et al. (2013) reported that an increase in the temperature and solid-liquid ratio (biomass: water) resulted in an increase in the yield of the crude biopolymers of *Spirulina* sp. extracted with hot water. After extraction, the PS were recovered from the supernatant by precipitation with 1% cetyltrimethylammonium bromide (CTAB) solution, followed by centrifugation and washing with saturated sodium acetate solution in 95% ethanol and absolute ethanol. Once the extraction conditions were optimized (single step, solid–liquid ratio of 1:45 m v^{-1}, 90 °C, 120 min), a yield of 8.3% (dry basis) of PS was obtained.

Alternative methods have been proposed for the extraction of bioactive compounds from microalgae, including extraction with subcritical water (SWE) (Castro-Puyana et al. 2013), ultrasound-assisted extraction (UAE), and microwave-assisted extraction (MAE) (Costa et al. 2020). Several studies have used these techniques to obtain PS from microalgae and cyanobacteria (Kurd and Samavati 2015; Sousa e Silva et al. 2018; Yu et al. 2019). For example, 13.6% of water-soluble PS was obtained from *S. platensis* by using UAE, followed by precipitation with 80% ethanol, washing with ethanol, and centrifuging under optimal conditions (85 °C, 25 min, 90 W, water: biomass ratio of 20 mL g^{-1}) (Kurd and Samavati 2015). Yu et al. (2019) investigated the efficiency of six extraction methods on the yield, composition (crude and monosaccharide extract), and in vitro and in vivo antioxidant activity of PS obtained from *C. vulgaris*. Among the methods evaluated, UAE yielded 17.1% of crude PS, and the crude extract of these biomolecules had higher levels of total sugars (57.5%), uronic acid (18.1%), sulfates (29.7%), and lower protein fractions (3.1%).

Sousa e Silva et al. (2018) applied the MAE technique using water as a solvent to extract PS from *A. platensis*. Before extraction, the biomass of the cyanobacterium was pretreated with ethanol to extract lipids and pigments. A single extraction stage

(20 min) was found to be sufficient for the recovery of these compounds, reusing the solvent. The highest PS yield was obtained using MAE (434 W) for 1 min with a ratio of biomass: solvent of 1:30 w v^{-1} and without the need for additional contact after microwave extraction. MAE proved to be a promising technique since it was both fast and energetically economical (99.7%) compared to conventional PS extraction methods.

Subcritical water extraction (SWE) and pressurized hot water extraction (PHWE) are also promising techniques for the extraction of bioactive compounds from microalgae. In addition to being efficient, these techniques are non-toxic and environmentally friendly. SWE is considered a "green" technique and is based on the use of water at high temperatures (above the boiling point and below the critical temperature) under high pressure to remain in a liquid state during the extraction process (Castro-Puyana et al. 2013). SWE (between 100 and 300 °C) was used to extract PS from *Chlorella* sp., with the highest PS yield (23.6%) obtained at 150 °C (Zakaria et al. 2017). SWE is based on the same principles as pressurized liquid extraction (PLE). However, PLE can use other solvents in the extraction process (Castro-Puyana et al. 2013). Santoyo et al. (2010) applied PLE from water, ethanol, and acetone at 150 °C, for 20 min (1500 psi) to obtain *C. vulgaris* extracts. From the lyophilized aqueous extract, PS were isolated using hot water (90 °C), followed by precipitation with cold ethanol (2 volumes) and dialysis for purification. In the PS-rich fraction isolated from the aqueous extract, the proportion of carbohydrates was 46%, composed of high concentrations of glucose, galactose, mannose, and rhamnose, as well as small fractions of arabinose, xylose, fucose, and myo-inositol.

Alcoholic precipitation is a widely used technique for the extraction of PS and EPS from microalgae. This technique is based on reducing the solubility of these biomolecules in aqueous media by changing the polarity when alcohol is added. During precipitation, factors such as temperature and alcohol polarity can influence the yield and purity of PS or EPS (Patel et al. 2013). Ethanol has been widely used for PS/EPS precipitation (Chaiklahan et al. 2014; Kurd and Samavati 2015; Chen et al. 2016; Barboríková et al. 2019; Zhang et al. 2019a), although methanol and isopropanol can also be used (Patel et al. 2013). This technique has several advantages, such as selectivity and the ability to reuse the solvent through distillation (Delattre et al. 2016). Unfortunately, it also has several disadvantages, such as the coprecipitation of non-sugar compounds and salts (Patel et al. 2013). However, additional steps can be used to eliminate these undesirable compounds and increase the purity of PS and EPS (Delattre et al. 2016; Belhaj et al. 2017; Yu et al. 2019).

Barboríková et al. (2019) extracted EPS from the concentrated culture medium of *Chlorella vulgaris* BEIJ. 1890 after 10 d of cultivation using acidified ethanol (96%, 1: 4 v v^{-1}) for precipitation. After obtaining the precipitate, the authors used dialysis for 2 to 3 d, followed by lyophilization. The EPS obtained was then purified using repeated ethanol precipitation. Zhang et al. (2019a) also used alcoholic precipitation to extract EPS from *C. zofingiensis* 30412 and *C. vulgaris* UTEX 395 cultures. First, the supernatant (1/5 of the original volume) was concentrated by heating in a vacuum (60 °C), followed by the removal of free proteins (addition of trichloroacetic acid) and small molecules (ultrafiltration with distilled water). After these steps,

ethanol (70% v v^{-1}) was added and maintained at 4 °C, followed by centrifugation and lyophilization to obtain purified EPS.

Chen et al. (2016) used the UAE technique for extract PS from biomass of *Chlorella pyrenoidosa* by inducing cell disruption, heating in water at 90 °C, and incubating in a rotary evaporator under vacuum (55 °C), followed by alcohol precipitation using different concentrations of ethanol (60, 70, and 85%). The authors verified the effect of ethanol concentration on the chemical composition and antioxidant activity of PS: the PS extract precipitated with 70% ethanol (CPP70) showed a higher concentration of total sugars and a higher molar ratio of monosaccharides, including D-glucose and D-xylose.

The supernatant/permeate containing the PS can be concentrated before alcoholic precipitation using ultrafiltration and evaporation. Chaiklahan et al. (2014) compared three separation/concentration methods of *Spirulina* of the PS from the culture medium: (1) precipitation with cetyltrimethylammonium bromide (1% CTAB), (2) concentration by vacuum evaporation and ethanol precipitation, and (3) concentration by ultrafiltration and ethanol precipitation. Although there was no significant difference between the methods, ultrafiltration was considered the most appropriate because of its lower energy consumption and its potential for applications at a larger scale. The authors also found that a sample: ethanol ratio of 1: 2 (v v^{-1}) was ideal for PS precipitation.

As discussed previously, membrane separation has been proposed as a method for the isolation and purification of PS and EPS from microalgae (Majdoub 2009; Li et al. 2011; Bafana 2013; Patel et al. 2013), and is considered an alternative to conventional methods, such as alcoholic precipitation (Delattre et al. 2016). This method is based on membrane processes driven by different pressures, acting as a molecular sieve for the separation of compounds in an efficient and environmentally friendly (Charcosset 2006; Li et al. 2011). However, one of the disadvantages of this process is the occurrence of membrane obstruction due to the high PS or EPS viscosity (Zhang and Santschi 2009; Delattre et al. 2016). In this sense, studies have been conducted to enable the use of membranes for the extraction and purification of PS and EPS.

Li et al. (2011) used a coupled microfiltration and ultrafiltration system to isolate and purify EPS from single-cell filamentous microalgae culture media. In their study, the process parameters in the ultrafiltration stage were optimized to promote a greater recovery efficiency and reduce the obstruction of the membranes. The authors obtained an EPS yield of 231.3 mg L^{-1} (36 d cultivation) for *S. platensis* and 73.8 mg L^{-1} (70 d cultivation) for *Haematococcus pluvialis*, demonstrating the feasibility of applying this system on a wide scale. Majdoub (2009) used ultrafiltration by membranes with a cut-off molecular weight of 100 kDa (elimination of low molecular weight compounds and salts) for the extraction and purification of EPS from *A. platensis*. The crude EPS was fractionated using anion exchange chromatography, and a sulfated spirulan-like component (PUF2) was verified. Bafana (2013) separated *C. reinhardtii* cells from the culture medium by centrifugation, after which the EPS in the supernatant was concentrated and dialyzed by tangential flow filtration, resulting in an EPS concentrate with a high molecular weight.

5 Uses of Polysaccharides or Extracellular Polymeric Substances from Microalgae in Biomedical Applications

The biological activity of the PS and EPS obtained from microalgae can be verified by the direct use of the extracts or purified compounds obtained. The biomolecules have previously demonstrated several beneficial effects, including antitumor (Zhang et al. 2019a), antioxidant (Rajasekar et al. 2019), antiviral (Mader et al. 2016), anti-inflammatory (Zampieri et al. 2020), and antimicrobial (Belhaj et al. 2017) properties.

5.1 Antitumoral

The antitumor activity of EPS extracted from *Nostoc sphaeroides* and *H. pluvialis* was verified by an in vitro cytotoxicity assay using the MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) method. The EPS of *N. sphaeroides* has previously shown dose-dependent inhibition (0.1–2.0 mg mL^{-1}), with the highest inhibition rates observed when applied to the highest concentration in human gastric carcinoma cell line BGC-823 (61%), human promyelocytic leukemia cell line HL60 (39%), human acute lymphoblastic leukemia cell line MOLT-4 (34%) and human erythromyeloblastoid leukemia cell line K562 (53%). Similarly, EPS isolated from *H. pluvialis* has been found to inhibit cancer cells BGC-823 and HL60 in dose-dependent effects, with 68% maximum rates (BGC-823) and 40% (HL60) observed for 2 mg EPS mL^{-1} (Li et al. 2011).

PS CPPS Ia and CPPS IIa extracted from *C. pyrenoidosa* are mainly composed of rhamnose, mannose, glucose, galactose, and an unknown monosaccharide. Galactose is found at higher concentrations in fraction Ia (46.5%), while rhamnose is found at higher concentrations in fraction IIa (37.8%). These two PS have shown antitumor activity against human lung adenocarcinoma A549 cells in in vitro assays using the MTT cell viability method. Cancer cell growth inhibition was found to be dependent on the applied dose of each PS, with a maximum inhibition rate of 68.7% with 1000μg mL^{-1} for CPPS Ia. This is comparable to the 5-Fu control (68.5%) and was significantly higher than CPPS IIa (49.5%) (Sheng et al. 2007).

EPS extracted from the cultivation of *Chlorella zofingiensis* 30,412 (EPS-CZ) and *C. vulgaris* UTEX 395 (EPS-CV) showed antitumor activity against HCT8 colon cancer cells in in vitro cell viability assays using the MTT method. As a result, EPS-CZ (28.3%) showed an inhibitory activity higher than that observed for EPS-CV (18.0%) in HCT8 cells at 0.6 mg EPS mL^{-1}. In addition, using cell viability assays, no inhibitory effect was identified when the cytotoxicity of both kinds of EPS was evaluated against normal CCL1 cells at 0.6 mg EPS mL^{-1} (Zhang et al. 2019a).

5.2 Antioxidant

In the in vitro assay, the soluble fraction of EPS produced by the thermophilic microalgae *Graesiella* sp. (AEPS, a hetero-sulfated-anionic PS) was found to exhibit

a total antioxidant capacity of ~90% relative to the value considered high for this parameter. The addition of 1 and 2 mg mL^{-1} of AEPS was higher and equal, respectively, as a potential inhibitor of the OH^- radical compared to ascorbic acid. In addition, 0.5 and 1.0 mg AEPS mL^{-1} showed an iron-chelating activity 1.2-fold greater compared to the same concentration of EDTA. These results can be attributed to the AEPS composition in uronic acid (23%) and ester sulfate (11%) (Trabelsi et al. 2016).

In vitro assays showed that PS extracted from the biomass of *C. pyrenoidosa* (CPP60, CPP70, and CPP85) showed antioxidant activity, which was found to be influenced by the final concentration of ethanol used in the precipitation process. Comparing the three PS fractions obtained using different ethanol concentrations, 1.6 mg L^{-1} of CPP70 showed a greater OH^- radical elimination activity (92.7%), while 2 mg mL^{-1} of CPP70 showed greater elimination activities of the DPPH radical (1,1-diphenyl-2-picrylhydrazyl) (39.7%) and superoxide radical (76.8%), as well as a greater reducing power (0.328 AU) (Chen et al. 2016).

PS crude extract from *P. versicolor* (CPv-PS), which has been identified as hetero-sulfated-anionic PS, was found to be comprised of (% m m^{-1}): carbohydrate (79.4), sulfate (6.8), and uronic acid (4.4). An in vitro evaluation showed that the antioxidant capacity of CPv-PS followed a dose-dependent pattern (0.078–2.5 mg mL^{-1}). With 0.625 mg CPv-PS mL^{-1} its capacity to eliminate the DPPH radical was 57.3%; and with 2.5 mg CPv-PS mL^{-1} the reduce Fe^{3+} to Fe^{2+} was 90% compared to that obtained when using vitamin C. This result was attributed to the presence of hydroxyl in the composition of the PS. In addition, 1.25 mg CPv-PS mL^{-1} was found to prevent β-carotene bleaching, demonstrating its antioxidant power and ability to prevent membrane lipid oxidation (Belhaj et al. 2017).

Sulfated EPS isolated from *S. platensis* was found to be mainly composed of (% m m^{-1}): carbohydrates (38.7), sulfate (21.3), uronic acid (7.9), and protein (7.1). At a concentration of 5 mg EPS mL^{-1}, EPS demonstrated a potent antioxidant activity against the radical DPPH (76.5%), reducing power (1.3 AU), hydrogen peroxide elimination (66.3%), OH^- elimination (68.6%), nitric oxide (81.4%), and total antioxidant activity (1.66 AU). These results were slightly lower than those obtained for L-ascorbic acid in terms of DPPH (11.0%), reducing power (19.0%), and nitric oxide (7.0%) (Rajasekar et al. 2019).

The EPS extracted from *C. zofingiensis* 30412 (EPS-CZ) and *C. vulgaris* UTEX 395 (EPS-CV) were found to exert antioxidant activity in in vitro assays for the elimination of DPPH and OH^- radicals in concentration-dependent effects. For radical DPPH, the maximum elimination capacity was observed at 3.0 mg mL^{-1}, reaching values of 71.5% for EPS-CZ and 59.6% for EPS-CV. The highest elimination of OH^- was achieved with 1.0 mg mL^{-1} EPS-CV (70.4%) and EPS-CZ (44.5%) (Zhang et al. 2019a).

5.3 Antiviral

Sulfated PS extracted from the cell wall of *Porphyridium* sp., *P. aerugineum*, and *Rhodella reticulata* have been found to exert activity against Herpes simplex viruses

(HSV) 1 and 2 and Varicella zoster virus (VZV). However, the PS extracted from *Porphyridium* sp. inhibited the cytopathic effect caused by HSV and VZV more effectively in Vero cell culture. In vitro and in vivo assays using the PS obtained from these microalgae showed that these viruses were prevented from initiating infection by PS hampering their penetration, adhesion, or fusion in the tested cells. In addition, in the more advanced stages of the virus, protein synthesis was found to be prevented (Huleihel et al. 2001; Talyshinsky et al. 2002). This is attributed to the higher degree of sulfation of the PS obtained, which proving the correlation between the sulfation level and the antiviral activity of PS (Huleihel et al. 2001). This hypothesis was corroborated by the EPS of *P. cruentum*, in which the highest antiviral activity was observed with the highest sulfation degree (Raposo et al. 2014).

S. platensis sulfated PS (known as calcium spirulan, Ca-Sp) has been found to exert antiviral activity against the replication of certain envelope viruses, such as measles, mumps, influenza A, and herpes simplex 1 (HSV-1). The molecular retention conformation by chelating the calcium ion with sulfate groups has been suggested as indispensable to this antiviral effect. Ca-Sp has also been found to exert antiviral activity against human herpes virus type 8 and prevent infection with the HSV-1 virus (Mader et al. 2016). Among the main findings of this study, the binding of HSV-1 to human keratinocytes was found to be inhibited in an in vitro experiment, highlighting the comparable potency of Ca-Sp to that of acyclovir. In a clinical model of herpes exacerbation with 198 volunteers, the prophylactic use of Ca-Sp in lip cream was higher than that of acyclovir cream (Mader et al. 2016).

5.4 Anti-Inflammatory

Crude PS extracts obtained from *Chlorella stigmatophora* and *Phaeodactylum tricornutum* have been found to be mainly composed of reducing sugars (23.4% and 30.8%), sulfates (8.2% and 7.5%), and uronic acids (4.6% and 6.3%), respectively. These PS extracts have shown strong anti-inflammatory activity in vivo compared to indomethacin, by reducing carrageenan-induced edema in the paws of rats (Guzmán et al. 2003).

The sulfated EPS fraction extracted from *A. sacrum* (Sacran) showed anti-inflammatory effects on the ears and paws of rats in in vivo assays. The most effective concentration of Sacran (0.05% m v^{-1}) was higher than that of BPAA (4-biphenyl acetic acid), a reference nonsteroidal anti-inflammatory (NSAID), in the inhibition of edema caused by carrageenan. In its topical application, the same concentration of Sacran was found to significantly suppress the formation of paw edema caused by kaolin and dextran. Sacran (0.05% m v^{-1}) showed the highest inhibitory effect against the animal's ear swelling induced by TPA (12-O-tetradecanoylpholia-13-acetate). Sacran solutions showed a negligible cytotoxicity up to 0.25% m v^{-1} (Motoyama et al. 2016).

The EPS of *Phormidium* sp. ETS05 demonstrated anti-inflammatory and pro-resolution activities induced chemically and by injury in in vivo models of zebrafish (*Danio rerio*). However, in vitro (human skin fibroblasts) and in vivo

assays did not show toxicity in these biomolecules, including increasing cell viability, in the first case, with treatments of 25–100μg mL^{-1} of EPS. This study found that the EPS of this cyanobacteria was among the most important molecules present in the mud of the Euganean Thermal District (Italy) (Zampieri et al. 2020).

5.5 Other Activities

In delayed hypersensitivity and phagocytic activity assays in vivo and in vitro, *c*rude aqueous extracts of PS obtained from *C. stigmatophora* and *P. tricornutum* showed immunomodulatory effects, with an emphasis on the immunosuppressive activity of the crude extract of PS from *C. stigmatophora* and on the immunostimulatory activity of PS from *P. tricornutum* (Guzmán et al. 2003). PS of storage β-1,3-glucan was found in the *Chlorella* genus and is considered to be the most important molecule for human health, acting as an active immunostimulatory, free radical scavenger, and blood lipid reducer (Iwamoto 2004).

The sulfated EPS of *S. platensis* in in vitro assays showed a dose-dependent relationship in antimicrobial activity against *Vibrio vulnificus*, a bacterium that can cause serious health issues in humans, including fulminant systemic infection and septicemia. The bioassay indicated that, with 100μg mL^{-1} EPS, little or no bacterial colony was observed (Rajasekar et al. 2019). The disk diffusion method (Ø - diameter of the inhibition zone) with PS extracted from *P. versicolor* (CPv-PS) (2.5 mg mL^{-1}) showed moderate antimicrobial activity against Gram-positive bacteria (Ø 12.0 to 14.1 mm), compared to the positive control with ampicillin (Ø 19.0 to 21.0 mm). Antimicrobial activity against Gram-negative bacteria was considered low for *Salmonella enterica* (CIP 8039) (Ø 7.0 mm) and *Pseudomonas aeruginosa* (ATCC 9027) (Ø 7.2 mm), and moderate (Ø 11.1 mm) for *Escherichia coli* (ATCC 8739) compared to ampicillin (Ø 19.0 to 28.0 mm). On the other hand, when the CPv-PS extract was applied to yeasts and fungi, a strong antimicrobial activity (Ø 15.0 to 17.3 mm) was observed in comparison to amphotericin (control) (Ø 20.0 to 28.0 mm). Thus, CPv-PS extract is considered to exert both antibacterial and antifungal activities (Belhaj et al. 2017).

6 Final Considerations

In this chapter, the extensive biomedical applications of PS and EPS of microalgae and cyanobacteria were highlighted, as well as their potential for comparison with reference drugs. However, the class of biomolecules presented here will need to be studied further regarding the maximization of the production method and, most importantly, in terms of their clinical use. Therefore, this chapter showed that PS and EPS obtained from microalgae, in particular those with a high sulfate concentration in their composition, have the potential to become leading bioactive molecules in the fields of health and biomedicine as alternatives to synthetic compounds,

providing not only greater clinical benefits than their synthetic counterparts, but also promoting a higher quality of life in humans.

Acknowledgments The authors would like to thank the Coordination for the Improvement of Higher Education Personnel (CAPES) – Finance Code 001, National Council for Scientific and Technological Development (CNPq), the Ministry of Science, Technology, and Innovations (MCTI), and the Foundation for Research Support of the State of Rio Grande do Sul (FAPERGS) for financial support. This research was developed within the scope of the Capes-PrInt Program (Process # 88887.310848/2018-00). Besides, G. M. Rosa and L. Moraes would like to thank the postdoctoral fellowships from CNPq, process numbers 161006/2019-1, and 160030/2018-8, respectively.

References

Bafana A. Characterization and optimization of production of exopolysaccharide from *Chlamydomonas reinhardtii*. Carbohydr Polym. 2013;95:746–52.

Barboríková J, Šutovská M, Kazimierová I, et al. Extracellular polysaccharide produced by *Chlorella vulgaris* – chemical characterization and anti-asthmatic profile. Int J Biol Macromol. 2019;135:1–11.

Belhaj D, Frikha D, Athmouni K, et al. Box-Behnken design for extraction optimization of crude polysaccharides from Tunisian *Phormidium versicolor* cyanobacteria (NCC 466): partial characterization, *in vitro* antioxidant and antimicrobial activities. Int J Biol Macromol. 2017;105:1501–10.

Bhunia B, Prasad Uday US, Oinam G, et al. Characterization, genetic regulation and production of cyanobacterial exopolysaccharides and its applicability for heavy metal removal. Carbohydr Polym. 2018;179:228–43.

Castro-Puyana M, Herrero M, Mendiola JA, Ibáñez E. Subcritical water extraction of bioactive components from algae. In: Domínguez H, editor. Functional ingredients from algae for foods and nutraceuticals. Oxford: Woodhead Publishing; 2013. p. 534–60.

Chaiklahan R, Chirasuwan N, Triratana P, et al. Polysaccharide extraction from *Spirulina* sp. and its antioxidant capacity. Int J Biol Macromol. 2013;58:73–8.

Chaiklahan R, Chirasuwan N, Triratana P, et al. Effect of extraction temperature on the diffusion coefficient of polysaccharides from *Spirulina* and the optimal separation method. Biotechnol Bioprocess Eng. 2014;19:369–77.

Charcosset C. Membrane processes in biotechnology: an overview. Biotechnol Adv. 2006;24:482–92.

Chen L, Li D, Song L, et al. Effects of salt stress on carbohydrate metabolism in desert soil alga. J Integr Plant Biol. 2006;48:914–9.

Chen Y-X, Liu X-Y, Xiao Z, et al. Antioxidant activities of polysaccharides obtained from *Chlorella pyrenoidosa* via different ethanol concentrations. Int J Biol Macromol. 2016;91:505–9.

Cheng Q, Jiang Y, Jin Z, et al. Enhanced excretion of extracellular polymeric substances associated with nonylphenol tolerance in *Dictyosphaerium* sp. J Hazard Mater. 2020;395:122644.

Chentir I, Hamdi M, Doumandji A, et al. Enhancement of extracellular polymeric substances (EPS) production in *Spirulina* (*Arthrospira* sp.) by two-step cultivation process and partial characterization of their polysaccharidic moiety. Int J Biol Macromol. 2017;105:1412–20.

Costa JAV, Freitas BCB, Moraes L, et al. Progress in the physicochemical treatment of microalgae biomass for value- added product recovery. Bioresour Technol. 2020;301:122727.

De Philippis R, Vincenzini M. Exocellular polysaccharides from cyanobacteria and their possible applications. FEMS Microbiol Rev. 1998;22:151–75.

Delattre C, Pierre G, Laroche C, Michaud P. Production, extraction and characterization of microalgal and cyanobacterial exopolysaccharides. Biotechnol Adv. 2016;34:1159–79.

El-Naggar NEA, Hussein MH, Shaaban-Dessuuki SA, Dalal SR. Production, extraction and characterization of *Chlorella vulgaris* soluble polysaccharides and their applications in AgNPs biosynthesis and biostimulation of plant growth. Sci Rep. 2020;10:1–19.

Ge H, Xia L, Zhou X, et al. Effects of light intensity on components and topographical structures of extracellular polysaccharides from the cyanobacteria *Nostoc* sp. J Microbiol. 2014;52:179–83.
Guzmán S, Gato A, Lamela M, et al. Anti-inflammatory and immunomodulatory activities of polysaccharide from *Chlorella stigmatophora* and *Phaeodactylum tricornutum*. Phyther Res. 2003;17:665–70.
Han P, Sun Y, Jia S, et al. Effects of light wavelengths on extracellular and capsular polysaccharide production by *Nostoc flagelliforme*. Carbohydr Polym. 2014;105:145–51.
Huleihel M, Ishanu V, Tal J, Arad S. Antiviral effect of red microalgal polysaccharides on *Herpes simplex* and *Varicella zoster* viruses. J Appl Phycol. 2001;13:127–34.
Iwamoto H. Industrial production of microalgal cell-mass and secondary products – major industrial species – *Chlorella*. In: Richmond A, editor. Handbook of microalgal culture: biotechnology and applied phycology. Oxford: Blackwell; 2004. p. 255–63.
Kurd F, Samavati V. Water soluble polysaccharides from *Spirulina platensis*: extraction and *in vitro* anti-cancer activity. Int J Biol Macromol. 2015;74:498–506.
Lee RE. Basic characteristics of the algae. In: Lee RE, editor. Phycology. 4th ed. Cambridge: Cambridge University Press; 2008. p. 3–30.
Lee J-B, Hayashi T, Hayashi K, et al. Further purification and structural analysis of calcium spirulan from *Spirulina platensis*. J Nat Prod. 1998;61:1101–4.
Li H, Li Z, Xiong S, et al. Pilot-scale isolation of bioactive extracellular polymeric substances from cell-free media of mass microalgal cultures using tangential-flow ultrafiltration. Process Biochem. 2011;46:1104–9.
Liu Y, Wang W, Zhang M, et al. PSII-efficiency, polysaccharide production, and phenotypic plasticity of *Scenedesmus obliquus* in response to changes in metabolic carbon flux. Biochem Syst Ecol. 2010;38:292–9.
Luo X, Zhang H, Li Q, Zhang J. Effects of static magnetic field on *Chlorella vulgaris*: growth and extracellular polysaccharide (EPS) production. J Appl Phycol. 2020;32:2819–28.
Lupi FM, Fernandes HML, Tomé MM, et al. Influence of nitrogen source and photoperiod on exopolysaccharide synthesis by the microalga *Botryococcus braunii* UC 58. Enzym Microb Technol. 1994;16:546–50.
Mader J, Gallo A, Schommartz T, et al. Calcium spirulan derived from *Spirulina platensis* inhibits herpes simplex virus 1 attachment to human keratinocytes and protects against herpes labialis. J Allergy Clin Immunol. 2016;137:197–203.e3.
Majdoub H, Mansour MB, Chaubet F, et al. Anticoagulant activity of a sulfated polysaccharide from the green alga *Arthrospira platensis*. Biochim Biophys Acta. 2009;1790:1377–81.
Motoyama K, Tanida Y, Hata K, et al. Anti-inflammatory effects of novel polysaccharide sacran extracted from cyanobacterium *Aphanothece sacrum* in various inflammatory animal models. Biol Pharm Bull. 2016;39:1172–8.
Myklestad SM. Release of extracellular products by phytoplankton with special emphasis on polysaccharides. Sci Total Environ. 1995;165:155–64.
Ozturk S, Aslim B. Modification of exopolysaccharide composition and production by three cyanobacterial isolates under salt stress. Environ Sci Pollut Res. 2010;17:595–602.
Patel AK, Laroche C, Marcati A, et al. Separation and fractionation of exopolysaccharides from *Porphyridium cruentum*. Bioresour Technol. 2013;145:345–50.
Phélippé M, Gonçalves O, Thouand G, et al. Characterization of the polysaccharides chemical diversity of the cyanobacteria *Arthrospira platensis*. Algal Res. 2019;38:101426.
Rajasekar P, Palanisamy S, Anjali R, et al. Isolation and structural characterization of sulfated polysaccharide from *Spirulina platensis* and its bioactive potential: *in vitro* antioxidant, antibacterial activity and Zebrafish growth and reproductive performance. Int J Biol Macromol. 2019;141:809–21.
Raposo MFDJ, de Morais AMMB, de Morais RMSC. Influence of sulphate on the composition and antibacterial and antiviral properties of the exopolysaccharide from *Porphyridium cruentum*. Life Sci. 2014;101:56–63.
Rossi F, De Philippis R. Exocellular polysaccharides in microalgae and cyanobacteria: chemical features, role and enzymes and genes involved in their biosynthesis. In: Borowitzka M, Beardall J, Raven J, editors. The physiology of microalgae. Developments in applied phycology. Cham: Springer International Publishing; 2016. p. 565–90.

Santoyo S, Plaza M, Jaime L, et al. Pressurized liquid extraction as an alternative process to obtain antiviral agents from the edible microalga *Chlorella vulgaris*. J Agric Food Chem. 2010;58: 8522–7.

Sheng J, Yu F, Xin Z, et al. Preparation, identification and their antitumor activities *in vitro* of polysaccharides from *Chlorella pyrenoidosa*. Food Chem. 2007;105:533–9.

Sivaramakrishnan R, Suresh S, Pugazhendhi A, et al. Response of *Scenedesmus* sp. to microwave treatment: enhancement of lipid, exopolysaccharide and biomass production. Bioresour Technol. 2020;312:123562.

Sousa e Silva A, Magalhães WT, Moreira LM, et al. Microwave-assisted extraction of polysaccharides from *Arthrospira* (*Spirulina*) *platensis* using the concept of green chemistry. Algal Res. 2018;35:178–84.

Talyshinsky MM, Souprun YY, Huleihel MM. Anti-viral activity of red microalgal polysaccharides against retroviruses. Cancer Cell Int. 2002;2:1–7.

Tiwari ON, Muthuraj M, Bhunia B, et al. Biosynthesis, purification and structure-property relationships of new cyanobacterial exopolysaccharides. Polym Test. 2020;89:106592.

Trabelsi L, Ben Ouada H, Zili F, et al. Evaluation of *Arthrospira platensis* extracellular polymeric substances production in photoautotrophic, heterotrophic and mixotrophic conditions. Folia Microbiol (Praha). 2013;58:39–45.

Trabelsi L, Chaieb O, Mnari A, et al. Partial characterization and antioxidant and antiproliferative activities of the aqueous extracellular polysaccharides from the thermophilic microalgae *Graesiella* sp. BMC Complement Altern Med. 2016;16:1–10.

Vo HNP, Ngo HH, Guo W, et al. Selective carbon sources and salinities enhance enzymes and extracellular polymeric substances extrusion of *Chlorella* sp. for potential co-metabolism. Bioresour Technol. 2020;303:122877.

Yim JH, Kim SJ, Ahn SH, Lee HK. Characterization of a novel bioflocculant, p-KG03, from a marine dinoflagellate, *Gyrodinium impudicum* KG03. Bioresour Technol. 2007;98:361–7.

Yu M, Chen M, Gui J, et al. Preparation of *Chlorella vulgaris* polysaccharides and their antioxidant activity *in vitro* and *in vivo*. Int J Biol Macromol. 2019;137:139–50.

Zakaria SM, Mustapa Kamal SM, Harun MR, et al. Extraction of antioxidants from *Chlorella* sp. using subcritical water treatment. IOP Conf Ser. Mater Sci Eng. 2017;206:012035.

Zampieri RM, Adessi A, Caldara F, et al. Anti-inflammatory activity of exopolysaccharides from *Phormidium* sp. ETS05, the most abundant cyanobacterium of the therapeutic Euganean thermal muds, using the zebrafish model. Biomolecules. 2020;10:582.

Zhang S, Santschi PH. Application of cross-flow ultrafiltration for isolating exopolymeric substances from a marine diatom (*Amphora* sp.). Limnol Oceanogr Methods. 2009;7:419–29.

Zhang J, Liu L, Chen F. Production and characterization of exopolysaccharides from *Chlorella zofingiensis* and *Chlorella vulgaris* with anti-colorectal cancer activity. Int J Biol Macromol. 2019a;134:976–83.

Zhang J, Liu L, Ren Y, Chen F. Characterization of exopolysaccharides produced by microalgae with antitumor activity on human colon cancer cells. Int J Biol Macromol. 2019b;128:761–7.

Zhao J, Liu S, Liu N, et al. Accelerated productions and physicochemical characterizations of different extracellular polymeric substances from *Chlorella vulgaris* with nano-ZnO. Sci Total Environ. 2019;658:582–9.

Fungal Polysaccharide Production for Dermatological Purposes

18

Carla Maísa Camelini, Márcio José Rossi, Francielle Tramontini Gomes de Sousa, and Admir Giachini

Contents

C. M. Camelini (✉)
Sole Vitae® Cosméticos Brazil and Chile, Palmital, SP, Brazil

M. J. Rossi
Departamento de Microbiologia, Imunologia e Parasitologia, Universidade Federal de Santa Catarina, Florianopolis, SC, Brazil
e-mail: marcio.rossi@ufsc.br

F. T. G. de Sousa
Division of Infectious Diseases and Vaccinology, School of Public Health, University of California-Berkeley, Berkeley, CA, USA

A. Giachini
Ressacada de Pesquisas em Meio Ambiente, Universidade Federal de Santa Catarina, Florianopolis, SC, Brazil
e-mail: admir.giachini@ufsc.br

J. M. Oliveira et al. (eds.), *Polysaccharides of Microbial Origin*,
https://doi.org/10.1007/978-3-030-42215-8_64

Abstract

Bioactive polysaccharides such as β-glucans are important structural component of cell wall in practically all fungi. To obtain these substances is essential to guarantee isolation, culturing, and preservation of the fungal specimen, especially for species like *Agaricus subrufescens* for which science has demonstrated high biotechnological potential due to its medicinal properties related mainly to its β-(1 → 6)-(1 → 3)-glucan and β-(1 → 2)-gluco-β-(1 → 3)-mannan. Many health benefits have been attributed to β-glucans, including immunomodulatory, hypoglycemic, antioxidant, and antitumor properties. Moreover, β-glucans also exhibit a promising dermatological activity on the skin, scalp, and hair. For research and industrial/commercial purposes, the submerged cultivation of *A. subrufescens* mycelium in bioreactors is a superior way of standardization of the polysaccharidic product (s) allowing more stable and controlled conditions and a faster pace production than solid cultivation. In this chapter we will describe some of these production approaches, bringing detailed information into the realm of *A. subrufescens* cultivation and the biotechnological applications of its polysaccharides for dermatological purposes through their formulation in cosmeceuticals.

Keywords

Agaricus brasiliensis · *Agaricus subrufescens* · Cosmeceutical · Dermatological activity · β-glucan · Polysaccharide · Skin immunity · Submerged cultivation

1 Introduction

1.1 Fungal Conservation to Protect Species of Biotechnological Interest

1.1.1 Ecological Role and Human Interaction

Fungi play several distinctive roles in ecosystems. They are capable of decaying organic matter, acting as parasites of plants and animals, developing mutualistic relationships to several phototrophic organisms (e.g., cyanobacteria and algae), and becoming mycorrhizal partners of vascular plants (Fabian et al. 2017). They exist in a wide range of habitats, including fresh water and the sea, soil, litter, decaying remains of animals and plants, in stool, living plants, and animals (Webster and Weber 2010). Fungi help maintain a sustainable ecosystem for the other living entities that share the same habitat.

The life strategies of fungi vary according to the environment and the availability of resources. Those strategies can go from rapid exploitation during primary resource capture to a phase of assimilation and utilization, consolidation and defense. Depending on the strategy, fungi may produce different morphological structures that help them grow and reproduce throughout their life cycle. These forms are endowed of several structural and active components found both on single-celled as

well as filamentous species. Some of the most biological active compounds exploited by humans from fungi are those located within the cell wall, namely, chitosan and glucans, although many other compounds have been utilized from the primary and secondary metabolism (ergosterol and other steroids, vitamins, glycoproteins and proteoglucans) (Itoh et al. 2008; Wisitrassameewong et al. 2012).

In order to consider large scale production of fungal biomass, one must first isolate the organism of interest. Isolation is foreseen considering the particularities of each species and includes finding the most suitable in vitro conditions for the organism to thrive. Not all species grow in vitro and many, even if established in axenic conditions, will not grow following minimal requirements. In that respect, finding or developing the best set of conditions for the organism of interest is one of the first steps in culturing a candidate with biotechnological potential. Culture collections are a way of maintaining and preserving the germplasm of fungi for that and other purposes. Preservation is an essential early step for research and industrial applications of natural resources as well as to exploit their genetic and ecological functions (Cameotra 2007). Preservation protocols are widely available for many species, including *Agaricus subrufescens* (Camelini et al. 2012).

Considering structural polysaccharides as the compound of interest, as they are part of the cell walls, the larger the amounts of cells produced, the greater is the productivity. In that respect, fruiting bodies or the production of large amounts of biomass (yeast-like cells or mycelia) have been the objectives of those exploiting the active compounds from fungi for wide-ranging applications.

1.1.2 *Agaricus subrufescens*, and Important Biotechnological Species

Glucans are frequently present in the cell wall of fungi like mushrooms and yeast, cereals like oats, and bacteria. These polysaccharides are polymers built of glucose units linked by α- (alpha) and/or β- (beta) type glycosidic bonds and have complex chemical structure, which perform various physiological and structural functions. The major β-glucans used for pharmaceutical preparations in various Asian countries are obtained from the mushrooms *Lentinus edodes*, *Schizophyllum commune*, *Trametes versicolor*, and *Grifola frondosa*. Their structures and functionality vary widely depending on the species source and extraction method (Khan et al. 2018). Another important species exploited industrially is the *A. subrufescens* Peck (= *Agaricus brasiliensis* Wasser, Didukh, de Amazonas & Stamets) identified previously as *A. blazei* (Kerrigan 2005), a culinary-medicinal fungus native to the Americas and one of the most important basidiomycetes commercially produced in many countries, being Brazil its larger worldwide producer (Camelini et al. 2012; Wisitrassameewong et al. 2012).

Consumption of this fungus has increased in recent years mainly because of its nutritional and pharmaceutical properties, which are mainly attributed to the cell wall β-glucans from fruiting bodies resulting from the reproductive phase (Camelini et al. 2005a). Nevertheless, these polysaccharides can also be found in the mycelium (vegetative phase) due to their structural functions in the cell wall, or obtained from extracellular components secreted by the fungus in the culture medium (Lindequist et al. 2005).

The nutritional composition of *A. subrufescens* fruiting bodies contain 89–91% water, with almost 48% of its total dry matter consisting of crude protein and 18% of carbohydrates, with low lipid content (0.5%), and high levels of valuable minerals, such as potassium, phosphorus, calcium, magnesium, and zinc (Wisitrassameewong et al. 2012).

β-Glucans are present in the nutritional composition as carbohydrates, or a dietary fibers, with a highly ordered structure formed mainly by β-D-glucose units with high molecular weight. The differences in the type of connection between the units of the sugars of the main chain and the lateral branches give them specific structural characteristics and distinct biological actions, according to the species of origin or even in different life phases of the same species. Through ^{1}H and ^{13}C Nuclear Magnetic Resonance (NMR) analysis of *A. subrufescens* water soluble polysaccharides obtained from fruiting bodies a β-(1 → 6)-(1 → 3)-glucan was characterized, and the principal polysaccharide from the mycelium was a β-(1 → 2)-gluco-β-(1 → 3)-mannan. The average molecular weight these polysaccharides was estimated to be 609 and 310 kDa, respectively (Cardozo et al. 2013a).

Many health benefits have been attributed to β-glucans, including immunomodulatory, anti-inflammatory, antitumor, antimutagenic, and antimicrobial activities. Furthermore, these molecules are known to act as biological response modifiers because they interact and modify the immune response of the host (bioregulation) (Silveira et al. 2012). These polysaccharides can also be used for cholesterol management, prevention of cancer, diabetes, hyperlipidemia, arteriosclerosis, and chronic hepatitis (Lindequist et al. 2005).

Antitumor activity has been found in the lipid fractions of *A. subrufescens*, although the main antitumor substances are polysaccharide-enriched extracts and protein–bound polysaccharide complexes (Wisitrassameewong et al. 2012). Other substances as blazein steroid have also been isolated from the species and can induce DNA fragmentations in in vitro culture cell of human lung cancer and stomach cancer cells (Itoh et al. 2008). Agaritine is another compound prone to be used as an antitumor drug since it presented positive effects against in vitro leukemic tumor cells (Endo et al. 2010). Furthermore, polysaccharide fractions of *A. subrufescens* prevent DNA damages induced by known genotoxic chemicals (Angeli et al. 2009).

Agaricus subrufescens can also act in lymphocyte balance through the activation of dendritic cells (Førland et al. 2010). It has also shown to stimulate cytokine production, through induction of interleukin-12 (IL-12), interferon-gamma (IFN-γ), and natural killer (NK) activity, the latter recognized as important for immune surveillance for tumor cells and pathogens (Smyth et al. 2002; Yokoyama and Scalzo 2002). The protein-bound polysaccharide proteoglucan has been cited as a valuable compound extracted from *A. subrufescens* that increases defense against invasive organisms of both prokaryotic and viral origins. Therefore, it has been proposed as an alternative to the use of regular antibiotics (Wisitrassameewong et al. 2012).

In conclusion, there are plenty of reports indicating the positive effects brought by the structural and biochemical composition of *A. subrufescens*, which has triggered several studies devoted to decipher its properties as well as to master the conditions for proper cultivation and biomass recovery. This chapter will scratch the surface of

some of these approaches, bringing further information into the realm of *A. subrufescens* cultivation and the biotechnological potential applications of its products for dermatological purposes.

2 Laboratory Fungal Cultivation and Scale-Up

2.1 Biomass Production

The cultivation of basidiomycetes with the objective of producing biomass and metabolites for medicinal and cosmetic applications is difficult on a laboratory scale, being a much greater challenge when aiming at scaling. Filamentous fungi quickly adjust their metabolism to environmental changes, which requires strict process control for significant and reproducible results (Zhong and Tang 2004; Vrabl et al. 2019). Considering that the most suitable process for this control is through submerged cultivation in bioreactors, two particularities are responsible for most of the challenges, and both are related to the nature of these fungi. Because their cells are much larger than those of prokaryotes and also because they develop in a filamentous manner, the biomass doubling time is more than an order of magnitude greater than that of potentially contaminating bacteria. Additionally, because the cellular development is on a mycelial form, variations occur mainly in the macromorphology of these fungi, with important consequences for the development of the production process. Cells that undergo morphological transformations are more likely to undergo metabolic changes as well (Camelini et al. 2012; Musoni et al. 2015; Berovic and Podgornik 2016).

The fragmentation of biomass in blenders to form suspensions of small mycelium propagules to be used as inoculants (Rossi et al. 2017a), is an approach little explored, but it has important consequences for providing conditions suitable for the physiology of fungal growth, fundamental to the scaling process in bioreactors and product quality. This is due, in essence, to the nature of these fungi that sporulate only sexually through fruiting bodies, and the fact that these specialized structures do not form in liquid culture flasks or in submerged culture bioreactors. The fragmentation of biomass adds potentially harmful steps to the process, such as the risk of contamination by bacteria and fungi that produce asexual spores, such as conidia. Another problem associated to fragmentation is a possible decrease or even loss of cell viability (Rossi et al. 2017a, b), which is suspected to be the reason why researchers do not use this important step in the process (Lee et al. 2004; Hamedi et al. 2007; Souza Filho et al. 2017).

Submerged cultivation in a bioreactor is a special way of standardizing a biotechnological product (Zhong and Tang 2004). In addition to being faster, it is possible to maintain conditions much more stable than in other types of cultivation, such as those carried out on solid substrate, for example. In this format it is possible to maintain the level of oxygen above critical concentrations, temperature, pH, and controlled luminosity, in addition to control nutrient input in proportions and concentrations suitable for primary or secondary metabolism (Musoni et al. 2015;

Rossi et al. 2016). Added to the difficulty of controlling the main process variables in solid-state systems, their solid matrix makes it difficult to separate and purify the product. All possible means in submerged cultivation, and their standardization, generate more reproducibility, contributing to keep the fungus' metabolic processes in a stable and efficient way (Zhong and Tang 2004), resulting in products with a more uniform composition, increasing the guarantees of their functional properties.

With the exception of glycogen, which is a reserve polysaccharide, all other polysaccharides are basically structural components of the fungal cell wall (internal polysaccharides), although several species also produce soluble exopolysaccharides (EPS). Both are classified as secondary metabolites (Ohno 2007), although internal polysaccharides are related to primary metabolism, that is, it is intended to growth, development and reproduction of the organism. Thus, their production in bioreactors is equivalent to the production of biomass, and the efforts to produce polysaccharides must be directed to promote a high specific growth rate of fungi and, consequently, high productivity.

Because the metabolism for obtaining energy in basidiomycetes is exclusively respiratory, the substrate to biomass conversion factors ($Y_{X/S} = dX/dS$) are elevated in submerged cultures, generally above 40%, mainly when conducted in bioreactors (Lee et al. 1999, 2004; Hamedi et al. 2007). This characteristic is very positive, considering that, on the contrary, the yields are very low in anaerobic processes. However, as the respiratory metabolic pathways are long and complex, involving a complex set of enzymes, the specific growth rates are much lower than those achieved in anaerobic or fermentative processes. This characteristic makes the production processes of these fungi long and at high risk, considering that a longer cultivation time represents a greater potential for contamination. Although this was not the purpose of Lee et al. (1999), the cultivation strategy starting at low pH, and after a few days increasing to higher values, is also an efficient way to avoid bacterial contamination. Regarding many studies and discussions on the effects of pH and temperature on the cultivation of basidiomycetes, when the objective is the production of biomass and polysaccharides, these variables must simply be controlled to their optimal values. It is the few variables, on the contrary, for example of dissolved oxygen (DO), that are possible to have an efficient control.

A characteristic inherent to the submerged cultivation of basidiomycetes, like many other filamentous fungi, is the growth morphology. This morphology is dependent on several process conditions, such as the forms and intensities of agitation and aeration, pH, among others. The mycelium can develop freely, or in the form of more or less dense clusters, generally referred as pellets (Zhong and Tang 2004). The free or dispersed form results in more viscous broths, with a rheological behavior of non-Newtonian fluids, while pellets form less viscous suspensions (Gibbs et al. 2000). Higher viscosities make it extremely difficult to transfer oxygen in the bioreactors.

In the submerged culture in which pellet formation occurs, the most common morphology in pneumatic bioreactors (Fig. 1a and b), several implications can interfere with mass transfer phenomena. The pellets increase in size over the time of cultivation through the folding of the external hyphae, promoted by the swirling

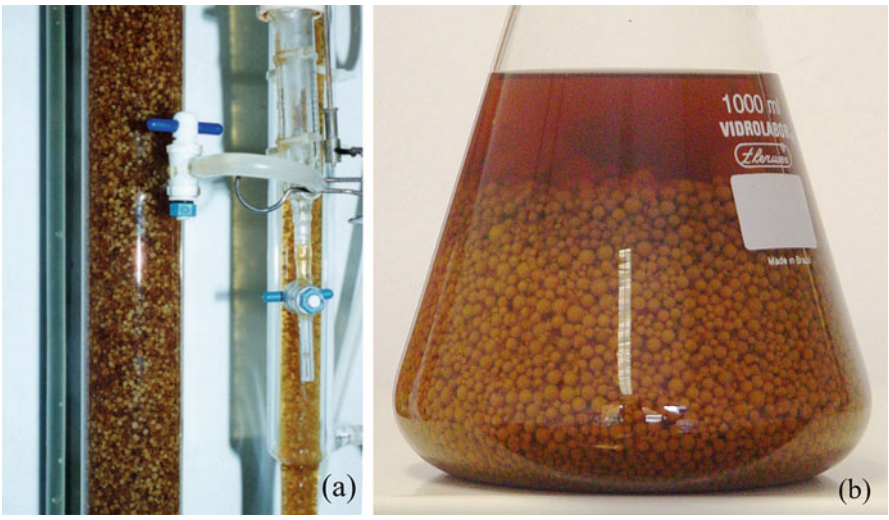


Fig. 1 (**a**) Appearance of basidiomycete mycelium pellets grown in a bench-top airlift bioreactor. (**b**) Similar morphology occurs in cultures conducted in flasks that received compressed air injection

motion of the fluid in the bioreactor (Rossi et al. 2002). Thus, over time, larger pellets occupy more space within the bioreactor, causing an increase in the rate of collisions between them, leading to a progressive compaction of the pellets. This compaction has consequences for mass transfer and cell viability. Compressed pellets are denser and remain longer at the bottom of the bioreactor, decreasing homogeneity. It is also more difficult to transfer mass to the hyphae inside the pellets, such as oxygen and other nutrients. In this situation critical conditions can be established, with loss of cell viability (Fig. 2a), production of secondary metabolites and possibly also molecular variations in the composition of the polysaccharides, which may alter the desired bioactivity (Rossi et al. 2002, 2017b; Zhong and Tang 2004). Even the transfer of oxygen from the air bubbles to the culture medium is reduced with the increase in size of the pellets since they occupy space in the fluid differently than the dispersed mycelium, reducing the space for the movement of air bubbles. This reduction in space promotes greater coalescence of bubbles, decreasing the surface area for oxygen transfer and, in addition, larger air bubbles leave the system more quickly, without the proper exchange of gases. This effect is more significant in pneumatic bioreactors (Rossi et al. 2016), and may anticipate the change in the bubble regime for lower air flows than predicted. All of this impairs the transfer of oxygen, and, in addition to the internal conditions in the pellets, critical conditions that limit growth and induce unwanted metabolic pathways can easily be achieved.

As oxygen is the much less soluble component of all nutrients necessary for the development of microorganisms, and physiologically the most important (Gibbs et al. 2000), its transfer to the culture medium becomes the focus of attention for the choice or development of bioreactors (Zhong and Tang 2004). The issue is

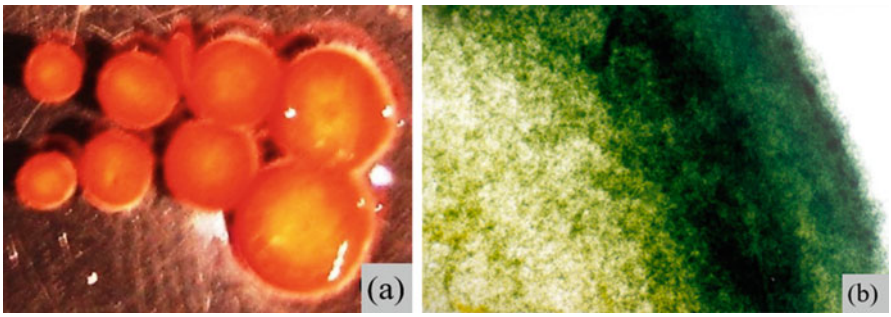


Fig. 2 (**a**) Viability assay using a vital dye (TTC) that shows viable hyphae intensely red. Biomass pellets of a basidiomycete fungus with diameters from 1.6 to 4 mm. From left to right pellets sampled at 4, 7, 9, and 11 days of cultivation in an airlift bioreactor. Light colored in central regions indicate lower metabolic activity. (**b**) Microtome section of a 4 mm pellet mycelium of a basidiomycete fungus cultured in an airlift bioreactor showing three different zones of hyphae compaction. (Images from Rossi (2006) with authorization)

investigated through the volumetric oxygen transfer coefficient (k_La), because improving oxygen transfer in a bioreactor is equivalent to improving k_La. The design (type and geometry of the bioreactor), the system (fluid properties), and the operation (gas and liquid velocity) must be studied in order to optimize the k_La, with a minimum energy requirement. The maintenance of k_La values, predetermined as adequate in bench-top bioreactors, is also one of the main criteria used in the scale up of bioprocesses (Musoni et al. 2015).

The difficulty to manage the mass transfer, resulting from the formation of large and compacted pellets (Fig. 2b), can be overcome by planning the process properly. With more diluted culture media (20 g/L of carbon source, for example), the concentration of biomass is limited to lower values (<10 g/L), allowing the bioreactor to be operated at higher DO concentrations. In addition, using higher concentrations of inoculum and also adequately dispersed in small propagules, it is possible to perform the cultivation with the formation of smaller and more fluffy pellets, with less resistance to mass transfer and consequently greater growth rate (Zhong and Tang 2004). Blender fragmentation for 20 s, which promotes a good splintering of the fungal mycelium, and the use of initial concentrations of inoculum greater than 250 mg/L are recommended for a pneumatic airlift bioreactor. Fragmentation in blenders at 3600 rpm is not detrimental to the viability of the fungi, and times longer than 20 s, if necessary, can be used without loss of viability. In this type of disperser, small fragments of mycelium are no longer reached by the cutting blades when a critical size is established. For other types of homogenizers, care with the fragmentation time may be necessary to avoid pulverizing the inoculums (Rossi et al. 2016, 2017b).

Damage to hyphae by fragmentation in blenders during inoculum preparation is not a problem. When observing the fungal biomass fragments under a microscope, it is noticed that the damage is almost imperceptible, and it is possible to verify high viability when the fragmented mycelia is inoculated into Petri dish culture (Rossi

et al. 2017a). Basidiomycete hyphae are septated, so that the leakage of cytoplasmic material in fragmented hyphae is minimized. However, the fragmentation of the mycelium may, for certain species, induce an indirect loss of viability, justifying some fears related to fragmentation, and avoiding its application in studies of fungal cultivation (Lee et al. 2004; Hamedi et al. 2007). Fragmentation, in fact, releases metabolites and other substances from the cytoplasm to the saline solution (or buffer used in fragmentation), which were previously, at least in some way, under the control of the fungus. These released metabolites can undergo secondary reactions, such as the oxidation of lipids, which becomes toxic to the fungus. Viability losses occurring within a few hours after fragmentation have been observed in basidiomycete fungi (Rossi 2006). This issue is not yet a clarified topic, but a simple way to resolve any loss of viability due to fragmentation is through the use of activated charcoal (0.2%, e.g.) after fragmentation (Silveira et al. 2012; Rossi et al. 2017a). Charcoal can also be added to the culture medium for storage, as it promotes a longer useful life, increasing maintenance intervals (Camelini et al. 2012). However, it is not indicated for a bioreactor, as it is not a selective adsorbent and can cause an imbalance in the composition of micronutrients and limitations to growth. In addition, charcoal turns the culture medium dark, making direct observation difficult, and it also becomes an obstacle in the separation of products.

2.2 Culture Media and Inoculation

The composition of the cultivation substrate differs between authors and this is quite understandable if one considers the different species and objectives of each study (Berovic and Podgornik 2016). Basidiomycetes notably use various sources of carbon and nitrogen, and studies to optimize the composition of the culture media have been conducted for submerged cultivation (Lee et al. 2004, Hamedi et al. 2007). Although these two examples of studies show different results, possibly because different species of fungi were used, the use of simple and cheaper sources is the right choice (Mahapatra and Banerjee 2013). Basidiomycetes tolerate high concentrations of sugars, and glucose is the simplest and most suitable source of carbon and energy to use. Peptones and plant extracts are important to provide nutrients necessary for the biosynthesis pathways, since essential amino acids are not produced by inorganic sources of nitrogen (Berovic and Podgornik 2016), but can be used only in moderate concentrations (less than 0.5%) for cost reasons. Inorganic nitrogen (N) sources, such as ammonium phosphate or ammonium nitrate, are also suitable (Mahapatra and Banerjee 2013), complementing the culture medium with the addition of micronutrients.

Relatively simple media such as classic Melin-Norkrans Medium (MNM) or Pridham-Gottlieb modified by Kuek medium (PGKM) are examples of media with the appropriate substrates for the production of basidiomycete biomass. Filamentous fungi, unlike bacteria and even unicellular fungi such as yeasts, have less need for nitrogen. The ratio between carbon and nitrogen in the average composition of filamentous fungi biomass is 6:1 (Rossi and Oliveira 2011). Then, considering a

feasible 45% conversion rate ($Y_{X/S} = 0.45$), a culture medium with 20 g/L of glucose allows the production of 9.0 g/L of biomass (dry basis), requiring approximately 1.5 g/L of the N source. This balance is important to promote the complete use of macronutrients in the medium with maximum conversion into biomass. The MNM was not exactly designed for productions in bioreactors, so it is necessary to supplement it with N up to the recommended ratio of 6:1 (C:N). It is important to use the carbon source as a limitation of cultivation, since excess carbon causes other essential nutrients to be depleted in advance, leading to an unrecognized change in the dynamics of fungal growth and its physiology (Vrabl et al. 2019), being able to de-characterize the desired product(s).

For the production of fungal polysaccharides for use in humans, it is not appropriate to use residual substrates, such as agro-industrial residues. The residues are not standardized and compromise the purity of the final products. Sugar-based culture media are inexpensive means for the purposes of scaling production, especially considering the high added value of polysaccharides for medicinal and cosmetic purpose. Simple sugars are highly soluble, promoting easy access and direct use in the metabolic pathways, also allowing a perfect separation of biomass for later extraction of polysaccharides, quite contrary to the difficulty of extraction and purification that occurs in solid matrix production processes.

A common fact observed in studies on the production of basidiomycetes for the obtainment of biomass and EPS is related to the preparation of the inoculum (Lee et al. 2004; Hamedi et al. 2007). After a preculture in agitated flasks, where fungi are inoculated with mycelium-agar discs obtained from plate cultures, the biomass obtained in the form of pellets is used to inoculate bioreactors from a previous mycelium fragmentation in blenders. It is understood that the homogenization of the biomass used as an inoculum is important for the process, as previously discussed, and also facilitates its appropriate quantification. Inoculum size is believed to be important for both biomass and polysaccharide production (Hamedi et al. 2007; Berovic and Podgornik 2016). However, the dosage of the inoculum from a pelleted biomass, that is, not homogenized, based on the relationship between the volume of the inoculum and the volume of the medium in the bioreactor is difficult to apply, and there may be a lot of variation in the concentration. The correct dimensioning of the inoculum must be done by concentration and not by relation between volumes.

The initial concentration of biomass in a given cultivation should not interfere with the specific growth rate (μX), as this instantaneous rate, by definition, considers the cell concentration at that time. The size of the inoculum should have an effect only on the duration of the cultivation, as observed in single-celled microorganisms, such as bacteria and yeasts. In this scenario, *quorum sensing* phenomena are not being considered, since in bioreactors a small size of the inoculum still contains a very large concentration of cells, far beyond what would be found in a natural environment. However, Rossi et al. (2017b) demonstrated that the size of the inoculum interferes with the specific growth rate of mycelial cells in a bioreactor. This is the result of limitations in the transfer of oxygen that can occur specifically in large and dense pellet morphologies. These, in turn, are the products of the size of the

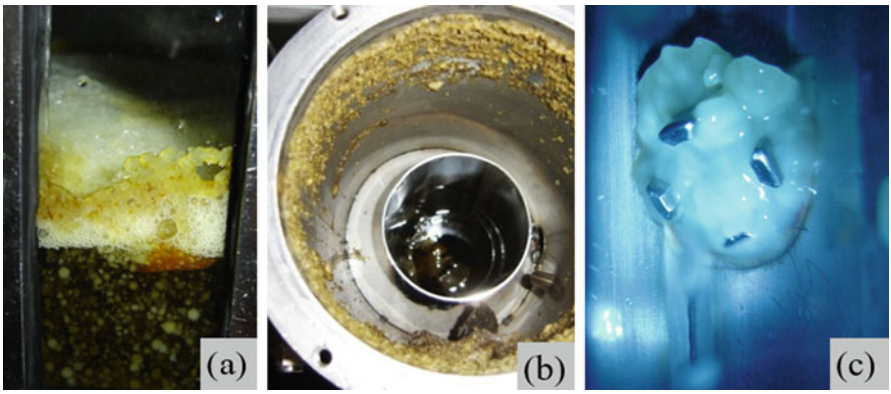


Fig. 3 (**a**) Foaming in the gas separator. (**b**) Basidiomycete mycelium adhered to the gas separator and (**c**) completely obstructing the pH sensor in an airlift bioreactor after a 7-day batch cultivation

inoculum and the size of its propagules. The lack of fragmentation of the biomass used in the studies by Lee et al. (2004) can explain the production performance in biomass and lower EPS of an airlift bioreactor in relation to the performance in a stirred tank bioreactor (STR). In the latter, mechanical agitation causes the dispersion of the mycelium, eliminating limitations in the transfer of oxygen to the cells On the contrary, in the airlift bioreactor, limiting conditions were established in half the cultivation time, providing less cell yield, since the entire carbon source was totally consumed (Lee et al. 2004).

An adequate dimensioning of both the concentration of nutrients and biomass (inoculum), and its fragmentation into small propagules, allows that concentrations of DO above critical can be maintained throughout the time of cultivation (Rossi et al. 2016). This can allow, in pneumatic bioreactors with recirculation, yields and growth rates equal and even higher than the STR, since the shear damages are less intense in airlifts, with significantly lower implantation, operation and maintenance costs (Gibbs et al. 2000; Garcia-Ochoa and Gomez 2009; Berovic and Podgornik 2016). In addition, less shear can also be more favorable to the production of EPS (Zhong and Tang 2004).

One of the common problems in submerged cultivation, especially in systems with mechanical agitation, is the formation of foam (Fig. 3a). Foam drags culture medium and biomass to the outlet line and gases and compromises the entire process. Thus, the use of anti-foaming agents is almost indispensable. Despite the availability of many additives for this purpose, the use of vegetable oils can be considered, which besides being inexpensive, can stimulate fungal growth (Lee et al. 1999; Yang et al. 2000).

2.3 Bioreactors for Submerged Cultivation of Basidiomycetes

It is not intended here to discuss the different types of bioreactors for submerged cultivation, which can be used to produce biomass and fungal polysaccharides. In

general, both agitated (STR) and pneumatic bioreactors with liquid recirculation (airlift) can be used (Zhong and Tang 2004; Garcia-Ochoa and Gomez 2009; Berovic and Podgornik 2016). Due to the complexity of the STR and greater difficulty in relation to the tightness of dynamic seals and also greater difficulties with scale-up, pneumatic bioreactors may be more convenient. If the production project starts from the installation of the plant, pneumatic bioreactors must be considered carefully. They are simpler and, therefore, cheaper to build, and also simpler to operate. Another important aspect is the fact that it is possible to minimize the damage to the fungi hyphae that occurs in STR through the agitation system, since pneumatic systems use only air to aerate and agitate the culture medium, dispensing turbines (Zhong and Tang 2004; Garcia-Ochoa and Gomez 2009). It is important to clarify that a momentary damage to the hyphae during the fragmentation stage, previously presented as an important stage of the preparation of the inoculum, differs from the fragmentation resulting from the shear stress imposed constantly by the oxygen transfer system in a mechanical agitation system. Obviously, a STR can be set to operate with milder shear forces, but this greatly impairs oxygen transfer. The shear forces in these bioreactors are fundamental for breaking air bubbles and increasing the mass transfer area and, consequently, k_La. In addition, the height to diameter ratio (usually 2) causes air bubbles to be retained in contact with the liquid for longer periods of time, which generally impairs the cultivation in STR. In airlift bioreactors the design is accommodating, that is, very large proportions of height/diameter, up to 10, or greater, diffuse the problem related to air bubble retainment in the system (Rossi et al. 2016).

In airlift bioreactors, the absence of moving mechanical parts for agitation also reduces the risk of contamination, as it facilitates cleaning and sterilization. The gas injection serves to agitate and aerate, eliminating the energy expense for this, promoting an increase in the mass and heat transfer capacity, making it attractive to use in aerobic processes. Another advantage of airlifts over other bioreactors, related to less shear force and, consequently, less damage to cells, is the fact that shear field is also more homogeneous, being relatively constant throughout the bioreactor, presenting better defined flow patterns. Even with random movements overlapping, there is total directionality of the flow of the liquid. A less turbulent flow appears to have a positive effect on the production of shear-sensitive cells. Even in highly viscous media, the use of airlift bioreactors can be advantageous (Rossi et al. 2016, 2017b).

Although the presented advantages, the application of airlift bioreactors on an industrial production scale is more limited in relation to STR. Airlifts are less flexible for process changes than STR, since geometric parameters are determined for a given process during the project; the speed of the gas flow is, in principle, the only parameter that can be adjusted during operation. Therefore, airlifts are less adaptable to other processes with a very different need for mixing and agitation, gas distribution and mass transfer characteristics, compared to STR, which have independent controls for aeration and agitation. However, in addition to the greater complexity of construction and operation of the STRs already mentioned, one of the major challenges is to maintain production efficiency in the scale-up, which results in

production and yields lower than those achieved on a bench scale (Zhong and Tang 2004). This is one of the most important advantages that pneumatic bioreactors have considering recirculation (airlift) compared to STR.

2.4 Operation of Bioreactors for the Cultivation of Basidiomycetes

Studies on the production of basidiomycetes are carried out in batch-operated cultures, because it is extremely difficult to operate a continuous system for the cultivation of these fungi. In addition to the classic problems, related to the initial investment and the possibility of selecting unwanted mutants, there are also the problems of contamination and the difficulty of reaching a steady state due to the low growth speed and the small volume of the bioreactors. Another problem, possibly one of the biggest, is the growth of mycelium trapped in the walls (Fig. 3b), supply, and outlet pipes of the bioreactor (Rossi 2006; Musoni et al. 2015). The latter can be observed when the cultivation time is extended for several days and often also represents a problem in batch cultivation.

The location points where the biomass attaches to the airlift bioreactor are the air distributor, the weld pores or rough edge, places where air bubbles are trapped, sight glass, gas separators and sensors (Fig. 3c), places where the speed of flow is decreased (at the connections), and at the base of the gas separator, where the absence of bubbles makes the flow more laminar. In airlift bioreactors with internal circulation, this problem would possibly be aggravated by obstacles to flow, such as the supports that hold the riser in position, or by the holes in the concentric tube, when this model of riser is used with the objective of increasing the mass transfer (Fu et al. 2003), or even when a device is inserted in the riser for that same purpose (Nikakhtari and Hill 2005).

Despite the problems pointed out, it would be important to use a system operating in a fed-batch mode for cultures of basidiomycete fungi. It could be used to minimize the effects of metabolism control, to prevent inhibition by substrate or precursors, to minimize the formation of toxic metabolism products, and for kinetic studies, where maximum speeds could be found in these circumstances. However, for airlift bioreactors with external circulation, fed-batch can be difficult to apply, since almost the entire volume must be filled at the beginning of the cultivation to create the conditions for the circulation of the liquid. If the volume fed is small enough to vary only at the top of the bioreactor (allowing circulation), such as the feeding of precursors or inhibitors, or concentrated nutrients, this type of operation would also be possible in an airlift bioreactor with external circulation. For airlift bioreactors with internal circulation, using the perforated concentric tube (Fu et al. 2003), it is possible to operate in a fed-batch mode starting from small initial volumes, since the perforated tube allows the circulation of the liquid in any volume. The fed-batch operation system, added to the use of diluted culture media (Rossi et al. 2017b), can be an alternative to obtain greater productivity and viability of the mycelium in

cultivation of basidiomycete fungi (Zhong and Tang 2004), especially those sensitive to the products of their own metabolism.

Although k_La is an important process parameter, it should not be controlled, because in addition to varying even for pre-set aeration and agitation conditions, a fixed k_La value may not meet the demands for a batch process. The correct way is to keep the DO concentration value above the critical value, so that the agitation and/or aeration is automatically changed depending on the biological demand (for this, a reliable DO sensor is required). In the cultivation of filamentous fungi, a certain DO value over critical can become critical over time with the increase in size and density of the pellets. Thus, to maintain non-limiting process conditions in relation to oxygen, it is necessary to know its transfer and consumption kinetics (Rossi et al. 2017b), which can be obtained through dynamic assays (Garcia-Ochoa and Gomez 2009).

Considering the advantages of airlift bioreactors, especially with scale-up reproducibility, Rossi et al. (2016) present the geometric relations for the construction of an airlift bioreactor with external circulation, where hydrodynamics and oxygen transfer coefficient (k_La) are characterized. Additionally, a correlation obtained experimentally between k_La and the superficial gas velocity is presented, which can be used for scale-up. This correlation serves to establish the air flow for operation in larger bioreactors, necessary to reach the k_La values established in the bench. Rossi et al. (2017b) also present a proposal for scheduling the process considering the inline fragmentation of the mycelium pellets between the process steps. The mycelium produced in flasks, after fragmentation in blenders, is used to inoculate a small bioreactor, and this, in turn, is used to inoculate a larger bioreactor, and so on. Each stage requires prior fragmentation of the pellets to allow for a large number of propagules that form a large number of small new pellets, avoiding oxygen limitations throughout the process and maintaining high and constant specific growth rates (exponential growth).

2.5 Mechanical Dispersion of Pellets During Cultivation

One way to improve mass transfer during the cultivation of basidiomycete fungi is the in-line dispersion of hyphae through the fragmentation of pellets (Rossi et al. 2017b). This could be done using a disperser coupled to the bioreactor, which would be used only at a certain time during cultivation. This equipment could also be installed only at the time of use, through aseptic ports, allowing a disperser tool to be used for several bioreactors. There are models of this equipment in various sizes available on the market, such as the one in Fig. 4 developed by IKA® Works Inc. for laboratory use, or Ystral GmbH for industrial use. Aside from being compact, these devices have a high processing capacity. In addition to improving mass transfer, this strategy can be a safe and quick way to start a new batch by directly inoculating another bioreactor (Fig. 5a). Choosing the right position for the doors would allow

Fig. 4 IKA® particle size reduction. In-line disperser with a DK 50.11 flow chamber (https://www.artisantg.com/Scientific/62285-1/IKA-50-Basic-Inline-ULTRA-TURRAX-Crusher-Disperser)

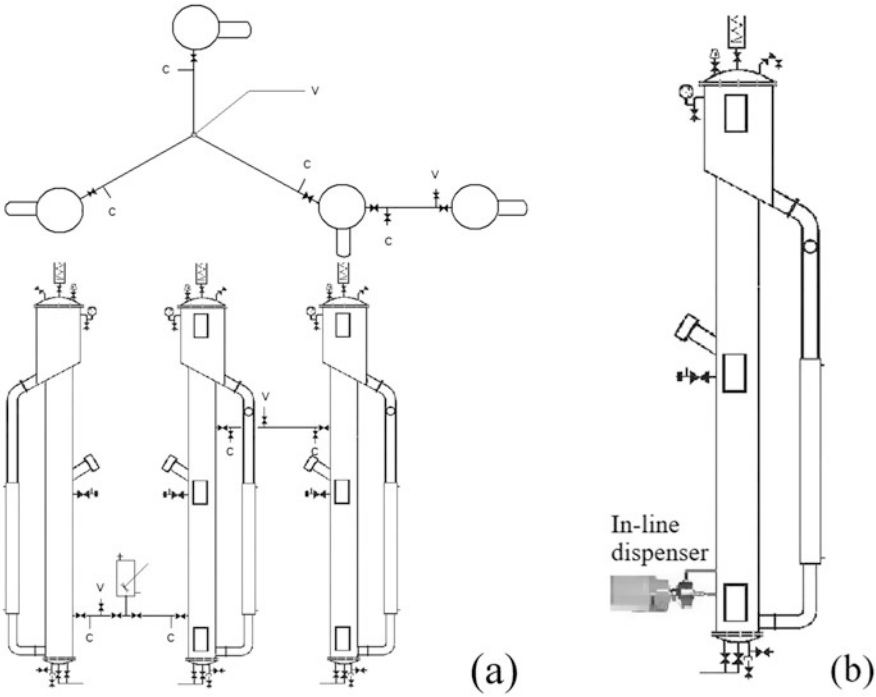


Fig. 5 (**a**) Example of a configuration (using four bioreactors) to produce biomass and EPS from basidiomycete fungi. (**b**) The use of an in-line fragmentation system is a strategy to improve growth kinetics, in addition to allowing a safe and fast way to start new cultivation through direct inoculation. The volume of the bioreactors can be different, as in a production scale-up process. (Images from Rossi (2006) with authorization)

operations to be carried out only with the use of gravity (Fig. 5b), eliminating the need for pumps (Rossi 2006).

3 Bioactive Polysaccharides

3.1 Dermatological Activities

Fungal polysaccharides have many biological activities in skin cells that can be developed into dermatological products. The main skin cells are keratinocytes, fibroblasts, and melanocytes with their various immune receptors, among others, in addition to those known immune cells that are also present in the epidermis and dermis (Clark et al. 2005; Reemann et al. 2014; Hesselink et al. 2017). Beyond the skin cells, the epidermis has specialized dendritic cells (DCs) known as Langerhans cells and CD8 cytotoxic T cells. The dermis is also home to diverse specialized immune cells including antigen-presenting dermal DCs, T cells, B cells, and NK cells, as well as mast cells, monocytes, and macrophages (Clark et al. 2005; Pasparakis et al. 2014; Egbuniwe et al. 2015).

There are some receptors already identified for β-glucans on the immune cells of animals. Among them complement receptor 3 (CR3), dectin-1 and lactosilceramide found mainly in leukocytes, macrophages, monocytes, neutrophils, and NK cells, as well as in non-immune cells such as endothelial and fibroblast cells. The CR3, for example, is responsible for various in vitro and in vivo biological activities, stimulating the secretion of cytokines such as tumor necrosis factor (TNF-α), IFN-α, IFN-γ, and IL-6 in NK cells, known as modulators of vessel formation (Brown and Gordon 2003; Bamias and Dimopoulos 2003).

In addition, β-glucans have effects on human keratinocytes by the stimulation of interleukin production (IL-8, IL-6, and IL-1α), especially under simulated conditions of skin damage and infection. Dectin-1 was also detected on these keratinocytes and may have an important role in the cell response to β-glucan. These are important for the host as first line defense against fungal pathogens and augment the immune system response upon disruption of the epidermal horny cell layer (Hau et al. 2011). Fibroblasts also have receptors for β-glucans on their surface and these enhance the dermal fibroblast migration directly, stimulating the synthesis of collagen, further influencing macrophage activation to release more TNF-α cytokine, indirectly enhancing wound healing (Son et al. 2007).

On the other hand, β-glucans from *Ganoderma lucidum* showed immunomodulating functions and promoted hematopoietic stem/progenitor cell homing for tissue repair, down regulating the expression of both angiogenic vascular endothelial growth factor receptor-3 (VEGFR-3) and endoglin (CD105) molecules in the presence of angiogenic factors. This modulation is very important and contributes to the normal wound healing vasculogenesis, which is regulated by well-balanced angiogenic and angiostatic factors (Chen et al. 2010).

The vascular endothelial growth factor (VEGF) expressed by many cell types and essential factors for normal and aberrant angiogenesis, can also participate in

physiological and pathological conditions in the epidermis as an important keratinocyte-derived factor, principally with receptors for VEGF-A type. This factor acts in both autocrine and paracrine manner, affecting epidermal cells and dermal tissues in wound healing, psoriasis, and other states of increased skin angiogenesis. These factors respond also to ultraviolet (UV) irradiation. Although keratinocytes account for the main epidermal protection, melanocytes situated at the basal layer of the epidermis are responsible for melanin synthesis and skin color (melanogenesis). On the other hand, the VEGF extends beyond the classical roles in blood vessel formation and their receptors (VEGFR-1 and VEGFR-2) have been found in the melanocytes, functionally involved in melanogenesis. Both VEGFRs can be induced by UVB-irradiation besides the VEGFs modulation mode, demonstrating another important role of these factors as potent melanogenic activators. They can also decrease tyrosinase activity and melanin production via inhibiting activation of VEGFRs by some bioactive substances (Zhu et al. 2020).

Saad et al. (2018) investigated the anti-melanogenesis and anti-inflammatory activity of various fungal polysaccharidic extracts for dermatological purpose. The capacity to reduce the tyrosinase activity is widely investigated to indicate target anti-melanogenesis molecules for the treatment of hyperpigmentation, such as melasma, age spots, and freckles. The authors found that the biggest decrease of this intracellular enzyme activity in melanoma cells was obtained with the polysaccharide extract from brown *A. bisporus*. The extract was also effective in reducing melanin production with no toxic effects. The inflammatory activity that can be caused by sun UVB-irradiation has an important negative effect on skin appearance, increasing age spots. Polysaccharides from the *A. bisporus* extract also reduce the expression of pro-inflammatory mediators, as nitric oxide (NO) and TNF-α, when macrophages were stimulated by endotoxin (lipopolysaccharide, LPS), i.e., with a nitric oxide synthase inducible (iNOS) highly expressed.

Other interesting use of glucans is in association with chitin, which together can be used as nano/microfibrous fungal-scaffold for tissue engineering and regenerative medicine applications. The treatment of fungal-scaffolds using β-mercaptoethanol improved the in vitro hemo- and bio-compatibility with respect to the adhesion and proliferation of human keratinocytes. This raw material may be a sustainable alternative to synthetic cell scaffolds for tissue engineering, due to similarities with extracellular matrix and their intricate network of collagens, proteoglycans/glycosaminoglycans, elastin, fibronectin, laminins, and other glycoproteins for cells support (Narayanan et al. 2020).

3.2 Cosmeceuticals

The term “cosmeceutical” was created because of the necessity of an interphase between cosmetics and pharmaceuticals, since it was demonstrated that a product applied to the skin produces changes, originating a third category of products described as cosmeceuticals. This term was explained for the first time by Albert

Kligman in 1984 at the National Scientific Meeting of the Society of Cosmetic Chemists, gleaming products formulated with bioactive ingredients, topically applied on the skin to produce a medical drug-like benefit, while also improving skin structure and function (Kligman 2005).

There are numerous products from fungi that are being exploited as cosmeceuticals or also as nutricosmetics (oral supplementation for skin care). Cosmetic companies from Europe, Asia, and America have been using fungal extracts or ingredients in several applications, ranging from exfoliant to anti-inflammatory, stimulating faster skin renewal, increasing skin elasticity, and serving as skin brighteners. The most used fungal extracts in cosmetic products are obtained from the species *L. edodes*, *G. lucidum*, *S. commune*, *Tremella fuciformis*, *Cordyceps sinensis*, *Inonotus obliquus,* and *A. subrufescens* (Hyde et al. 2010; Wu et al. 2016).

β-Glucans are one of the most important substances obtained from fungi in a cosmeceutical products, mostly for nourishing and improving skin appearance. These polysaccharides are also documented as effective agents for treating various dermatological conditions. Applications of β-glucan for skin health promotion are focused on antioxidant, anti-wrinkle, and anti-ultraviolet light activities, wound healing and moisturizing effect, deeply penetrated into the epidermis and dermis through intercellular space. β-Glucans also possess skin regenerative properties, can be involved in revitalizing the immune cells in skin, regenerating collagen-producing cells, strengthening the skin ability to deal with adverse environmental effects and promoting anti-skin-aging effects (Du et al. 2014). For example, a β-(1 → 6)-branched-β-(1 → 3)-glucan from *S. commune*, denominated schizophyllan, has been used as an ingredient of a marketed cosmetic with anti-inflammatory and moisturizing effects (www.macrocare.net/en/products). In addition, β-glucans have antiallergic properties with potential use in prevention of dermatitis and to defend cells against different allergens by modulation of the cutaneous immune system (Jesenak et al. 2014, 2016). Other β-glucans can be found in cosmetic ingredients indicated as anti-aging, anti-redness, anti-wrinkle, and antioxidants (https://cosmetics.specialchem.com/inci/beta-glucan).

There are many requirements to prove that products have cosmeceutical activity. In addition to the characteristics of formulation, such as stability and compatibility, the most importantly feature is the harmless effects upon use, no side effects proven in toxicological studies, and positive effects proven in clinical trials (Wu et al. 2016; Taofiq et al. 2017).

The clinical potential effects of β-glucan 1% essence on skin of 10 young women were analyzed via a non-invasive skin testing instrument and sensorial evaluations. The results, after 28 days of β-glucan administration twice a day on face skin, showed higher average skin moisture content around cheek and lips, reduced skin oil (sebum) content and decreased melanin index. Furthermore, assessment with survey questionnaires of subjects before and after using β-glucan essence indicated a higher frequency of “very good” and “good” for absorption, skin moisture level, refreshment or comfort in use, feel at foundation makeup, compatibility to color makeup, and satisfaction. These results were all positive and did not cause irritation and erythema tested instrumentally by an index (Park et al. 2003).

Glucomannans also have been used as protective agents in cosmetic formulations for reduction of detrimental effects of solar radiation on skin, after exposure to short wavelength solar light and UV irradiation. This protection was verified in an in vitro model of UVB-irradiated human keratinocytes, which revealed a protective effect on the viability of these irradiated cells, connected with the suppression of their apoptosis and repair of DNA lesions. Furthermore, the glucomannans suppressed the gene expression of pro-inflammatory markers that were induced by UVB-irradiation, including down regulation of prostaglandin E2 and interleukin 1α release, more profound 48 h after the treatment. These photoprotective effects were confirmed on the skin of healthy human volunteers. The dermal reaction as a UVB-induced erythema formation was reduced and the enzymatic activity of β-glucocerebrosidase slightly increased, which contributes to repair of the UVB-damaged skin (Ruszova et al. 2008).

To demonstrate the efficacy of the hydrogel formulations with soluble β-(1 → 3), (1 → 6)-glucan and carbopol in different concentrations on wound healing, a model similar to the mechanisms of human wound closure that depends equally on both contraction of wound margins and skin reepithelialization was used in diabetics mice. The treatment with low carbopol (0.25%, w/w) and high β-glucan content (1%, w/w) increased wound reepithelialization in comparison to the formulations with higher (0.5%) levels of carbopol. This treatment also increased wound contractions compared to water treatment on days 20 and 24. Furthermore, the rheological characterization of all formulations confirmed the gels as stable, and indicates that the inclusion of β-glucan increases the hydrogels firmness more than carbopol, contributing to augment the gel strength in a dose-dependent manner. The formulations showed high stability and not deteriorate over 26 weeks, considering its usage in a sprayable device destined for wound treatment, as it was acting as a fluid under higher share stress (spraying) and returning to a gel in the wound (Grip et al. 2017).

That experiment using carbopol as a carrier of β-glucan interfered negatively in the wound heling, so the same scientific group developed a new β-glucan-loaded nanofiber wound dressing by manufacturing a method of needle-free electrospinning, chosen because of the possibility to industrial scale-up. Firstly, they demonstrated that β-glucan and nanofiber excipients (hydroxypropyl methylcellulose, polyethylene oxide and ethanol) did not exhibit any toxicity to keratinocytes, and provide information that this nanofibers would not affect the cell viability during the proliferation phase of wound healing. All nanofiber treatments containing three different doses of β-glucan were better than the negative control (water treatments) and provided improved healing conditions similar to a positive control (since the 8th day of treatment). The nanofibers collaborated for the reduction of the wound area, considered the contraction and reepithelization, even with the lowest β-glucan dose, i.e., of 190 μg/nanofiber (Grip et al. 2018).

Other formulations with an aqueous mixture of soluble β-glucan and polyvinyl alcohol (PVA) were cast into films for wound dressing, varying from 7% to 50% β-glucan concentrations. The hydrophilicity is important for evaluating the wound dressing materials, and in this case the characteristic tests of wetting ratio and water vapor transmission rate increased with the glucan content, close to the appropriate

ranges for maintaining a proper fluid balance on the skin wound, which can facilitate cellular migration and enhance reepithelialization. In addition, the blending of glucan with linear PVA turned the films more flexible and elastic, and when contacting water, the percent release of glucan increased with the glucan content, indicating that glucan was not covalently bonded with PVA. Observing the wound healing of rat skin, the wound contraction ratio was accelerated from 21 to 11 days when treated with PVA/glucan films. This accelerating effect was attributed to the release of glucan applied as wound dressing, without causing skin irritation (Huang and Yang 2008).

An important antioxidant activity focused for skin health promotion was found to the hot water crude polysaccharide extract from fruiting bodies of *G. lucidum* and its partially purified fraction. These polysaccharidic extracts contained principally β-glucans that were effective inhibitors of lipid peroxidation, with an almost twofold free radical blocking than ascorbic acid, which may help the strength in the skin's barrier. In addition, the extracts have a skin-lightening effect as inhibitors of tyrosinase, and for the anti-collagenase and anti-elastase activities to help restore skin elasticity and tensile strength. Moreover, no obvious toxicity was observed in human keratinocytes. These diverse functionalities indicate that β-glucans from *G. lucidum* may be additive in cosmeceutical formulations as anti-aging agents (Kozarski et al. 2019).

The antiallergic activity was described for a cream formulation containing β-glucan isolated from *Pleurotus ostreatus* as a supportive complementary therapy of atopic dermatitis in children and adults during 6-months. This topical treatment was successful to decrease the duration and intensity of atopic dermatitis flares and attenuated the pruritus intensity within a few days of regular application, with good tolerability and low frequency of unwanted adverse effects. In addition, it was observed a reduction of objective scores as an eczema area and severity index on the site of β-glucan cream application (Jesenak et al. 2016). A review published by Zhou et al. (2014) describing Chinese Food and Drug Administration approved fungal glycan-based drugs describe anti-alopecia activity of the pachymaran, a linear β-(1 → 3)-glucan extracted from *Poria cocos* mycelium formulated as injectable with no less than 84% of the polysaccharide. They also describe lentinan, the β-(1 → 3)-(1 → 6)-glucan extracted from *L. edodes*, used in formulations to treat urticaria.

By the other hand, polysaccharides are also part of new generation hair care compounds used to minimize hair breakage or other signs of hair damage, and serve as agents of thickening, film forming, suspending, dispersing, stabilizing, slow releasing, emulsion stabilizers, and rheology modifiers in cosmetic formulations. The nonionic β-glucans obtained from *S. commune* and *Sclerotium* species, respectively called schizophyllan and scleroglucan, have been used as rheology modifiers and stabilizers in cosmetic preparations as they stabilize the oil-in-water emulsions along with surfactants, with high thermal stability and compatibility with thickening agents, and all other ingredients (Sajna et al. 2015).

Many of these properties are involved in the conditioning and moisturizing capacity of polysaccharides for skin and hair care, rehydrating and preventing water loss. In addition, some patents describe the important properties of β-glucan from the mycelium of *Phellinus linteus* to promote hair regeneration and growth by

activation of hair follicles. Hair regrowth formulations are used to stimulate the immunity of hair follicles in a long period of rest due to hair loss in the hair cycle, and promotion of blood circulation. That is, it activates hair follicles by immunomodulatory effects on the scalp, and stimulates hair follicle cell division by simultaneously supplying nutrients to activate hair follicles. β-Glucans extracted from basidiomycetes generally are highly water soluble and have good absorption ability to the scalp as well as better hair follicle activating effect. These great properties are summed to the safety for human body and do absent side effects in consideration of various problems of the commonly marketable hair regrowth agents (Sang-Muk 2000; Bong 2006).

3.3 Dermatological Use of *Agaricus subrufescens* Polysaccharides

Several trademarks, patents and scientific publications describe the properties and the dermatological use of *A. subrufescens* polysaccharides with antiallergic, wound healing, antiherpetic, antitumoral on skin papillomas, protective activities of damage caused to the skin by pollution and radiation, and also in the whitening of stains and signs (Uchiyama and Haramaki 2002; Kozuka et al. 2005; Cardozo et al. 2013b). Another important activity is due to the high hygroscopicity of these biopolymers that could be used as humectants in moisturizing dermal formulations, and also due to their high solubility in water without forming gel or hydrocolloids (Hyde et al. 2010; Cunha et al. 2019).

Various polysaccharide fractions obtained from *A. subrufescens* mycelia showed great in vitro antioxidant ability. The most active antioxidant fraction extracted by using hot water (100 °C) and ethanol precipitation resulted in a high carbohydrate fraction mainly composed by 757 kDa polysaccharides. This polysaccharidic fraction at a dosage of 5 mg/mL showed excellent free radical scavenging capability compared to the reference control of citric acid (35%). In terms of the absolute chelating power, it also had an excellent activity, principally explained by the mole numbers of ferrous ions chelated that were inversely related with the mean molecular mass of the polysaccharides in each fraction. In addition, the antioxidant activities had been associated with the solubility of the polysaccharidic fractions (Ker et al. 2005).

The preclinical in vitro and in vivo efficacy and safety of *A. subrufescens* polysaccharides supported the continuity of their clinical evaluation. Our in vitro results have shown that high molar mass fruiting body polysaccharides displayed biological activities with no geno- or cytotoxicity. In fact, we found that *A. subrufescens* fruiting body polysaccharidic extract and isolated β-glucan displayed antigenotoxic activity in assays where HepG2 cell DNA damage was induced by substances such as H_2O_2, bleomycin, or doxorubicin in a concentration dependent manner (Angeli et al. 2009). For this same strain, β-glucans from both fruiting bodies and mycelium had no cytotoxicity to VERO or GMK AH1 cells and no in vitro anticoagulant activity (Cardozo et al. 2013a).

We also found that these β-glucans from fruiting bodies and mycelium activated macrophages in vitro, shown by an increase in the expression of TNF-α and iNOS

mRNAs. In addition, the fruiting body polysaccharide with higher molecular weight (627 KDa) was also able to stimulate B cells, the main effector cell of the humoral immune response (Silveira et al. 2012). B Lymphocytes and macrophages are involved in different physiological and pathological processes, such as tissue remodeling during embryogenesis and repair of lesions (wound healing), removal of senescent or apoptotic cells subsequent to an injury or infection, hematopoiesis and homeostasis, as well as the recognition of neoplastic cells (Hetland et al. 2008). Furthermore, emerging evidence indicates that skin-associated B cells play important roles in skin homeostasis and disease, with both pro-inflammatory and immunoregulatory activities (Egbuniwe et al. 2015).

β-Glucans from *A. subrufescens* also have been used orally to heal wounded skin. The supplementation of animals fed with β-glucans accelerated burn wound repair and decreased a pro-inflammatory interleukin IL-1β production in skin macrophages of burn wound-treated rats. Recovery rate of wound skin increased with increasing doses, indicating that these polysaccharides could also be useful as potential anti-inflammatory agents to promote burned skin healing (Sui et al. 2010).

A polysaccharidic extract from a medicinal fungal (mushroom) blend including *A. subrufescens* also increased the production of four specific cytokines and chemokines that are specifically associated with antiviral activities and cellular recruitment of IFN-γ, monocyte chemoattractant protein-1 and macrophage inflammatory proteins 1α and 1β (Davis et al. 2020). Even though the antiviral activities of polysaccharides are related mainly with an immunological activation, it is possible that structural modifications structure, such as sulfation, increases negatively charged (polyanionic) moieties enhancing the direct antiviral action by blocking the early stages of virus infection, cellular attachment and penetration (He et al. 2020). We have previously shown that a sulfated derivative, denominated MI-S (Mw = 86 kDa), obtained by chemical sulfation of the β-(1 → 2)-gluco-β-(1 → 3)-mannan (Mw = 310 kDa) isolated from *A. subrufescens* mycelium, displayed in vitro anti-herpes simplex virus (HSV) activity by inhibiting virus-cell attachment and entry, as well as virus cell-to-cell spread (Cardozo et al. 2011).

We also assessed the in vivo antiviral activity of MI-S in murine cutaneous and genital HSV infection models. Although cutaneous application of MI-S did not reduce HSV-1 (strain KOS) skin lesions, cutaneously infected mice treated orally with MI-S had significantly reduced disease scores between days 9 and 13 post infection (p.i.), suggesting that healing was accelerated. Topical administration of MI-S in the vaginal mucosa right before (20 min) HSV-2 (strain 333) challenge reduced severity of genital herpes, with significant lower disease scores on days 5 to 9 p.i. and decreased mortality in comparison to the control groups (untreated and vehicle treated). These results show that MI-S may be useful as an oral agent to reduce the severity of cutaneous herpetic lesions and as a topical microbicide to block sexual transmission of HSV-2 genital infections (Cardozo et al. 2013b). A topical application of a cream containing sulfated polysaccharide obtained from the *Spirulina platensis* blue-green microalga was significantly more potent than topical acyclovir cream in preventing reactivation of herpes labialis (HSV-1) episodes after permanent lip makeup procedures in humans (Mader et al. 2016).

Another important activity of *A. subrufescens* β-glucans was demonstrated on in vivo pro-vasculogenic assays. In this experiment, discs containing β-glucan (1 to 20 μg/disc) were implanted in the vesicles (yolk-sac) of healthy chicken embryos. Results showed up to 30% increase in the number of vessels and growth of the embryo bodies (15%) in comparison to the untreated controls, with no evidence of toxicity to the embryos. We hypothesize that the stimulation of both, blood vessel formation and embryo growth, may have occurred in response to an immunomodulatory activity incited by the glucans (Camelini et al. 2005b).

On the other hand, a low molecular weight (48 kDa) polysaccharide isolated from *A. subrufescens* down-regulated VEGF derived from cells surrounding Sarcoma 180 tumors, which play critical roles in tumor angiogenesis and development (Niu et al. 2009). Differently, β-glucans from *G. frondosa* promoted a vessel formation (angiogenesis) by means of the activation of immune cells with increase in TNF-α and VEGF production, resulting in an important antitumoral activity (Matsui et al. 2001). These findings demonstrate that fungal polysaccharides can modulate vascularization processes by different mechanisms depending on the pathological or physiological condition, and also dependent on the polysaccharidic chemical structure. Angiogenesis is essential to several processes such as hair growth, ovulation, ulcer healing, tissue repair, ischemic organ revascularization, and regeneration. Vasculogenesis involves the formation of new blood vessels from those preexisting ones formed during the embryonic period or by recruitment of bone-marrow-derived circulating endothelial progenitor cells throughout the adult life. Both mechanisms of vessel formation as neoangiogenesis and neovascularization may occur in pathophysiological processes, for example, in ischemic organs or solid tumor development (Carmeliet 2003; Zammaretti and Zisch 2005).

All results of in vitro and in vivo assays using *A. subrufescens* polysaccharides showed that these substances had no toxicity, in addition to indicating probable biological activities for clinical and dermatological testing in anti-aging skin care and to prevent hair loss. For these reasons, the clinical evaluation for efficacy and safety was carried out in a project supported by the National Council for Scientific and Technological Development in Brazil (CNPq-Brazil). In this project, cosmetic formulations (facial cream and shampoo) were developed containing the polysaccharides. Both products showed skin compatibility without causing irritations or sensations of discomfort, acceptable for repeated applications. In addition, the efficacy of the facial cream for anti-aging and the shampoo to stimulate hair growth and anti-hair loss has also been proven subjective and instrumental.

The accelerated stability test was performed for both facial cream and shampoo formulations that presented similar characteristics before and after storage in different temperatures and luminosity conditions, over 90 days. The aspect of the cream presented light beige color, characteristic aroma, mild pH changes between 5.5 and 6.0, and density of 0.96 to 1.00. The appearance of the shampoo formulation resulted in a viscous liquid, also light beige color, with characteristic aroma, pH between 5.3 and 6.0, and density of 0.98 to 1.00. According to the results obtained in this period of time, there were no relevant changes for the cream or the shampoo, and both could be considered stable for 24 months shelf life.

The formulations with pH between 4.0 and 6.0 can be considered to be favorable in a clinical skin care to maintain the acid mantle and the integrity of the stratum corneum (outermost layer of the epidermis), with evidences that the skin surface pH is a key factor in barrier homeostasis and antimicrobial defenses since most pathogenic bacteria thrive at neutral pH levels (Ali and Yosipovitch 2013).

β-Glucans also have hydrophilic functional groups in their chemical structure, such as hydroxyl (–OH), that when dissolved in water releases a positive hydrogen ion (H+), principally in a pH solution value less than 7. In acidic solutions, the excess of cations can be readily available for reaction with basic species. Because skin and hair can have reductions in their acidity by endogenous or exogenous factors resulting from, for example, chemical treatments, they can become slightly anionic, causing its physical-chemical affinity with cationic components (Velasco et al. 2009; Ali and Yosipovitch 2013). Then, the polysaccharides dissolved in water can be deposited on the hair or skin and provide conditioning on their own.

Skin aging is characterized by wrinkling, loss of elasticity, laxity, and rough-textured appearance, caused from both intrinsic and extrinsic aging factors. When a cosmetic product is proved to augment these features (skin appearance improvement and regeneration), it can be indicated as an anti-aging as an cosmeceutical product (Mohiuddin 2019). Topical polysaccharides also improved tissue regeneration through the increment of fibroblast proliferation, collagen synthesis, and vascularization (Camelini et al. 2005b; Son et al. 2007; Du et al. 2014).

The clinical trials for an anti-aging facial cream with 0.2% of *A. subrufescens* β-glucans, evaluated in a project founded by CNPq, provided an improvement of 24% in elasticity and 29% in firmness, in 28 days of use, instrumentally verified by skin suction through a Cutometer® equipment. Regarding the subjective evaluation, 85% of the participants suggested improvement of hydration and skin texture after use of the product for 28 consecutive days. They also described improved signs against aging in the face, such as reduction in wrinkles and lines of expressions (data not shown).

Other biomolecules as tocopherols, fatty acids and organic acids can also be extracted by ethanol from *A. subrufescens* with the intention of contributing to skin health and included in cream formulations. Taofiq et al. (2019) showed preliminary safety tests on human keratinocytes for both the ethanolic extract and its formulations prepared on a semi-solid base cosmetic cream, which presented a light-yellow color and a pH of 4.3, considered suitable for skin contact purposes and cosmeceutical applications.

To evaluate the anti-hair loss effect of a shampoo with 0.2% of *A. subrufescens* β-glucans, also evaluated in a project founded by CNPq, an instrument-based mechanical test approach (modified dynamometer) was used to probe the physical properties of individual hair fibers for tensile strength and resistance. The participants were evaluated at moment T0 (D0), after 30 days (D30) without use, and after using the shampoo at least three times a week during 30 days (D60). The results showed that in the D30/D60 period, tensile hair strength increased on the average 0.12 g and the resistance by 1.03 cm, i.e., up to 77% in stretch length more than D0/D30. Participants also related hair improvement in softness, shine, hydration,

reduction of hair loss, and increased hair growth, in addition to a pleasant perfume, with no dermal irritations (data not shown).

During hair growth, the cuticle and cortex proteins are synthesized with sulfur in the reduced state as cysteinyl residues forming keratin. In the presence of fatty acids, manly anteiso-18-methyleicosanoic acid, which are covalently linked to the cuticle surface, new hair cells are formed. Keratin is a laminated complex formed by different structures, being the cuticle formed principally by the A-layer, exocuticle and endocuticle, which gives the hair characteristics as strength, flexibility, durability, and functionality (Rogers 2019). The cuticle covers all the hair fibers from the scalp to the end as an overlapping layer and it may be more or less affected by cosmetic treatments, interfering in these characteristics. In the inner part it has a thin membrane-like structure that covers the cortex in a highly chemically resistant structure that acts as a barrier (Takahashi and Yoshida 2017). Hence, if the shampoo of *A. subrufescens* containing polysaccharides increased hair stretching and resistance, softness, shine, and hydration, it proves to be effective for fiber recuperation acting on damages done to the cuticle. These could be acting on opening and breakage of scales, as well as an effect of cleaning dust particles and scalp secretions built up over threads, in addition to stimulating hair growth.

The rheological properties are the most common reason for the use of polysaccharides in cosmetics, but a new generation of hair care treatment uses various polysaccharides from biotechnological compounds to minimize breakage or other signs of hair damage (Sajna et al. 2015). Hair chemical treatments with alkaline relaxers used to straighten curly hair often cause considerable hair damage resulting in loss of tensile strength and hair swelling. The cationic and non-ionic polymers have been applied to repair and reduce these damages that occur mainly due to breakage of disulfide and hydrogen bonds, and also to prevent swelling during hair relaxing (Syed et al. 2002).

This stimulating of hair growth and the anti-hair loss activity in the scalp can also be related to the pro-vasculogenesis activity of *A. subrufescens* polysaccharides found in a normal physiological process of embryogenesis (Camelini et al. 2005b). The cyclic life of hair follicles consists of recurring phases of growth, generally 3–6 years long (anagen), decay (catagen) and rest (telogen). Hair growth is also dependent of induction of angiogenesis in the anagen phase correlated with the upregulation of the VEGF mRNA expression by follicular keratinocytes of the outer root sheath. This improved follicle vascularization promotes an increase of nutritional support for rapid cell division and hair growth. Overexpression of VEGF during the growth phase contributes to increase bulb diameter, thickness of fully developed hair shafts, and also enlarged hair follicle, vessels size, total vascular mass, and accelerate the growth of hair (Yano et al. 2001).

Wolff et al. (2016) describe the hair loss as a symptom caused by a variety of hair growth disorders that can involve a range of genetic, endocrine, immune, and inflammatory processes, each of which calls for its own form of treatment. However, androgenetic alopecia, the most common type of hair loss that affects more men but also women, is related to a reduction of the expression levels of the growth factor

VEGF in the scalp skin in both genders compared with healthy individuals (Kubanov et al. 2017). Further, the immune cells have impact on epithelial hair follicle. A new phase of hair growth, an anagen, can be activated through resting hair follicle stem cells by the skin resident macrophages. Castellana et al. (2014) demonstrated that this process involves the death and activation of a fraction of resident macrophages, releasing ligands that in turn activate hair follicle stem cells. On the other hand, T cells can damage hair follicles (caused by an autoimmune disease) resulting in hair loss known as alopecia areata that can be generally treated with immune modulators (Wolff et al. 2016).

Yoon et al. (2019) demonstrated that the cutaneous lymphatic vessels also have a physiological role in the normal hair cycle, promoting the prolongation of anagen hair follicle growth, increasing the levels of the lymphangiogenesis growth factor VEGF-C. This process can be also involved in pathological hair loss, and the activation of VEGF-C secretion might serve as a potential new treatment option for alopecia. While blood vessels supply peripheral tissues with oxygen and nutrients, a major function of lymphatic vessels is the drainage of tissue fluid and cells from the periphery and their recirculation, principally in the transport of immune cells to the lymph nodes, resulting in a regulation of the humoral immunity by the activation of VEGFR-3 (Thomas et al. 2012).

A polysaccharide fraction obtained from *G. lucidum* promotes hematopoietic stem/progenitor cell homing for better human tissue protection, reducing disease progression and health. This polysaccharide was able to modulate the stimulatory effects on blood endothelial progenitor colony formation, with concomitant reduction of CD105 and VEGFR-3 marker expression in the presence of angiogenic factors (Chen et al. 2010). VEGFR-3 is the main receptor of lymphatic growth factors VEGF-C and VEGF-D, but not for VEGF-A, and regulates vascular and lymphatic endothelial cell function (Shibuya and Claesson-Welsh 2006).

Other cosmetic products with *A. subrufescens* extracts in their formulas were previously marketed in Brazil, claimed to provide softness and movement to hair (shampoo), while moisturizing facial creams were recommended to renew and revitalize the skin (Hyde et al. 2010; Wu et al. 2016). The high hygroscopicity of polysaccharides also contributes to the moisturizing and humectant characteristics in dermal formulations for skin and hair, including scalp care.

4 Conclusion

All biological activities described in this chapter for *A. subrufescens* turned this species biotechnologically very important, and therefore it is necessary to preserve and maintain the species in order to consider large scale production of fungal biomass to allow the use of polysaccharides, which includes their dermatological applications. The development of cosmeceuticals containing these bioactive polysaccharides contributes to bring beauty, with efficacy and safety, through products dermatologically tested and environmentally sound since the producing organism (fungi) can be cultivated in large-scale bioreactors without the burden of exploiting nature's natural sources to the extreme. Learning about the whole spectrum of these

active compounds and polishing the ways to obtain large volumes of raw material can be the break-through technological cutting-edge for a broader use of these molecules.

References

Ali SM, Yosipovitch G. Skin pH: from basic science to basic skin care. Acta Derm Venereol. 2013;93(3):261–7. https://doi.org/10.2340/00015555-1531.

Angeli JPF, Ribeiro LR, Camelini CM, Mendonça MM, Mantovani MS. Evaluation of the anti-genotoxicity of polysaccharides and β-glucans from *Agaricus blazei*, a model study with the single cell gel electrophoresis/Hep G2 assay. J Food Compos Anal. 2009;22(7–8):699–703. https://doi.org/10.1016/j.jfca.2009.03.003.

Bamias A, Dimopoulos MA. Angiogenesis in human cancer: implications in cancer therapy. Eur J Intern Med. 2003;14:459–69. https://doi.org/10.1016/j.ejim.2003.10.003.

Berovic M, Podgornik BB. Cultivation of medicinal fungi in bioreactors. In: Petre M, editor. Mushroom biotechnology-developments and applications. Amsterdam: Academic; 2016. p. 155–71. https://doi.org/10.1016/B978-0-12-802794-3.00009-6.

Bong SS. Compositions for promoting hair growth containing complexes of stabilized vitamin C or vitamin C derivatives. KR Patent KR100796904B, 3 May 2006. 2006.

Brown GD, Gordon S. Fungal β-glucans and mammalian immunity. Immunity. 2003;19:311–5. https://doi.org/10.1016/S1074-7613(03)00233-4.

Camelini CM, Maraschin M, Mendonça MM, Zucco C, Ferreira AG, Tavares LA. Structural characterization of β-glucans of *Agaricus brasiliensis* in different stages of fruiting body maturity and their use in nutraceutical products. Biotechnol Lett. 2005a;27(17):1295–9. https://doi.org/10.1007/s10529-005-0222-6.

Camelini CM, Mendonça MM, Dias PF, Maraschin M. β-Glucanas do cogumelo *Agaricus subrufescens* Peck eck (sinonímia *Agaricus blazei* Murrill sensu Heinemann = *Agaricus brasiliensis* Wasser, Diduck, de Amazonas & Stamets): extração, análise de rendimento, estrutura química e atividade biológica. Biotecnologia cienc desenvolv. 2005b;35:36–47. https://www.yumpu.com/pt/document/read/12835909/glucanas-do-cogumelo-biotecnologia.

Camelini CM, Pena DA, Gomes A, Steindel M, Rossi MJ, Giachini AJ, Mendonça MM. An efficient technique for *in vitro* preservation of *Agaricus subrufescens* (=A. brasiliensis). Ann Microbiol. 2012;62:1279–85. https://doi.org/10.1007/s13213-011-0373-8.

Cameotra SS. Preservation of microorganisms as deposits for patent application. Biochem Biophys Res Commun. 2007;353:849–50. https://doi.org/10.1016/j.bbrc.2006.12.069.

Cardozo FT, Camelini CM, Mascarello A, Rossi MJ, Nunes RJ, Barardi CR, Mendonça MM, Simões CM. Antiherpetic activity of a sulfated polysaccharide from *Agaricus brasiliensis* mycelia. Antivir Res. 2011;92(1):108–14. https://doi.org/10.1016/j.antiviral.2011.07.009.

Cardozo FTGS, Camelini CM, Cordeiro MNS, Mascarello A, Malagoli BG, Larsen I, Rossi MJ, Nunes RJ, Braga FC, Brandt CR, Simões CMO. Characterization and cytotoxic activity of sulfated derivatives of polysaccharides from *Agaricus brasiliensis*. Int J Biol Macromol. 2013a;57:265–72. https://doi.org/10.1016/j.ijbiomac.2013.03.026.

Cardozo FTGS, Larsen IV, Carballo EV, Jose G, Stern RA, Brummel RC, Camelini CM, Rossi MJ, Simões CM, Brandt CR. In vivo anti-herpes simplex virus activity of a sulfated derivative of *Agaricus brasiliensis* mycelial polysaccharide. Antimicrob Agents Chemother. 2013b;57(6):2541–9. https://doi.org/10.1128/AAC.02250-12.

Carmeliet P. Angiogenesis in health and disease. Nat Med. 2003;9:653–60. https://doi.org/10.1038/nm0603-653.

Castellana D, Paus R, Perez-Moreno M. Macrophages contribute to the cyclic activation of adult hair follicle stem cells. PLoS Biol. 2014;12(12):e1002002. https://doi.org/10.1371/journal.pbio.1002002.

Chen W-Y, Yang W-B, Wong C-H, Shih DT-B. Effect of Reishi polysaccharides on human stem/progenitor cells. Bioorg Med Chem. 2010;18:8583–91. https://doi.org/10.1016/j.bmc.2010.10.016.

Clark RA, Yamanaka K-I, Bai M, Dowgiert R, Kupper TS. Human skin cells support thymus-independent T cell development. J Clin Invest. 2005;115(11):3239–49. https://doi.org/10.1172/JCI24731.

Cunha MAA, Santos VAQ, Calegari GC, Luna WNS, Marin SLA, Dekker RFH, Barbosa-Dekker AM. Structure and biological properties of lasiodiplodan: an uncommon fungal exo-polysaccharide of the (1→6)-β-D-glucan type. In: Cohen E, Merzendorfer H, editors. Extracellular sugar-based biopolymers matrices. Biologically-inspired systems, vol. 12. Cham: Springer; 2019. p. 409–32. https://doi.org/10.1007/978-3-030-12919-4_10.

Davis R, Taylor A, Nally R, Benson KF, Stamets P, Jensen GS. Differential immune activating, anti-inflammatory, and regenerative properties of the aqueous, ethanol, and solid fractions of a medicinal mushroom blend. J Inflamm Res. 2020;13:117–31. https://doi.org/10.2147/JIR.S229446.

Du B, Bian Z, Xu B. Skin health promotion effects of natural beta-glucan derived from cereals and microorganisms: a review. Phytother Res. 2014;28:159–66. https://doi.org/10.1002/ptr.4963.

Egbuniwe IU, Karagiannis SN, Nestle FO, Lacy KE. Revisiting the role of B cells in skin immune surveillance. Trends Immunol. 2015;36(2):102–11. https://doi.org/10.1016/j.it.2014.12.006.

Endo M, Beppu H, Akiyama H, Wakamatsu K, Ito S, Kawamoto Y, Shimpo K, Sumiya T, Koike T, Matsui T. Agaritine purified from *Agaricus blazei* Murrill exerts anti-tumor activity against leukemic cells. Biochim Biophys Acta. 2010;1800:669–73. https://doi.org/10.1016/j.bbagen.2010.03.016.

Fabian J, Zlatanovic S, Mutz M, Premke K. Fungal–bacterial dynamics and their contribution to terrigenous carbon turnover in relation to organic matter quality. ISME J. 2017;11:415–25. https://doi.org/10.1038/ismej.2016.131.

Førland DT, Johnson E, Tryggestad AM, Lyberg T, Hetland G. An extract based on the medicinal mushroom *Agaricus blazei* Murrill stimulates monocyte-derived dendritic cells to cytokine and chemokine production *in vitro*. Cytokine. 2010;49:245–50. https://doi.org/10.1016/j.cyto.2009.09.002.

Fu CC, Wu WT, Lu SY. Performance of airlift bioreactors with net draft tube. Enzym Microb Technol. 2003;33:332–42. https://doi.org/10.1016/S0141-0229(03)00151-0.

Garcia-Ochoa F, Gomez E. Bioreactor scale-up and oxygen transfer rate in microbial processes: an overview. Biotechnol Adv. 2009;27:153–76. https://doi.org/10.1016/j.biotechadv.2008.10.006.

Gibbs PA, Seviour RJ, Schmid F. Growth of filamentous fungi in submerged culture: problems and possible solutions. Crit Rev Biotechnol. 2000;20:17–48. https://doi.org/10.1080/07388550091144177.

Grip J, Engstad RE, Skjæveland I, Škalko-Basnet N, Holsæter AM. Sprayable carbopol hydrogel with soluble beta-1,3/1,6-glucan as an active ingredient for wound healing – development and in-vivo evaluation. Eur J Pharm Sci. 2017;107:24–31. https://doi.org/10.1016/j.ejps.2017.06.029.

Grip J, Engstad RE, Skjæveland I, Škalko-Basnet N, Isaksson J, Basnet P, Holsæter AM. Beta-glucan-loaded nanofiber dressing improves wound healing in diabetic mice. Eur J Pharm Sci. 2018;121:269–80. https://doi.org/10.1016/j.ejps.2018.05.031.

Hamedi A, Vahid H, Ghanati F. Optimization of the medium composition for production of mycelia biomass and exo-polysaccharide by *Agaricus blazei* Murill DPPh 131 using response-surface methodology. Biotechnology. 2007;6(4):456–64. https://doi.org/10.3923/biotech.2007.456.464.

Hau CS, Tada Y, Shibata S, Uratsuji H, Asano Y, Sugaya M, Kadono T, Kanda N, Watanabe S, Tamaki K, Sato S. High calcium, ATP, and Poly(I:C) augment the immune response to β-glucan in normal human epidermal keratinocytes. J Invest Dermatol. 2011;131(11):2255–62. https://doi.org/10.1038/jid.2011.201.

He X, Fang J, Guo Q, Wang M, Li Y, Meng Y, Huang L. Advances in antiviral polysaccharides derived from edible and medicinal plants and mushrooms. Carbohydr Polym. 2020;229:115548. https://doi.org/10.1016/j.carbpol.2019.115548.

Hesselink JMK, Kopsky DJ, Bhaskar AK. Skin matters! The role of keratinocytes in nociception: a rational argument for the development of topical analgesics. J Pain Res. 2017;10:1–8. https://doi.org/10.2147/JPR.S122765.

Hetland G, Johnson E, Lyberg T, Bernardshaw S, Tryggestad AMA, Grinde B. Effects of the medicinal mushroom *Agaricus blazei* Murill on immunity, infection and cancer. Scand J Immunol. 2008;68:363–70. https://doi.org/10.1111/j.1365-3083.2008.02156.x.

Huang M-H, Yang M-C. Evaluation of glucan/poly(vinyl alcohol) blend wound dressing using rat models. Int J Pharm. 2008;346(1–2):38–46. https://doi.org/10.1016/j.ijpharm.2007.06.021.

Hyde KD, Bahkali AH, Moslem MA. Fungi: an unusual source for cosmetics. Fungal Divers. 2010;43(1):1–9. https://doi.org/10.1007/s13225-010-0043-3.

Itoh H, Ito H, Hibasami H. Blazein of a new steroid isolated from *Agaricus blazei* Murrill (himematsutake) induces cell dealth and morphological change indicative of apoptotic chromatin condensation in human lung cancer LU99 and stomach cancer KATO III cells. Oncol Rep. 2008;20:1359–61. https://doi.org/10.3892/or_00000152.

Jesenak M, Banovcin P, Rennerova Z, Majtan J. β-Glucans in the treatment and prevention of allergic diseases. Allergol Immunopathol. 2014;42(2):149–56. https://doi.org/10.1016/j.aller.2012.08.008.

Jesenak M, Urbancek S, Majtan J, Banovcin P, Hercogova J. β-Glucan-based cream (containing pleuran isolated from *Pleurotus ostreatus*) in supportive treatment of mild-to-moderate atopic dermatitis. J Dermatolog Treat. 2016;27(4):351–4. https://doi.org/10.3109/09546634.2015.1117565.

Ker Y-B, Chen K-C, Chyau C-C, Chen C-C, Guo J-H, Hsieh C-L, Wang H-E, Peng C-C, Chang C-H, Peng RY. Antioxidant capability of polysaccharides fractionated from submerge-cultured *Agaricus blazei* mycelia. J Agric Food Chem. 2005;53(18):7052–8. https://doi.org/10.1021/jf0510034.

Kerrigan RW. *Agaricus subrufescens*, a cultivated edible and medicinal mushroom and its synonyms. Mycologia. 2005;97:12–24. https://doi.org/10.3852/mycologia.97.1.12.

Khan AA, Gani A, Khanday FA, Masoodi FA. Biological and pharmaceutical activities of mushroom β-glucan discussed as a potential functional food ingredient. Bioact Carbohydr Diet Fibre. 2018;16:1–13. https://doi.org/10.1016/j.bcdf.2017.12.002.

Kligman A. The future of cosmeceuticals: an interview with Albert Kligman, MD, PhD. Dermatol Surg. 2005;31(7):890–1. https://doi.org/10.1111/j.1524-4725.2005.31737.

Kozarski M, Klaus A, Jakovljević D, Todorović N, Wan-Mohtar WAAQI, Nikšić M. *Ganoderma lucidum* as a cosmeceutical: antiradical potential and inhibitory effect on hyperpigmentation and skin extracellular matrix degradation enzymes. Arch Biol Sci. 2019;71(2):253–264. https://doi.org/10.2298/ABS181217007K.

Kozuka M, Oyama M, Tokuda H, Nishino H, Lee KH. Cancer preventive agents 3: antitumor promoting effects of *Agaricus blazei*. Pharm Biol. 2005;43(6):568–72. https://doi.org/10.1080/13880200500220979.

Kubanov AA, Gallyamova YA, Korableva OA. The study of growth factors in patients with androgenic alopecia. Biomed Pharmacol J. 2017;10(3):1219–28. https://doi.org/10.13005/bpj/1224.

Lee KM, Lee SY, Lee HY. Bistage control of pH for improving exopolysaccharide production from mycelia of *Ganoderma lucidum* in an air lift fermentor. J Biosci Bioeng. 1999;88(6):646–50. https://doi.org/10.1016/s1389-1723(00)87094-2.

Lee BC, Bae JT, Pyo HB, Choe TB, Kim SW, Hwang HJ, Yun JW. Submerged culture conditions for the production of mycelial biomass and exopolysaccharides by the edible basidiomycete *Grifola frondosa*. Enzym Microb Technol. 2004;35(5):369–76. https://doi.org/10.1016/j.enzmictec.2003.12.015.

Lindequist U, Niedermeyer THJ, Jülich WD. The pharmacological potential of mushrooms. eCAM. 2005;2(3):285–99. https://doi.org/10.1093/ecam/neh107.

Mader J, Gallo A, Schommartz T, Handke W, Nagel CH, Günther P, Reich K. Calcium spirulan derived from *Spirulina platensis* inhibits herpes simplex virus 1 attachment to human keratinocytes and protects against herpes labialis. J Allergy Clin Immunol. 2016;137(1):197–203. https://doi.org/10.1016/j.jaci.2015.07.027.

Mahapatra S, Banerjee D. Fungal exopolysaccharide: production, composition and applications. Microbiol Insights. 2013;6:1–16. https://doi.org/10.4137/MBI.S10957.

Matsui K, Kodama N, Nanba H. Effects of maitake (*Grifola frondosa*) D-fraction on the carcinoma angiogenesis. Cancer Lett. 2001;172:193–8. https://doi.org/10.1016/S0304-3835(01)00652-8.

Mohiuddin AK. Skin aging & modern edge anti-aging strategies. Int J Dermatol Skin Care. 2019;1: 08–62. https://doi.org/10.36811/ijdsc.2019.110002.

Musoni M, Destain J, Thonart P, Bahama J-B, Delvigne F. Bioreactor design and implementation strategies for the cultivation of filamentous fungi and the production of fungal metabolites: from traditional methods to engineered systems. Biotechnol Agron Soc Environ. 2015;19(4):430–42.

Narayanan KB, Zo SM, Han SS. Novel biomimetic chitin-glucan polysaccharide nano/microfibrous fungal-scaffolds for tissue engineering applications. Int J Biol Macromol. 2020;149:724–31. https://doi.org/10.1016/j.ijbiomac.2020.01.276.

Nikakhtari H, Hill GA. Enhanced oxygen mass transfer in an external loop airlift bioreactor using a packed bed. Ind Eng Chem Res. 2005;44:1067–72. https://doi.org/10.1021/ie0494925.

Niu YC, Liu JC, Zhao XM, Wu XX. A low molecular weight polysaccharide isolated from *Agaricus blazei* suppresses tumor growth and angiogenesis *in vivo*. Oncol Rep. 2009;21:145–52. https://doi.org/10.3892/or_00000201.

Ohno N. Analysis of glycans; polysaccharide functional properties. In: Kamerling JP, Boon G-J, editors. Comprehensive glycoscience from chemistry to systems biology, vol 2. Amsterdam: Elsevier; 2007.

Park E-K, Kang S-M, Leem M-H. A study on the variation of skin moisture, oil (sebum), melanin and erythema index after application of β-glucan. Asian J Beauty Cosmetol. 2003;1:83–94.

Pasparakis M, Haase I, Nestle F. Mechanisms regulating skin immunity and inflammation. Nat Rev Immunol. 2014;14:289–301. https://doi.org/10.1038/nri3646.

Reemann P, Reimann E, Ilmjärv S, Porosaar O, Silm H, Jaks V, Vasar E, Kingo K, Kõks S. Melanocytes in the skin – comparative whole transcriptome analysis of main skin cell types. PLoS One. 2014;9(12):e115717. https://doi.org/10.1371/journal.pone.0115717.

Rogers GE. Known and unknown features of hair cuticle structure: a brief review. Cosmetics. 2019;6(2):32. https://doi.org/10.3390/cosmetics6020032.

Rossi MJ. Tecnologia para produção de inoculantes de fungos ectomicorrízicos utilizando cultivo submerso em biorreator airlift. Thesis, Universidade Federal de Santa Catarina, 2006. http://www2.enqufsc.br/teses/d035.pdf

Rossi MJ, Oliveira VL. Growth of the ectomycorrhizal fungus *Pisolithus microcarpus* in different nutritional conditions. Braz J Microbiol. 2011;42(2):624–32. https://doi.org/10.1590/S1517-838220110002000027.

Rossi MJ, Souza JAR, Oliveira VL. Inoculum production of the ectomycorrhizal fungus *Pisolithus microcarpus* in an airlift bioreactor. Appl Microbiol Biotechnol. 2002;59(2–3):175–81. https://doi.org/10.1007/s00253-002-0999-3.

Rossi MJ, Nascimento FX, Giachini AJ, Oliveira VL, Furigo A Jr. Airlift bioreactor fluid-dynamic characterization for the cultivation of shear stress sensitive microorganisms. J Adv Biotechnol. 2016;5:640–51. https://doi.org/10.24297/jbt.v5i2.4846.

Rossi MJ, Nascimento FX, Brandão PMFDR, Camelini CM, Giachini AJ. Activated charcoal increases the viability of ectomycorrhizal fungi during the stages of cultivation aiming the production of large-scale inocula via submerged cultivation. Adv Biotech Micro. 2017a;6(4): 555692. https://doi.org/10.19080/AIBM.2017.06.555692.

Rossi MJ, Nascimento FX, Giachini AJ, Oliveira VL, Furigo A Jr. Transfer and consumption of oxygen during the cultivation of the ectomycorrhizal fungus *Rhizopogon nigrescens* in an airlift bioreactor. Appl Microbiol Biotechnol. 2017b;101(3):1013–24. https://doi.org/10.1007/s00253-016-7854-4.

Ruszova E, Pavek S, Hajkova V, Jandova S. Photoprotective effects of glucomannan isolated from *Candida utilis*. Carbohydr Res. 2008;343(3):501–11. https://doi.org/10.1016/j.carres.2007.11.010.

Saad HM, Sim KS, Tan YS. Antimelanogenesis and anti-inflammatory activity of selected culinary-medicinal mushrooms. Int J Med Mushrooms. 2018;20(2):141–53. https://doi.org/10.1615/2018025463.

Sajna KV, Gottumukkala LD, Sukumaran RK, Pandey A. White biotechnology in cosmetics. In: Pandey A, Höfer R, Taherzadeh M, Nampoothiri KM, Larroche C, editors. Industrial

biorefineries and white biotechnology. Elsevier; 2015. p. 607–52. https://doi.org/10.1016/B978-0-444-63453-5.00020-3.

Sang-Muk K. The hair-growing agent composition which is contained the extract from natural *Phellinus linteus* and preparation method using the same. KR Patent KR20000053859A, 5 Sept 2000. 2000.

Shibuya M, Claesson-Welsh L. Signal transduction by VEGF receptors in regulation of angiogenesis and lymphangiogenesis. Exp Cell Res. 2006;312(5):549–60. https://doi.org/10.1016/j.yexcr.2005.11.012.

Silveira DB, Celmer ÁJ, Camelini CM, Rossi MJ, Petrus JCC, Mendonça MM, Zanetti CR. Mass separation and *in vitro* immunological activity of membrane-fractionated polysaccharides from fruiting body and mycelium of *Agaricus subrufescens*. Biotechnol Bioproc E. 2012;17(4):804–11. https://doi.org/10.1007/s12257-011-0645-y.

Smyth MJ, Hayakawa Y, Takeda K, Yagita H. New aspects of natural-killer-cell surveillance and therapy of cancer. Nat Rev Cancer. 2002;2(11):850–61. https://doi.org/10.1038/nrc928.

Son HJ, Han D-W, Baek HS, Lim HR, Lee MH, Woo YI, Park J-C. Stimulated TNF-a release in macrophage and enhanced migration of dermal fibroblast by β-glucan. Curr Appl Phys. 2007;7S1:e33–6. https://doi.org/10.1016/j.cap.2006.11.010.

Souza Filho PF, Zamani A, Taherzadeh MJ. Production of edible fungi from potato protein liquor (PPL) in airlift bioreactor. Fermentation. 2017;3(1):12. https://doi.org/10.3390/fermentation3010012.

Sui ZF, Yang RY, Liu B, Gu TM, Zhao ZL, Shi DF, Chang DQ. Chemical analysis of *Agaricus blazei* polysaccharides and effect of the polysaccharides on IL-1 β mRNA expression in skin of burn wound-treated rats. Int J Biol Macromol. 2010;47:155–7. https://doi.org/10.1016/j.ijbiomac.2010.05.006i.

Syed AN, Habib WW, Kuhajda AM. Water-soluble polymers in hair care. In: Amjad Z, editor. Water soluble polymers – solution properties and applications. Boston: Springer; 2002. p. 231–44. https://doi.org/10.1007/0-306-46915-4_18.

Takahashi T, Yoshida S. A highly resistant structure between cuticle and cortex of human hair. International Int J Cosmet Sci. 2017;39:327–36. https://doi.org/10.1111/ics.12380.

Taofiq O, Heleno SA, Calhelha RC, Alves MJ, Barros L, González-Paramás AM, Barreiro MF, ICFR F. The potential of *Ganoderma lucidum* extracts as bioactive ingredients in topical formulations, beyond its nutritional benefits. Food Chem Toxicol. 2017;108:139–47. https://doi.org/10.1016/j.fct.2017.07.051.

Taofiq O, Rodrigues F, Barros L, Peralta RM, Barreiro MF, Ferreira ICFR, Oliveira MBPP. *Agaricus blazei* Murrill from Brazil: an ingredient for nutraceutical and cosmeceutical applications. Food Funct. 2019;10(2):565–572. https://doi.org/10.1039/c8fo02461h.

Thomas SN, Rutkowski JM, Pasquier M, Kuan EL, Alitalo K, Randolph GJ, Swartz MA. Impaired humoral immunity and tolerance in K14-VEGFR-3-Ig mice that lack dermal lymphatic drainage. J Immunol. 2012;189(5):2181–90. https://doi.org/10.4049/jimmunol.1103545.

Uchiyama S, Haramaki K. Use of Agaricus blazei Murill to prevent or treat skin and other disorders. US Patent 20020119164, 29 August 2002. 2002.

Velasco MVR, Dias TCS, Freitas AZ, Vieira Júnior ND, Pinto CASO, Kaneko TM, Baby AR. Hair fiber characteristics and methods to evaluate hair physical and mechanical properties. Braz J Pharm Sci. 2009;45(1):153–62. https://doi.org/10.1590/S1984-82502009000100019.

Vrabl P, Schinagl CW, Artmann DJ, Heiss B, Burgstaller W. Fungal growth in batch culture – what we could benefit if we start looking closer. Front Microbiol. 2019;10:2391. https://doi.org/10.3389/fmicb.2019.02391.

Webster J, Weber RWS. Introduction to fungi. 4th ed. Cambridge: Cambridge University Press; 2010. 841 p

Wisitrassameewong K, Karunarathna SC, Thongklang N, Zhao R, Callac P, Moukha S, Fe'randon C, Chukeatirote E, Hyde KD. *Agaricus subrufescens*: a review. Saudi J Biol Sci. 2012;19:131–146. https://doi.org/10.1016/j.sjbs.2012.01.003.

Wolff H, Fischer TW, Blume-Peytavi U. The diagnosis and treatment of hair and scalp diseases. Dtsch Arztebl Int. 2016;113:377–86. https://doi.org/10.3238/arztebl.2016.0377.

Wu Y, Choi M-H, Li J, Yang H, Shin H-J. Mushroom cosmetics: the present and future. Cosmetics. 2016;3(3):22. https://doi.org/10.3390/cosmetics3030022.

Yang FC, Ke YF, Kuo SS. Effect of fatty acids on the mycelial growth and polysaccharide formation by *Ganoderma lucidum* in shake-flask cultures. Enzym Microb Technol. 2000;27:295–301. https://doi.org/10.1016/S0141-0229(00)00213-1.

Yano K, Brown LF, Detmar M. Control of hair growth and follicle size by VEGF-mediated angiogenesis. J Clin Invest. 2001;107(4):409–17. https://doi.org/10.1172/JCI11317.

Yokoyama WM, Scalzo AA. Natural killer cell activation receptors in innate immunity to infection. Microbes Infect. 2002;4(15):1513–21. https://doi.org/10.1016/S1286-4579(02)00034-5.

Yoon SY, Dieterich LC, Karaman S, Proulx ST, Bachmann SB, Sciaroni C, Detmar M. An important role of cutaneous lymphatic vessels in coordinating and promoting anagen hair follicle growth. PLoS One. 2019;14(7):e0220341. https://doi.org/10.1371/journal.pone.0220341.

Zammaretti P, Zisch AH. Adult endothelial progenitor cells renewing vasculature. Int J Biochem Cell Biol. 2005;37:493–503. https://doi.org/10.1016/j.biocel.2004.06.018.

Zhong J-J, Tang Y-J. Submerged cultivation of medicinal mushrooms for production of valuable bioactive metabolites. Adv Biochem Engin/Biotechnol. 2004;87:25–59. https://doi.org/10.1007/b94367.

Zhou Z, Han Z, Zeng Y, Zhang M, Cui Y, Xu L, Zhang L. Chinese FDA approved fungal glycan-based drugs: an overview of structures, mechanisms and clinical related studies. Transl Med. 2014;4(141):1–11. https://doi.org/10.4172/2161-1025.1000141.

Zhu J-W, Ni Y-J, Tong X-Y, Guo X, Wu X-P. Activation of VEGF receptors in response to UVB promotes cell proliferation and melanogenesis of normal human melanocytes. Exp Cell Res. 2020;387(2):111798. https://doi.org/10.1016/j.yexcr.2019.111798.

Part III

Isolation, Extraction, Purification, and Production Processes

Exopolysaccharides from Lactic Acid Bacteria

19

Yousra Abid and Samia Azabou

Contents

Abstract

Lactic acid bacteria (LAB) are continuously arousing interest for their capacity to produce extracellular polysaccharides (EPS) without any health risks owing to their GRAS status. EPS have been found to be associated with multiple functions in microbial cells, essentially the protection of the microbial cells against both biotic and abiotic stress factors. Apart from their physiological roles, the wide diversity of the composition and functionality of EPS from LAB is broadening their industrial applications including pharmaceutical, cosmetic, medical, environmental, and food products. Structural features of EPS such as chain length, sugar composition, sugar linkages type, and the presence of substitutions may considerably affect their biological and technological properties. Indeed, EPS

Y. Abid · S. Azabou (✉)
ENIS, Laboratoire Analyse, Valorisation et Sécurité des Aliments, Université de Sfax, Sfax, Tunisia

J. M. Oliveira et al. (eds.), *Polysaccharides of Microbial Origin*,
https://doi.org/10.1007/978-3-030-42215-8_26

from LAB have extensively gained attention for their biological activities such as hypocholesterolemic, immunomodulatory, antioxidant, and antitumor activities. In addition, EPS from LAB have been applied as viscosity increasing agents, thickeners, gelling agents, emulsifiers, and stabilizers. Particularly, in the food industry, interactions between EPS and proteins are receiving rising interest since they demonstrated an ability to better control the texture and stability of food systems. Research development is constantly interested in the isolation, characterization, and applications of novel EPS as renewable resources.

Keywords

Exopolysaccharides · Lactic acid bacteria · Biosynthesis · Structure · Functionality · Applications · Protein–polysaccharide interactions

1 Introduction

Each year, tons of natural carbohydrates are biosynthesized mostly as polysaccharides and are widely applied in different fields of application including pharmaceutical, cosmetic, paper, packaging, and food industries (Alves et al. 2010). Natural polysaccharides can be isolated from animals (e.g., chitosan), plants (e.g., starch), or algae (e.g., alginate). Nevertheless, the limits of these macromolecules are their dependency on the availability of the source, the season of the year, and the climate conditions. Alternatively, they can be successfully recovered from various microorganisms with higher growth rates as well as possible higher production yields (Alves et al. 2010). Microbial polysaccharides are receiving special attention since they provide several advantages compared to other producing systems, according to Kambourova et al. (2015), including the possibility of using renewable resources like industrial by-products as carbon sources (e.g., molasses, glycerol, etc.), fast production (few days) unlike plants, production under controlled conditions with the possibility of genetic manipulation of the relatively simple bacterial genetic providing thus the required product composition and structure with a reproducible and optimized yield as well as easy recovery from the extracellular environment avoiding thus extra steps of purification (Morris and Harding 2009). Microbial polysaccharides can be obtained from either prokaryotic or eukaryotic groups including yeasts, microalgae, and bacteria. The majority of bacteria of all taxa are capable of producing polysaccharides with varied chemical and functional properties by using either simple or complex substrates. Three main classes of microbial polysaccharides can be distinguished depending on their location in the cell. Polysaccharides found in the cytoplasm are known as storage polysaccharides serving as carbon and energy sources for the cell; polysaccharides located in the cell wall, such as lipopolysaccharides, peptidoglycans and teichoic acids, are known as structural polysaccharides; and those located on the cell surface including capsular polysaccharides (covalently linked to the surface) or those loosely attached or completely exuded to the extracellular medium as exopolysaccharides (EPS) that are easier to isolate and to purify (Ruas-Madiedo and

Reyes-Gavilan 2005). Among the numerous EPS-producing bacteria, lactic acid bacteria (LAB) have received crucial attention. LAB are not only generally recognized as safe (GRAS) microorganisms but they have also great capacities to produce EPS with wide diversity of structures without any health issue (Freitas et al. 2011). LAB-EPS can be found as ropy or non-ropy EPS. Some bacteria can only produce capsular EPS, some others only ropy form, while in some cases, both forms of EPS can be produced (Mende et al. 2016). *Streptococcus*, *Pediococcus*, *Lactococcus*, *Leuconostoc*, *Lactobacillus,* and *Weissella* are among the most common EPS-producing LAB species (Patel and Prajapat 2013). EPS from LAB can be classified following several criteria. One of the most used criteria is based on their monosaccharide composition. Depending on the composition of the repeating units and biosynthesis pathway, they can be classified into two groups: homopolysaccharides (HoPS) or heteropolysaccharides (HePS) (Mende et al. 2016). Homopolysaccharide synthesis occurs through the enzymatic reactions of either glucansucrase or levansucrase using sucrose as substrate, while heteropolysaccharide synthesis requires four major steps including sugar transportation, sugar nucleotide synthesis, repeating units synthesis, and polymerization (De Vuyst and Degeest 1999). The increasing attention given to EPS from LAB essentially results from their beneficial health effects to the consumer including antitumor, antioxidant, anti-ulcer properties, cholesterol-lowering and immune-stimulating activities (Patel and Prajapat 2013). Furthermore, EPS from LAB showed ability to reduce formation of pathogenic biofilms (Abid et al. 2018a) to modulate adhesion to epithelial cells and to stimulate bifidobacteria growth and activity showing a prebiotic potential (Hongpattarakere et al. 2012). EPS-producing LAB showed great capacity to withstand technological stresses and to survive the passage through the gastrointestinal tract compared to nonproducing bacteria (Abid et al. 2018a). Besides their health benefits, EPS from LAB greatly enhance the rheology, viscosity and texture, reduce syneresis, and improve the mouth-feel of fermented food formulations in the food industries (Badel et al. 2011; Patel and Prajapat 2013). EPS-producing LAB are crucial in the development of functional foods and are often used as starter cultures or coadjutants in developing fermented foods such as cheese, yoghurt, and cereal-based products (Badel et al. 2011; Ruas-Madiedo and Reyes-Gavilan 2005; Zannini et al. 2016). Therefore, the use of EPS-producing starter cultures may give several advantages over nonproducing ones. However, alcoholic beverages (beers, wines, ciders, etc.) could be spoiled by EPS-producing LAB. For instance, this spoilage could occur in wine during vilification or after bottling, which may lead to a ropiness or an oilness characterized by a viscous and thick texture, and oily feel, which renders the product unpleasant causing significant economic loss. EPS from LAB applications have not been limited to the food industry. They have been widely used in different other fields including environmental field as bioflocculants, heavy metal removal agents, bio-absorbents, medical field as, for example, drug delivery agents, and cosmetic field as moisturizing and emulsifying agents (Kambourova et al. 2015; More et al. 2014). Indeed, the biological and techno-functional properties of EPS are closely dependent on the structural characteristics of these macromolecules including their molecular weight (Mw), charge, chain length, monomer composition, and linkage types.

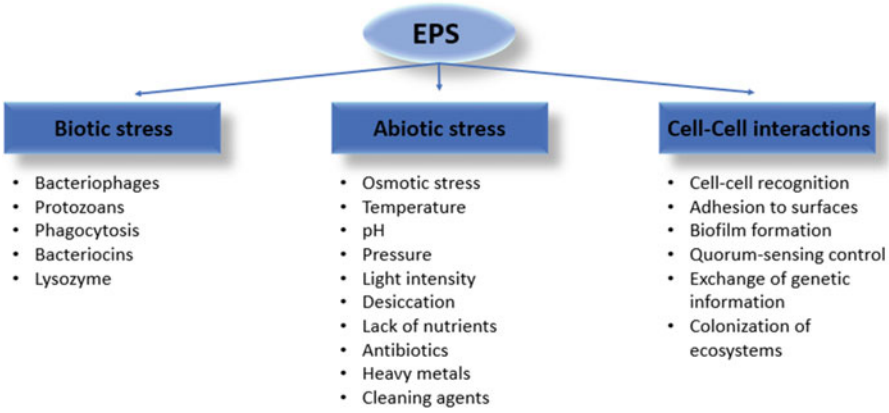


Fig. 1 Main physiological roles of bacterial EPS

This chapter reviews the physicochemical, functional, and biological properties as well as the applications of EPS produced by LAB.

2 Physiological Roles of Exopolysaccharides

EPS may exert various physiological functions (Fig. 1), which could vary among different microorganisms depending on the producing cells as well as their biotops (Patel and Prajapat 2013). EPS could enable:

- Cells protection against external environmental conditions (temperature, pH, pressure, light intensity, osmotic stress, dehydration, etc.)
- Nutrients and essential minerals sequestration from the surrounding environment owing to some EPS anionic nature
- Prevention against harmful substances access including macrophages, antibiotics bacteriophages, and toxic compounds such as heavy metals
- Cellular recognition, interactions between cells, adhesion to surfaces and biofilm formation

3 Chemical Classification of Exopolysaccharides

Monosaccharide composition is a key parameter to classify EPS into two main groups, namely HoPS and HePS:

- HoPS: Consisting of only one type of monomer that could be linked by either one linkage type or different linkage types with an average Mw of up to approximately 10^7 Da. HoPS could also be subdivided into linear and ramified chains. Depending on the carbon involved in the linkage, homopolysaccharides could be

further subdivided into four groups including: (i) α-D-glucans (dextran containing D-glucosyl units joined through α-(1,6) linkages, whereas the branching are linked through α-(1,3) and in some cases through α-(1,2) and α-(1,4), alternan (alterning α-(1,6) and α-(1,3) linkages), mutan (linear backbone containing α-(1,3) linkages with minor amount of α-(1,6)), and reuteran (composed of mainly α-(1,4) segments interconnected by α-(1,6) linkage)); (ii) β-D-glucans produced by *Streptococcus* sp. and *Pediococcus* sp., containing β-(1,3)-linked D-glucosyl units with β-(1,2) branching linkages; (iii) fructan subgroup (levan with β-(2,6) joined fructosyl units and inulin with β-(2,1) joined fructosyl units); and (iv) others like polygalactans formed by repeating units of galactose monomers with different glycosidic linkages (More et al. 2014). Generally, HoPS only differ in both glycosidic bonds types and molecular polymers sizes, which could be resulting from the unicity of the enzymes involved in the extracellular biosynthesis-dependent process;

- HePS: Consisting of branched repeating segments with more than one type of monosaccharide having an average Mw ranging from 10^4 to 10^6 Da, which is often lower than the average Mw of HoPS. Often, glucose, rhamnose, and galactose are found but in various ratios. Heteropolysaccharides could also contain other residues such as phosphate, succinate, pyruvate, sulfate, N-acetylgalactosamine, N-acetylglucosamine, glucuronic acid, and acetyl groups (Kambourova et al. 2015; More et al. 2014). This type of EPS could be either neutral, cationic, or anionic due to the presence or absence of uronic acids, acetate ester, formats, pyruvate ketals, succinates, phosphate, or sulfate (More et al. 2014). HePS could also be classified into neutral and acidic polymer. Indeed, acidic EPS include in their composition one or more uronic acids. The presence of acidic substituents like phosphorylation or sulfonation may also increase EPS acidity (Zhou et al. 2019).

Besides their differences in their chemical composition and types of linkages, homopolysaccharides and heteropolysaccharides differ also in their biosynthesis pathways.

4 Biosynthesis of EPS

EPS from LAB are generally biosynthesized by either an extracellular synthesis pathway or a Wzx/Wzy-dependent pathway.

4.1 The Extracellular Synthesis Pathway

The extracellular synthesis pathway consists of two major steps to synthesize HoPS in the extracellular environment, namely: the polymerization carried by specific enzymes: glucansucrase and fructansucrase, enabling the transfer of monosaccharide from substrates to the polysaccharide growing chains, followed by the

polymerized chains release into the extracellular environment. The common EPS that require dextransucrase or levansucrase for their biosynthesis and sucrose as a substrate are dextran and levan, respectively.

4.1.1 Levan Biosynthesis

Levan, a high Mw polymer (from 2 to 100 million Da), consists of D-fructofuranosyl units linked by β-(2–6) linkages and biosynthesized in the extracellular environment. It can be produced either by bacterial fermentation using sucrose as carbon source (such as by *Leuconostoc* species) or by enzymatic synthesis also using sucrose as substrate (Donot et al. 2012; Srikanth et al. 2015). The biosynthesis of this polymer closely depends on the extracellular levansucrase, also known as (EC 2.4.1.10), β-(2–6)-fructosyltransferase or as sucrose 6-fructosyltransferase. This enzyme is synthesized inside the cell, gets accumulated, and then adopts its final conformation before being excreted. A variation of the outer environmental pH or the presence of metallic ions such as calcium and iron could complete the signal for the excretion of levansucrase (Donot et al. 2012). This enzyme acts on the donor molecule (the substrate) and catalyzes the transfructosylation reactions. Then, the polymerization occurs. This step consists of a continuous fructose subunit addition to the growing levan polysaccharide chain (acceptor molecule) until the obtention of the final polymer macromolecule (Srikanth et al. 2015).

4.1.2 Dextran Biosynthesis

Dextran, a neutral and a highly branched high Mw homopolysaccharide (10–5000 kDa), consists of glucose units linked by α-(1–6) linkages. The branching chains can be attached either as α-(1–3), α-(1–4), or seldom α-(1–2) linkages. Dextrans are mostly synthesized by dextransucrases using sucrose as substrate mainly by *Streptococcus*, *Leuconostoc,* and *Lactobacillus* spp. Dextransucrase, also known as EC 2.4.1.5, is an extracellular glucosyltransferase, which is able to catalyze D-glucopyranosyl residues transfer from sucrose to dextran, while fructose is released. This enzyme is constitutively produced by *Streptococcus* spp., while its synthesis is usually inducible by growth on sucrose in wild-type strains of *L. mesenteroides*. Dextran is formed via glucosyl intermediates. Glucosyl moieties are transferred to the reducing end of a growing glucanosyl chain, which is covalently linked to the enzyme active site (Badel et al. 2011).

4.2 The Wzx/Wzy-Dependent Pathway

Compared with the extracellular synthesis pathway, the Wzx/Wzy-dependent pathway presents longer steps (five steps), more enzymes, and more interacting sites enabling thus the formation of a wide structure variability of EPS (exclusively HePS). The Wzx/Wzy-dependent pathway steps include, according to Zhou et al. (2019), the following:

- Monosaccharides and disaccharides transportation and phosphorylation either via phosphotransferase system (PTS)-assisted (which can synchronically provide the transport and the phosphorylation) or permease-assisted for the transport of monosaccharides or disaccharides additionally assisted by a specific kinase for further phosphorylation.
- Activation of monosaccharides through the formation of sugar nucleotides. Firstly, hexokinase phosphorylates glucose to obtain glucose-6-phosphate. Phosphoglucomutase intermediates then the transition from glucose-6-phosphate to glucose-1-phosphate, and then glucose-1-phosphate can be converted into UDP-glucose by UDP-glucose pyrophosphorylase.
- Synthesis of the repeating units of the biopolymer. Several glycosyltransferases catalyze repeating units using the activated sugar molecules as substrates and assemble the formed repeating units attached to undecaprenyl diphosphate anchor (C55), which is an isoprenoid lipid carrier located in the inner membrane surface to form repeating units.
- The transfer of repeating units from intracellular to extracellular surface by flippase (Wzx).
- Polymerization of the repeating units catalyzed by an outer membrane polymerization protein (Wzy) to obtain long chain and release of this latter.

Often, not only one type of EPS is produced as final product but a mixture of different polysaccharides is produced, each synthesized by a gene cluster. EPS structure and yield of production closely depend on the availability of precursors encoded by these genes. Gene clusters can be located either on the chromosome or on plasmids (which could explain the high production instability). It is worth pointing that different strains of the same specie could have different EPS gene clusters and various EPS characters (Zhou et al. 2019). Indeed, two groups of enzymes are implicated in EPS biosynthesis: not EPS-specific enzymes, which allow sugar nucleotides production (UDP-Glc, dTDP-Rha, and UDP-Gal); and EPS-specific enzymes regulated by specific genes whose organization is well studied for LAB (Badel et al. 2011). It is worth noting that EPS biosynthesis is an energy-dependent process and differs from one genus or specie to another.

5 Production and Isolation of Exopolysaccharides

Screening for EPS-producing bacteria could firstly occur by visualizing their phenotype (either ropy or mucoid). Strains grown on agar media plates and capable of forming a long filament when colonies are extended using an inoculation loop and those grown in liquid media and are resistant to flow through pipettes are known as ropy strains. Whereas, mucoid strains show glistening and slimy appearance on solid plates and are not able to form long filament when using the inoculation loop (Ruas-Madiedo and Reyes-Gavilan 2005).

As for the EPS production, bacteria are grown in liquid cultures in flasks for small-scale production or in fermenters for large-scale production (batch, fed-batch,

or continuous). Nevertheless, when using these fermentation processes, several challenges could be faced including the significant increase of the broth viscosity causing thus a nonuniform mixing and heat and oxygen transfer (De Vuyst and Degeest 1999).

As the carbon source of the fermentation broth represents the highest expense, the use of effective, eco-friendly, and cheap substrates such as agricultural and industrial by-products (e.g., vegetable and fruit pomaces, molasses, olive mill wastewaters, etc.) is of a great interest. Several EPS, including dextran and levan, have been produced using syrups and molasses as carbon sources since they are rich in sucrose and nutrients, readily available, and low-cost. Since the composition of the culture medium can notably affect both of the EPS production yield and its molecular characteristics, besides the possibility of interference between the medium components and the chemical analysis of EPS sample, it is crucial to adequately and carefully choose the composition of the culture medium for EPS production (e.g., carbon and nitrogen sources) (Ruas-Madiedo and Reyes-Gavilan 2005). The culture parameters optimization enables not only higher bacterial growth but also higher EPS production yield. Varying the fermentation conditions could cause different types of EPS synthesis that may differ either in their Mw or in their chemical composition. Nevertheless, prolonged incubation time could have adverse effects on the yield of EPS production because of their degradation by the produced glycosyl-hydrolases (Wang et al. 2014).

After microbial fermentation, different methods could be used in order to recover the produced EPS including chemical treatment (cation-exchange resin, ethylenediaminetetraacetic acid (EDTA), etc.), physical treatment (centrifugation, heating, ultrasound, etc.), or coupling chemical and physical methods, which could allow to improve the EPS isolation (e.g., coupling ultrasounds with varying pH, solvent, temperature, etc.) (Donot et al. 2012). Often, EPS isolation occurs by removing as a first step cellular biomass either by centrifugation or filtration. Then, proteins are removed by precipitation using either trichloroacetic acid (TCA) (final concentrations ranging between 4 and 20% (w/v)) or HCl (12 M) at 70 °C. Proteins could also be removed using proteases followed by an inactivation step via thermal treatment (Leroy and De Vuyst 2016). EPS is then precipitated using the adequate organic solvent (methanol, ethanol, acetone, isopropanol) and the recovered pellets are dissolved in ultrapure water and then dialyzed against ultrapure water for 2 to 4 days at 4 °C in order to remove salts and simple carbohydrates (such as the residual lactose co-precipitated with EPS). Other contaminants can be eliminated through dissolving EPS in 0.3 M NaOH followed by centrifugation. EPS is then lyophilized. If further purification is required, the crude EPS could be subjected to additional solvent precipitation, ion-exchange chromatography, size exclusion chromatography, followed by a final drying step (Leroy and De Vuyst 2016).

EPS, mostly constituted by carbohydrates, could contain several other components (proteins, DNA, and lipids) (Flemming and Wingender 2010):

- **Proteins**: The produced EPS often contain proteins and enzymes. Enzymes could be implicated in the degradation of EPS (either from the same producing strain or

from another enzyme-producing species) especially during starvation. Structural proteins as, for examples, lectins, cell-surface-associated proteins could be involved in the formation of the matrix network and could also facilitate the link between bacterial and extracellular surfaces (Flemming and Wingender 2010). Glycoproteins could also be detected, which could be involved in different cellular functions such as the maintenance of the structural integrity and the intercellular communication.

- **Extracellular DNA**: Extracellular DNA could be detected in the composition of EPS in variable amounts depending on the producing species. DNA could be involved in biofilm formation and in the horizontal gene transfer between cells of the biofilm (More et al. 2014).
- **Lipids**: Some EPS could have a hydrophobic nature due to the presence of lipids or lipid derivatives, which could be involved in the adhesion properties of the cells. Besides, they could have surface active properties (Flemming and Wingender 2010).

6 Chemical Characterization of Exopolysaccharides

The EPS structure determination is a crucial step to provide information about the physicochemical and biological properties of this macromolecule and its possible industrial applications. Preliminary assays are firstly carried out to estimate the contents of total carbohydrates using the method of phenol sulfuric acid with glucose as a standard as reported by Dubois et al. (1956), uronic acids using spectrophotometric method with glucuronic acid as a standard (Dische and Shettles 1948), and proteins using Lowry method as described by Lowry et al. (1951) with bovine serum albumin (BSA) as a standard.

The primary structure of the produced EPS can be obtained by combining various physical and chemical techniques. Chemical degradation and derivatization are generally used associated with chromatographic analyses together with mass spectrometry in order to assess the monosaccharide composition, the absolute configuration, as well as the presence of some possible noncarbohydrate substituents:

- **Monosaccharide composition:** The monomer composition of EPS is among the first required tests for the polymer structure identification. Both qualitative and quantitative identification of monomers, oligosaccharides, and uronic acids can be carried out by acid hydrolysis of the biopolymer followed by high-performance liquid chromatography (HPLC) with refractive index (RI)/UV detection (Kambourova et al. 2015). Gas chromatography associated with mass spectroscopy (GC-MS) is another sensitive and widely used method consisting of analyzing the produced alditol acetates. However, amino sugars that could be present in some EPS could be underestimated after the thermal decomposition step. This problem could be avoided when using alternative techniques for the determination of monosaccharide composition as, for example, the use of high

performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD).

- **Linkage analysis:** The type of linkages that unit monomers can be determined by methylation and GC-MS analyses. According to previous reports, it appears that the most common types of linkage found between monosaccharides are β-1,4 or β-1,3 exhibiting structural rigidity, whereas α-1,2 or α-1,6 mainly provide flexible structures (Kambourova et al. 2015).
- **FTIR analysis**: Fourier-transform infrared spectroscopy (FTIR) is commonly used to determine the functional groups and bonds in the studied EPS sample. Indeed, molecules show specific frequencies at which they are able to rotate or to vibrate. The interpretation of infrared spectra requires the correlation of the absorption bands of an unknown polymer with identified absorption frequencies for different bonds (Srikanth et al. 2015).
- **NMR spectra**: Results related to the linkages obtained by the GC-MS analysis are generally combined with those of NMR (Nuclear Magnetic Resonance) spectroscopy, which are also often used for the elucidation of the overall secondary structure of the polymer by providing glycosidic linkage types, ring conformation type (furanose or pyranose), anomeric configuration (α/β), and the relative orientations of each monomer with the other. Firstly, the anomeric proton chemical shifts are determined providing an initial estimation of the monosaccharides number. The number of the resonances in the ^{13}C 1D-NMR corresponding to the anomeric C1 may confirm the number of the different residues present in the polymer. If this latter comprises more than three monomers, the complexity of signals in the region of the ring proton can be avoided by using the 2D-NMR spectroscopy, which simplifies the most crowded regions of the spectrum. 2D-NMR analyses include:
  - Homonuclear (^{1}H-^{1}H) and heteronuclear (^{13}C-^{1}H or ^{31}P-^{1}H) correlation spectroscopies (Homonuclear Correlation Spectroscopy (COSY), Total Correlation Spectroscopy (TOCSY), Heteronuclear Single Quantum Correlation (HSQC), and Heteronuclear Multiple Bond Correlation (HMBC)) during which a transfer of magnetization from one spin to another occurs through the scalar coupling between them.
  - Through-space correlation spectroscopies (Nuclear Overhauser Effect Spectroscopy (NOESY) and Rotating-frame nuclear Overhauser Effect Spectroscopy (ROESY)) during which dipolar couplings between protons occur.

Accordingly, results obtained by both monodimensional and bidimensional spectra provide information about the anomeric configuration of each residue. Indeed, a $^{3}JH1,H2$ ranged between 2 and 3 Hz and a $^{3}JH1,C1 > 170$ Hz may indicate the presence of an α anomeric proton, whereas a $^{3}JH1,H2$ ranged between 8 and 10 Hz and a $^{3}JH1,C1 < 165$ Hz may indicate the presence of a β anomeric proton. Then, the glycosylated positions may be determined by comparison with reference values of unsubstituted monosaccharides and the linkage positions by the carbons chemical shift values. In fact, compared with standard values, carbons involved in the linkage present a downfield shift of 8–10 ppm, whereas the adjacent carbons are upfield shifted of 1–3 ppm.

- **Mw determination:** The Mw of EPS is commonly known as a contributing factor in its functionality (De Vuyst and Degeest 1999). Gel permeation chromatography has been often used for this purpose. However, this technique requires high quantity of EPS sample, which makes it more suitable to be used for the purification of EPS. Currently, high performance size exclusion chromatography with multi-angle laser light scattering (HP-SECMALLS) is being used for the determination of EPS-Mw. Both methods work with similar principle of weight and size separation. Nonetheless, when using HP-SECMALLS, the sample is run through smaller analytical scale columns before passing through UV light detectors that provide details about the presence of any residual nucleic acids or proteins in the polymer. The accurate Mw is then determined by a coupled system of MALLS and differential refractive index detectors without any requirement of standard materials (Badel et al. 2011).
- **X-ray spectra:** The crystalline or amorphous nature of a polysaccharide can be determined by X-ray powder diffraction (XRD), which is a rapid analytical method widely used for phase identification of EPS. Energy dispersive X-ray spectroscopy is among the variants of X-ray fluorescence spectroscopy that is used for the elemental analysis of the polysaccharide. It has been reported that crystalline domains are able to reinforce grid and to enhance the thermostability of EPS.

7 Techno-Functional Properties of Exopolysaccharides

EPS have been widely applied in different industrial sectors owing to their interesting functional properties (Table 1) such as:

- Water holding capacities: Syneresis problems in foods could be avoided by using EPS with high water-holding capacity (WHC), which could also be of great interest for the microbial safety of the product. EPS have been extensively used in dairy products, in particular cheese and yoghurt. EPS greatly affect textural and physical properties of yoghurt and improve sensory properties such as shininess, mouthfeel, ropiness, clean cut, and creaminess. EPS can enhance yoghurt WHC by interacting with proteins and micelles forming thus a three-dimensional network structure with a good ability to retain moisture, reduce the serum permeability through skim milk gel (Hassan 2008), decrease the syneresis, improve the stability of the product, and avoid textural and sensory defects in low-fat products (Mende et al. 2016).
- Emulsifiers and stabilizers: An efficient emulsifier is characterized by its ability to adsorb to the interface, significantly decrease the interfacial tension, and form an interfacial protective layer preventing droplets from flocculation. Numerous factors can affect food emulsions, including the aqueous phase rheology, droplet charge and size, the type of the used emulsifier, ionic strength, temperature, and pH. The dispersed polymer undergoes a rearrangement in order to orient its hydrophobic groups toward the oil phase and its polar groups to the aqueous phase, which could explain the importance of the biopolymer conformation and

Table 1 Examples of EPS from LAB

EPS	Monomer units	Main linkages (branching linkages)	Organism	Functional properties
Dextran	Glucose	α-1,6 (α-1,3, α-1,4, α-1,2)	*L. mesenteroides, L. amelibiosum, Lb. curvatus, L. citreum, P. pentosaceus, Lb. plantarum*	Thickener and gelling agent in syrups, moisture retention agent in confectionary, viscosifier, sugar crystallization inhibitor, desirable texture, and softness in bakery products
Reuteran	Glucose	α-1,4 α-1,6	*Lb. reuteri*	Thickener in dairy products, texturizer in bakery products
Inulin	Fructose	β-2,1 (β-2,6)	*St. mutans, St. salivarius, L. mesenteroides, Lb. reutri, L. citreum, Lb. johnsonii*	Prebiotic ingredient, fat replacer
Levan	Fructose	β-2,6 (β-2,1)	*L. pseudomesenteroides, St. salivarius, Lb. reutri, St. mutans, L. mesenteroides, Lb. sanfranciscensis*	Prebiotic ingredient, water holding agent, carrier of flavors, viscosifier and stabilizer in ice creams, beverages, and confectionary, desirable texture and taste of bakery products, emulsifier, thickener
Kefiran	Glucose, galactose	(1,6)-Glc,(1,3)-Gal,(1,4)-Gal, (1,4)-Glc & (1–2,6)-Gal with branching attached to O-2 of Gal and Glc residues in the end of the chain	*Lb. kefirgranum, Lb. parakefir, Lb. kefir, Lb. delbruekii, St. thermophilus, L. mesenteroides, Lc. lactis*	Viscosifier, antimicrobial agent, water holding agent, emulsifier, stabilizer

its molecular structure. Most of the polysaccharides are however hydrophilic open structures and with high Mw making them weak or non-surface-active polymers (McClements 2016). EPS can act to stabilize oil-in-water emulsions owing to their thickening and gelling properties by forming a macromolecular barrier between the dispersed droplets leading to an extended network in the

continuous phase. This latter becomes highly viscous and can even form a gel (Maalej et al. 2016). Emulsion stabilizers can be classified into non-adsorbing polysaccharides (without or with limited surface activity) enhancing the emulsion stability by gelling or increasing the viscosity of the aqueous phase and those displaying surface/interfacial activity able to adsorb at the oil droplet surface, which can be due to the presence of hydrophobic groups or the presence of proteins in their composition and then prevent droplet flocculation and coalescence through electrostatic and/or steric repulsive interactions.

- Rheological properties: EPS can provide unique rheological properties, making their use as functional ingredients in several industrial areas of great interest. These properties are highly influenced by the molecular and physicochemical properties of the macromolecule. For instance, the higher the Mw, the higher the viscosity is (Prasanna et al. 2012). EPS are known by their high thickening and shear thinning properties as well as their considerable intrinsic viscosities. Indeed, two rheological properties can be found: viscosity and elasticity. The viscosity describes the capacity of a solution to resist deformations, while the elasticity describes its ability to regain its initial conformation after a deformation occurs. Both viscosity and elasticity highly affect the product organoleptic properties including its appearance and mouthfeel. In addition, they are crucial for several processes such as mixing, pumping, and pouring during which different shear rates are applied. Polysaccharides having non-Newtonian shear thinning behavior are widely used in the food industry such as for cakes, syrups, puddings, dairy products, etc. (Jindal et al. 2011). When the biopolymer is added in sufficient amounts, most of the shear-thinning molecules (also known as hydrocolloids or gums) dispersions exert three viscous responses over a shear rate range: the biopolymer initially exhibits Newtonian properties at low-shear rates with a constant zero-shear viscosity ($\eta 0$), followed by a shear-thinning behavior according to the power law model where the viscosity decreases when increasing the shear rate and finally a constant infinite-shear-viscosity ($\eta \infty$) is attained at high shear rates. These three responses could originate from rearrangements occurred in the biopolymer conformation due to shearing. Numerous fermented dairy products containing EPS have shown their capacity to influence product rheology such as dahi, yoghurt, cheeses, sour cream, kefir, etc. (Patel and Prajapat 2013).

8 Biological Properties of Exopolysaccharides

EPS exhibit numerous great benefits on consumer health (Fig. 2) including:

- Antitumor activities: The antitumor activity of EPS could be associated with direct killing effects on tumor cells or indirect immunity regulating effects. The former has been observed for EPS produced by *Lb. plantarum* 70,810 that was able to significantly inhibit HepG-2, BGC-823 and HT-29 tumor cells (Wang et al. 2014). As for the indirect immunity regulating effects, some EPS showed an ability to indirectly activate macrophages to enhance their phagocytosis, facilitate

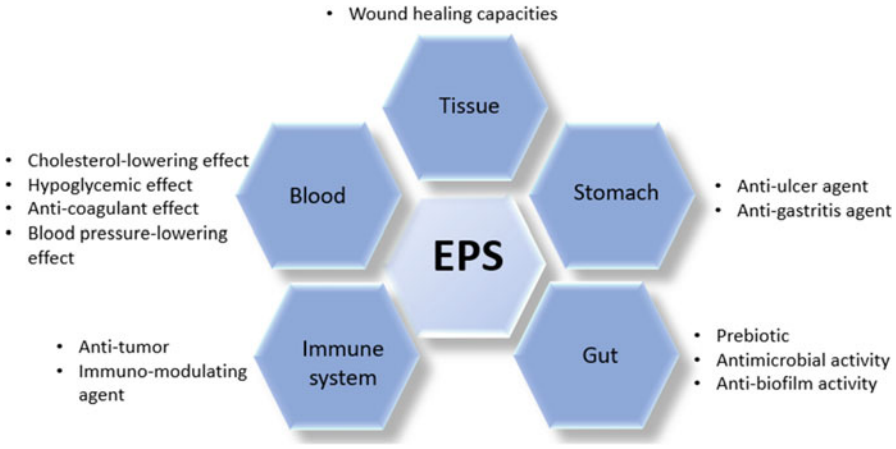


Fig. 2 Examples of the health benefits of EPS from LAB

interferons and pro-inflammatory factors secretion, and inhibit anti-inflammatory factors production besides an ability to stimulate interactions between immunocytes and tumor cells (Pan et al. 2015). EPS from *Lb. plantarum* 70,810 was able to significantly inhibit tumor cells proliferation in vitro (Wang et al. 2014). EPS from *Lb. plantarum* RJF4 showed a capacity to inhibit pancreatic cancer cells without affecting normal cells (Dilna et al. 2015).

- Immunomodulatory effects: Certain EPS produced by LAB have been characterized for their strong immunological reactions. Most of the EPS produced by LAB showed inflammation-regulating activities through modulating immune-related genes, stimulating the proliferation of lymphocytes, activating macrophages, and liberating interleukins, tumor necrosis factors, nuclear factors, and other cytokines (Zhou et al. 2019). Glucan produced by *P. parvulus* 2.6 showed an ability to provoke anti-inflammatory responses through downregulating interleukin-8 (IL-8), tumor necrosis factor-α (TNF-α) expressions, and myeloid differentiation factor-88 (MyD-88) (Pérez-Ramos et al. 2018).
- Antioxidant activities: EPS from LAB have been widely evaluated by both in vitro and in vivo tests. Dilna et al. (2015) showed that EPS produced by *Lb. plantarum* RJF4 exhibited good antioxidant properties. In vivo study of Pan and Mei (2010) proved that administration of EPS from *Lc. lactis* ssp. *lactis* 12 significantly increased the activity of antioxidant enzymes in serums (SOD and CAT) compared with the control, which could be associated with triggering of the gene expressions of both SOD and CAT enzymes. Thus, the complementary structure between EPS and these enzymes may contribute to their interactions. In vitro, EPS molecules are hydrolyzed at acidic environment leading to the production of active hemiacetal hydroxyls and simultaneously transferring electrons to radicals facilitating the conversion of free radicals into stable forms and thus decreasing radicals concentrations (Zhou et al. 2019).

- Cholesterol-lowering abilities: EPS from *Lb. plantarum* RJF4 was capable of lowering cholesterol level (Dilna et al. 2015). Based on both animal and in vitro tests, the mechanism involved in the cholesterol-lowering effect of EPS could include bile elimination, assimilation and conversion of the cholesterol, coprecipitation effect, and promotion of short fatty acids to reduce cholesterol (Ishimwe et al. 2015). These functions could be indirectly exhibited by the intestine colonization by EPS-producing probiotics. As a first step, about 5% of bile acids are synthesized from cholesterol leading thus to an effective decrease of the cholesterol level in blood serum (Begley et al. 2006). Cholesterol could also be absorbed by bacteria and forms one component of the cytoplasmic membrane or it could be converted into other intermediate substances such as 4-cholesten-3-one that could be removed with feces (Gérard 2013). Cholesterol and hydrolyzed bile salts could also coprecipitate at acidic conditions (caused by short-chain fatty acids-producing microorganisms) and be discharged in the feces (Begley et al. 2006). In addition, the key enzyme involved in the cholesterol conversion into acetate could be inhibited (Zhou et al. 2019).
- Prebiotic effect: Prebiotics are commonly defined as "selectively fermented ingredients allowing specific changes, both in the composition and/or the activity in the gastrointestinal microflora that confers benefits upon host wellbeing and health." Three main criteria are generally used to select a prebiotic, namely: its resistance to gastric acidity, hydrolytic enzymes, and gastrointestinal absorption; its fermentation by intestinal microbiota; and its capacity to selectively stimulate beneficial intestinal bacteria growth and/or activity (Roberfroid 2007). Health-promoting bacteria mainly *Lactobacillus* spp. and *Bifidobacterium* metabolize prebiotics as carbon sources to improve host immunity against pathogens (Hongpattarakere et al. 2012). While some EPS could be readily degraded, some others maintain integrity during gastric transit (Ryan et al. 2015). In the colon lumen, EPS must be able to stimulate health-promoting colonic bacteria growth to exert their effects on the host. Hongpattarakere et al. (2012) studied EPS from *W. cibaria* A2 and suggested that it could be served as a potential prebiotic ingredient in foods allowing the modulation of the intestinal microflora of consumers into health beneficial direction. Contrarily, purified EPS produced by *P. parvulus* did not exert prebiotic effects in a mouse model, despite the fact that consuming viable EPS-producing bacteria had an antagonistic effect on *Enterobacteriaceae* and enabled the maintenance of the microbiota homeostasis (Lindstrom et al. 2012). The aforementioned findings suggest that further investigations are required to confirm or refute the capacity of bacterial EPS to modulate the beneficial colonic microflora and associate this modulation with the health effects on the host.

The biological activities of EPS could be enhanced by the presence of substituent groups in the EPS composition including:

- Sulfonation: The most studied modification is sulfating, which was found to be effective and conducive to different biological activities. Antioxidant activity can

be significantly improved by sulfating as the cases of sulfated EPS from *St. thermophilus* GST-6 and *St. thermophilus* ASCC1275. In addition, several sulfated EPS have shown to provide higher inhibiting effects to multiple pathogens than non-sulfated ones (Zhang et al. 2016).

- Phosphorylation: Previous researches demonstrated that the naturally phosphorylated EPS from *Lb. delbrueckii* ssp. *bulgaricus* stimulated mitogenic process of murine splenocytes and Peyer's patches and macrophage cytostatic activity indicating that phosphorylated groups have positive effects on immunity-regulating activities. In fact, it has been reported that the high antitumor and immunity-enhancing activities displayed by sulfated or phosphorylated EPS might be originating from their high affinity to tumor and immunity cells (Liu et al. 2012).
- Acetylization: Acetylated EPS from *Lb. plantarum* 70,810 displayed better antioxidant, anti-HepG-2, and anti-HT-29 activities (Wang et al. 2015).

9 Industrial Applications and Commercialization of Exopolysaccharides

Considerable interest is being paid in screening and developing new bacterial EPS with novel industrial applications in various fields owing to their unique structure and physical properties leading to the production of substantial EPS in the global market such as dextran, levan, etc. For instance, bacterial hyaluronic acid (HA), which was discovered in 1970 is receiving growing interest and is currently the most expensive polymer used in the medical field (US $40,000–60,000 per kg) (Morris and Harding 2009). Interestingly, *Streptococcus* spp. are capable of producing HA identical to that isolated from human or animal bodies (Kambourova et al. 2015).

Several EPS are characterized by their biocompatibility and nontoxicity, which make them promising to be applied in various medical applications and of great interest since the mid-twentieth century. Dextran was the first to be tested as plasma expander in clinical trials and has been then applied as efficient plasma substitute in cases of shock and loss of blood (Amspacher and Curreri 1953).

EPS have been extensively used as emulsion stabilizer, binder, skin conditioner, and viscosifying agent in cosmetic products. They have been likewise used in baby skin products formulations. For instance, levan is known by its excellent moisturizing and skin proliferating capacities (Srikanth et al. 2015). HA is likewise used as an excellent hydrating polymer in cosmetic moisturizers and can also serve to fill tissue spaces in cosmetic surgeries (Morris and Harding 2009).

Bacterial polysaccharides could also be used as depolluting agents since they present sustainable ecofriendly and cost-effective substitutes of chemical flocculants. Application of bacterial EPS in the treatment of wastewater and sludge have been reviewed by More et al. (2014). The anionic nature of some EPS resulting from the presence of anionic functional groups (e.g., sulfhydryl, phosphoryl, carboxyl groups) allows them to capture and form complexes with heavy metals. The presence

of hydrophobic regions could also allow EPS to adsorb various organic pollutants like humic acids, phenanthrene, and benzene.

Before their use in food products, bacterial EPS should occur GRAS status. The wide structural diversity of the produced EPS and their GRAS status (despite their low production yields in some cases) make them extensively and easily used in food industries. Hence, they can be used without any health issues in a wide range of food products including salad dressings, toppings, sauces, processed cheese, and instant foods (Kambourova et al. 2015). Among the best-known EPS approved as food additives under E numbers are:

- Levan: Levan is currently produced by different companies around the world and applied in cosmetics, medicine, feed, and foods. In the food industry, it is extensively applied in dairy products as an excellent emulsifier, stabilizer, as well as flavor enhancer. It can also be applied in nonalcoholic beverages (e.g., ultra-high-fructose syrups). Furthermore, it can be applied as carrier of flavors and fragrances, fat substitute, or as improver of bread texture and taste in bakery products (Zannini et al. 2016). Often, levan is enzymatically or chemically hydrolyzed to obtain small FOS that are also interestingly used in foods as dietary fiber, sweetener, or as prebiotics (Huang et al. 2013).
- Dextran: LAB are well-known dextran producers particularly *L. mesenteroides*, which excrete commercially exploited dextran. Dextran can be applied in ice creams, confectionary, and jams to improve the viscosity, moisture retention, to inhibit sugar crystallization, and to maintain desirable flavor and appearance. It can also be applied in bakery products to improve softness, crumb texture, and loaf volume and to compensate low protein content of wheat flour. Dextran has also been used as stabilizer of frozen and dried foods (meat and fish products, vegetables) or as coating agents against food oxidation (Patel and Prajapat 2013; Zannini et al. 2016).

Hence, those EPS could be directly added to food products as bio-ingredient enabling to choose a specific and desired amount of this polymer at a specific and desired time providing thus the desired physical and functional properties of the end product. Nonetheless, the ex-situ use of other bacterial EPS requires their listing as additives in the ingredients list. Additionally, consumers demand for products with fewer or without additives. Another limitation of the ex-situ use of bacterial EPS is the cost of the isolation and purification steps. Therefore, as an alternative, bacterial EPS could be produced in situ particularly in foods fermentation providing longer shelf life, greater sensory properties by increasing the viscosity, lower syneresis problems, more flavors, etc. (Mende et al. 2016; Zhou et al. 2019). This method could be used for:

- Dairy products: Although the EPS production yield by LAB is relatively low, the in situ produced EPS play a crucial role in fermented dairy products as they provide great low-fat dairy products quality, great texture for yogurt (creamy and smooth), etc. The presence of EPS-producing LAB in yogurt, including stirred yogurt,

provides better texture and higher viscosity compared with the presence of non-EPS-producing strains. In addition, the production of EPS with higher Mw and stiffer conformation provides higher viscosities, which would be interesting since it enables to avoid stabilizers addition that could be prohibited in some countries (Badel et al. 2011). The in situ production of EPS in low-fat mozzarella also showed better properties in terms of water retention and texture (Badel et al. 2011).

- Dough and bread: The use of EPS-producing starter cultures in cereal and bakery products is receiving growing interest since these polymers showed an efficient capacity to replace commercial hydrocolloids like hydroxypropyl methylcellulose (HPMC). EPS-producers are of particular interest to be applied in sourdough (SD), which is a result of a mixture of water and flour naturally fermented by cereal-associated microbiota, typically yeast and LAB. Beneficial effects of EPS producers are observed in dough and bread including higher water absorption, better dough rheology, better maintain of the bread structure, higher volume of loaf, higher crumb softness, and delayed bread staling, allowing thus longer shelf life (Lynch et al. 2018).
- Vegetable-based products: The use of EPS-producing LAB to ferment puréed carrot raw material showed significant changes in viscosity, perceived texture, or both (Juvonen et al. 2015). In the same vein, tomato juice fermentation with EPS-producing *Weissella* sp. showed greater viscosity (Di Cagno et al. 2009).

Hence, bacterial EPS have appreciable potential either when applied as food additives or produced in situ by starter cultures. Once present in a food product, EPS may interact with other food components (e.g., fat particles, minerals, proteins). Interactions of bacterial EPS with proteins and their effects on the final product properties are receiving particular interest.

10 Interactions Between EPS and Proteins

EPS and proteins interactions affect the product properties including its stability, thickening, water holding capacity, and gelling properties. These interactions have been widely discussed by several works (Corredig et al. 2011; Ghosh and Bandyopadhyay 2012). However, these interactions are still among the most challenging area to understand despite the wide advancement achieved in the recent past. Different parameters including the pH, ionic strength, biopolymers conformations, their concentrations, and their charge densities strongly influence biopolymers interactions, which could be either attractive or repulsive (Ghosh and Bandyopadhyay 2012) (Fig. 3):

- Repulsive interactions: When noninteracting polymers (neutral or carrying the same charge) are mixed, either one or two-phase systems could be obtained. The former occurs in dilute solutions and below a critical polysaccharide to protein molar ratio leading to a uniform distribution of the separate polymers throughout the solution. While the latter, known as thermodynamic incompatibility, occurs at higher

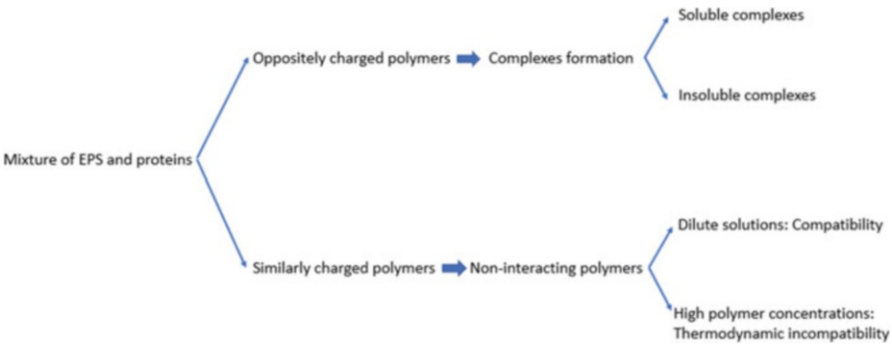


Fig. 3 The possible modes of interaction between polysaccharides and proteins

polymer concentrations through segregative phase separation (a polysaccharide enriched phase and a protein enriched one) resulting either from high repulsive interactions or high steric exclusion.

- Attractive interactions: When polysaccharides and proteins carry opposite charge, they form complexes, which can be either soluble (forming one single phase) or insoluble complexes that precipitate or coacervate to form 2-phase system (one phase is enriched with the formed complexes) through associative phase separation mechanism. These attractive interactions have been reported to be electrostatic and to be affected by the ionic strength and pH of the environment. Indeed, proteins carry positive charge when the pH became below its isoelectric point (pHi) and interact thus with negatively charged polysaccharides forming electrostatic stable complexes (Abid et al. 2019, 2018b) (Fig. 4). Contrarily, when the pH became higher than its pI, negatively charged proteins interact with anionic polysaccharides. Weaker complexes can be formed when the pH became almost equal to pHi of the protein since the surface charge of this latter became near to zero. Complex formation occurs mainly through electrostatic interactions with a secondary role of H bonding and hydrophobic interactions for the stability of the aggregates.

Interactions between polysaccharides and proteins have been reported to enhance the functional properties of protein such as surface activity, solubility, conformational stability, foaming, and emulsifying properties. Particularly, these interactions are of great interest in the dairy industries, as they may affect the structure and texture of dairy products. Different techniques have been used to provide information about the involved mechanisms during structuring of dairy products including rheological techniques, light scattering, spectroscopic, microscopic, nuclear magnetic resonance, and fluorescence recovery after photo-bleaching (Corredig et al. 2011). An example of particular interest that illustrates the changing dynamics during processing is the fermented milk gel by EPS-producing LAB. Indeed, unlike other polysaccharides used as an additive to stabilize the fermented product, LAB gradually produce EPS during milk fermentation, leading to a continuous change of

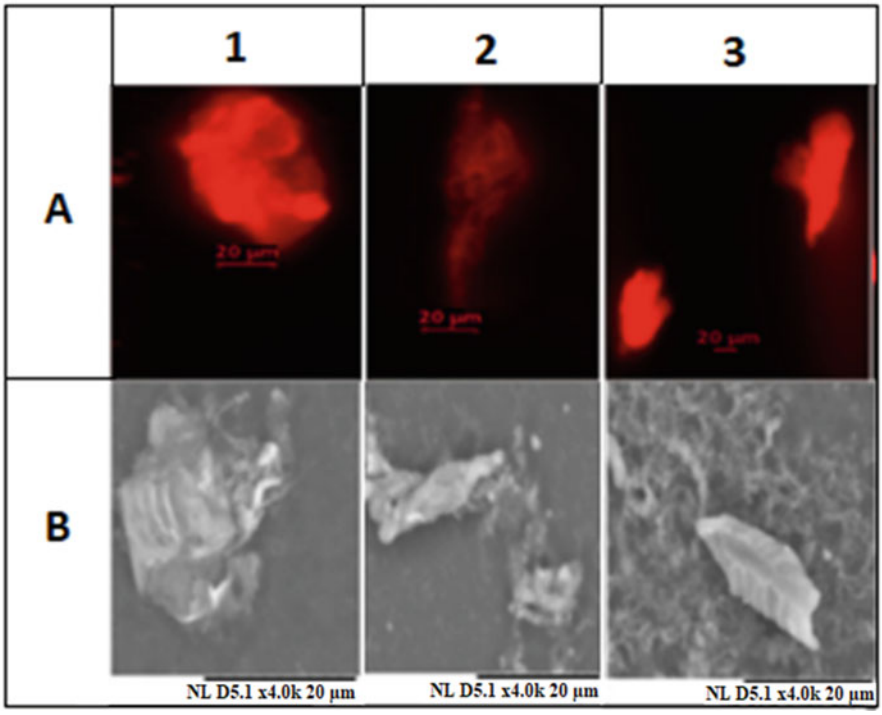


Fig. 4 Epifluorescence microscopic observations (A) and SEM observations of the formed complexes between sodium caseinate and (1): EPS from *Leuconostoc citreum*-BMS, (2): EPS from *L. mesenteroides*-TMS and (3): EPS from *Pediococcus pentosaceus*-DPS (Abid et al. 2018b)

the environmental conditions and of the interactions between both polymers, and both attractive and repulsive interactions may occur (Hassan 2008). It has been suggested that the gradually produced EPS interact with both bacterial cells and milk proteins, leading to continuous changes of the gel rheological properties and the stability of the final product (Hassan 2008). In the case of fermented milk gels, the produced EPS may be located within the protein network pores. Larger pores obtained in fermented milk gels could be attributed to depletion interactions between caseins and EPS, while smaller pores were obtained when using non EPS-LAB producer. These depletion interactions may also provide higher viscosity of the fermented milk. In addition, it has been reported that the presence of EPS enhances the water holding ability of the protein network. For instance, yogurt fermented by EPS-producing strains showed less syneresis and higher viscosity compared to that produced with EPS negative strains (Hassan 2008). These interactions may also influence the gelation pH during fermentation. It has been demonstrated by Girard and Schaffer-Lequart (2007) that EPS with high Mw results in higher gelation pH and higher gel firmness. The same authors found shorter time required for gel formation and higher gel firmness with negatively charged EPS, whereas neutral

EPS did not contribute to strengthening the gel network or in recovering the structure after shear.

Interactions and effects of EPS on food products are in fact very complex since they greatly depend on a wide number of parameters (EPS and protein concentration and their structural features, ionic strength, pH) that change for each type of EPS and each product. Therefore, model systems may only partially prove the multiple interactions in a product, but are still helpful tools to gain general insight.

11 Conclusion

LAB are well known for their capacity to produce and excrete in the extracellular environment EPS with great variety of chemical composition and structure providing thus unique technological properties and health-promoting effects allowing them to be used in various industrial fields. Particularly, EPS have been extensively applied in the food industry, either as safe-food additives or directly produced in situ by starter cultures, mainly as gelling agents, emulsifiers, stabilizers, and potential thickeners avoiding thus the addition of the common commercial stabilizers isolated from plants, animals, or from non-GRAS microbial origin, which could not be allowed in several countries. Nevertheless, the use of these valuable polymers is still limited because of their relatively low production yield. Accordingly, the research interest in EPS production by LAB is continuously focusing on the characterization and expression analyses of the EPS gene cluster, the use of low-cost substrates, the optimization of the culture fermentation conditions, as well as the use of modified strains producing higher levels of EPS.

References

Abid Y, Casillo A, Gharsallah H, et al. Production and structural characterization of exopolysaccharides from newly isolated probiotic lactic acid bacteria. Int J Biol Macromol. 2018a;108:719–28.

Abid Y, Joulak I, Ben Amara C, et al. Study of interactions between anionic exopolysaccharides produced by newly isolated probiotic bacteria and sodium caseinate. Colloids Surf B: Biointerfaces. 2018b;167:516–23.

Abid Y, Gharsallaoui A, Dumas E, et al. Spray-drying microencapsulation of nisin by complexation with exopolysaccharides produced by probiotic *Bacillus tequilensis*-GM and *Leuconostoc citreum*-BMS. Colloids Surf B: Biointerfaces. 2019;181:25–30.

Alves VD, Freitas F, Costa N, et al. Effect of temperature on the dynamic and steady-shear rheology of a new microbial extracellular polysaccharide produced from glycerol byproduct. Carbohydr Polym. 2010;79:981–8.

Amspacher WH, Curreri AR. Use of dextran in control of shock resulting from war wounds. AMA Arch Surg. 1953;66:730–40.

Badel S, Bernardi T, Michaud P. New perspectives for *Lactobacilli* exopolysaccharides. Biotechnol Adv. 2011;29:54–66.

Begley M, Hill C, Gahan CGM. Bile salt hydrolase activity in probiotics. Appl Environ Microbiol. 2006;72:1729–38.

Corredig M, Sharafbafi N, Kristo E. Polysaccharide-protein interactions in dairy matrices, control and design of structures. Food Hydrocoll. 2011;25:1833–41.
De Vuyst L, Degeest B. Heteropolysaccharides from lactic acid bacteria. FEMS Microbiol Rev. 1999;23:153–77.
Di Cagno R, Surico RF, Paradiso A, et al. Effect of autochthonous lactic acid bacteria starters on health-promoting and sensory properties of tomato juices. Int J Food Microbiol. 2009;128: 473–83.
Dilna SV, Surya H, Aswathy RG, et al. Characterization of an exopolysaccharide with potential health-benefit properties from a probiotic *Lactobacillus plantarum* RJF4. LWT Food Sci Technol. 2015;64:1179–86.
Dische Z, Shettles LB. A specific color reaction of methylpentoses and a spectrophotometric micromethod for their determination. J Biol Chem. 1948;175:595–603.
Donot F, Fontana A, Baccou JC, et al. Microbial exopolysaccharides: main examples of synthesis, excretion, genetics and extraction. Carbohydr Polym. 2012;87:951–62.
DuBois M, Gilles KA, Hamilton JK, et al. Colorimetric method for determination of sugars and related substances. Anal Chem. 1956;28:350–6.
Flemming HC, Wingender J. The biofilm matrix. Nat Rev Microbiol. 2010;8:623–33.
Freitas F, Alves VD, Reis MAM. Advances in bacterial exopolysaccharides: from production to biotechnological applications. Trends Biotechnol. 2011;29:388–98.
Gérard P. Metabolism of cholesterol and bile acids by the gut microbiota. Pathogens. 2013;3:14–24.
Ghosh AK, Bandyopadhyay P. Polysaccharide-protein interactions and their relevance in food colloids. In: Karunaratne DN, editor. The complex world of polysaccharides. London: IntechOpen; 2012. p. 395–408.
Girard M, Schaffer-Lequart C. Gelation and resistance to shearing of fermented milk: role of exopolysaccharides. Int Dairy J. 2007;17:666–73.
Hassan AN. ADSA Foundation Scholar Award: possibilities and challenges of exopolysaccharide-producing lactic cultures in dairy foods. J Dairy Sci. 2008;91:1282–98.
Hongpattarakere T, Cherntong N, Wichienchot S, et al. *In vitro* prebiotic evaluation of exopolysaccharides produced by marine isolated lactic acid bacteria. Carbohydr Polym. 2012;87: 846–52.
Huang MY, Lee CF, Ho ST, et al. High-yield levan produced by *Bacillus licheniformis* FRI MY-55 in high-sucrose medium and its prebiotic effect. J Pure Appl Microbiol. 2013;7:1585–99.
Ishimwe N, Daliri EB, Lee BH, et al. The perspective on cholesterol-lowering mechanisms of probiotics. Mol Nutr Food Res. 2015;59:94–105.
Jindal N, Singh DP, Khattar JIS. Kinetics and physico-chemical characterization of exopolysaccharides produced by the cyanobacterium *Oscillatoria formosa*. World J Microbiol Biotechnol. 2011;27:2139–46.
Juvonen R, Honkapää K, Maina NH, et al. The impact of fermentation with exopolysaccharide producing lactic acid bacteria on rheological, chemical and sensory properties of pureed carrots (*Daucus carota L.*). Int J Food Microbiol. 2015;207:109–18.
Kambourova M, Oner ET, Poli A. Exopolysaccharides from prokaryotic microorganisms – promising sources for white biotechnology processes. In: Pandey A, Hofer R, Taherzadeh M, et al., editors. Industrial biorefineries and white biotechnology. Amsterdam: Elsevier; 2015. p. 523–54.
Leroy F, De Vuyst L. Advances in production and simplified methods for recovery and quantification of exopolysaccharides for applications in food and health. J Dairy Sci. 2016;99:3229–38.
Lindstrom C, Holst O, Nilsson L, et al. Effects of *Pediococcus parvulus* 2.6 and its exopolysaccharide on plasma cholesterol levels and inflammatory markers in mice. AMB Express. 2012;2:66–75.
Liu J, Luo J, Ye H, et al. Preparation, antioxidant and antitumor activities *in vitro* of different derivatives of levan from endophytic bacterium *Paenibacillus polymyxa* EJS-3. Food Chem Toxicol. 2012;50:767–72.

Lowry OH, Rosebrough NJ, Farr AL, et al. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951;193:265–75.
Lynch KM, Coffey A, Arendt EK. Exopolysaccharide producing lactic acid bacteria: their techno-functional role and potential application in gluten-free bread products. Food Res Int. 2018;110: 52–61.
Maalej H, Hmidet N, Boisset C, et al. Rheological and emulsifying properties of a gel-like exopolysaccharide produced by *Pseudomonas stutzeri* AS22. Food Hydrocoll. 2016;52:634–47.
McClements D. Food emulsions. USA: CRC Press; 2016.
Mende S, Rohm H, Jaros D. Influence of exopolysaccharides on the structure, texture, stability and sensory properties of yoghurt and related products. Int Dairy J. 2016;52:57–71.
More TT, Yadav JSS, Yan S, et al. Extracellular polymeric substances of bacteria and their potential environmental applications. J Environ Manag. 2014;144:1–25.
Morris G, Harding S. Polysaccharides, microbial. In: Schaechter M, editor. Encyclopedia of microbiology. San Diego: Academic; 2009. p. 482–94.
Pan D, Mei X. Antioxidant activity of an exopolysaccharide purified from *Lactococcus lactis* subsp. *lactis* 12. Carbohydr Polym. 2010;80:908–14.
Pan D, Liu J, Zeng X, et al. Immunomodulatory activity of selenium exopolysaccharide produced by *Lactococcus lactis* subsp. *Lactis*. Food Agric Immunol. 2015;26:248–59.
Patel A, Prajapat JB. Food and health applications of exopolysaccharides produced by lactic acid bacteria. Adv Dairy Res. 2013;1:107–14.
Pérez-Ramos A, Mohedano ML, Pardo MÁ, et al. β-Glucan-producing *Pediococcus parvulus* 2.6: test of probiotic and immunomodulatory properties in zebrafish models. Front Microbiol. 2018;9:1684–92.
Prasanna PHP, Bell A, Grandison AS, et al. Emulsifying, rheological and physicochemical properties of exopolysaccharide produced by *Bifidobacterium longum* subsp. *infantis* CCUG 52486 and *Bifidobacterium infantis* NCIMB 702205. Carbohydr Polym. 2012;90:533–40.
Roberfroid M. Prebiotics: the concept revisited. J Nutr. 2007;137:830S–7S.
Ruas-Madiedo P, Reyes-Gavilan CGDL. Invited review: methods for the screening, isolation, and characterization of exopolysaccharides produced by lactic acid bacteria. J Dairy Sci. 2005;88: 843–56.
Ryan PM, Ross RP, Fitzgerald GF, et al. Sugar-coated: exopolysaccharide producing lactic acid bacteria for food and human health applications. Food Funct. 2015;6:679–93.
Srikanth R, Reddy CHSSS, Siddartha G, et al. Review on production, characterization and applications of microbial levan. Carbohydr Polym. 2015;120:102–14.
Wang K, Li W, Rui X, et al. Characterization of a novel exopolysaccharide with antitumor activity from *Lactobacillus plantarum* 70810. Int J Biol Macromol. 2014;63:133–9.
Wang K, Li W, Rui X, et al. Chemical modification, characterization and bioactivity of a released exopolysaccharide (r-EPS1) from *Lactobacillus plantarum* 70810. Glycoconj J. 2015;32:17–27.
Zannini E, Waters DM, Coffey A, et al. Production, properties, and industrial food application of lactic acid bacteria-derived exopolysaccharides. Appl Microbiol Biotechnol. 2016;100: 1121–35.
Zhang J, Cao Y, Wang J, et al. Physicochemical characteristics and bioactivities of the exopolysaccharide and its sulphated polymer from *Streptococcus thermophilus* GST-6. Carbohydr Polym. 2016;146:368–75.
Zhou Y, Cui Y, Qu X. Exopolysaccharides of lactic acid bacteria: structure, bioactivity and associations: a review. Carbohydr Polym. 2019;207:317–32.

Isolation of Microbial Polysaccharides

20

Namita Jindal, Jasvirinder Singh Khattar, and Davinder Pal Singh

Contents

Abstract

Microbial polysaccharides comprise of cellular, structural, and exopolysaccharides (EPS). Microbial EPS are a structurally very diverse class of polymers. A number of these molecules have found applications in different fields that extend from medicine, food, and cosmetics, on the one hand, to construction, drilling, and chemical industry, on the other hand. In view of this, identification of new microbial strains that produce maximum amount of novel polysaccharide has been a major area in the recent past. Observing the fact that nearly all microbes have the genetic and metabolic machinery for the production of polysaccharides under specific conditions, there is a need for high throughput screening techniques helpful for identifying novel variants of microbial EPS with properties superior to the already described ones, or even totally new ones. For the optimum cost-effective use of EPS in various applications, not only we should have EPS

N. Jindal · J. S. Khattar (✉) · D. P. Singh
Department of Botany, Punjabi University, Patiala, India

J. M. Oliveira et al. (eds.), *Polysaccharides of Microbial Origin*,
https://doi.org/10.1007/978-3-030-42215-8_27

producing microbial strains but the process optimization for the isolation, extraction, and purification is also very important so as to get purified EPS with its native structure. A number of physical and chemical methods available for this purpose are discussed in this chapter. No single method is suitable for different microbial EPS, and one has to select and optimize a suitable method for a particular EPS/microorganism.

Keywords

Isolation · Microbial · Polysaccharides · Purification · Screening

1 Introduction

Conventionally higher plants and macroalgae are good sources of polysaccharides which include functional starch, galactomanan, pectin, carrageenan, agar, etc. (Vroman and Tighzert 2009). Compared to higher plant polysaccharides, polysaccharides from microorganisms can be produced in short time, and there is no requirement of large land area for cultivation of these microorganisms. The major microbial sources of these biopolymers include microalgae, bacteria, and fungi. Out of these, till date, bacteria have proved to be the best source of polysaccharides having different structures with different functional properties and varied applications in different industries. Xanthan, gellan, alginate, glucans, hyaluronan, succinoglycan, and levan are the polysaccharides of microbial origin (Zhao et al. 2017).

Microbial polysaccharides are high-molecular weight compounds and are commonly found in three forms, i.e., exocellular polysaccharides, structural polysaccharides, and intracellular ones. Exocellular ones are so named because these are secreted outside the cell wall and are thus easy to isolate. In this chapter, we will mainly focus on exocellular or extracellular polysaccharides (EPS); these polysaccharides have tremendous applications in food, pharmaceutical, cosmetic, biomedical, etc. industries and have potential for biotechnological applications (Sutherland 1998). This has led to keen interest in production, isolation, and purification of polysaccharides produced by different microorganisms. The production of microbial polysaccharides is increasing rapidly because of growing demand for biobased polymers. Polysaccharides derived from microbes have proved to be a good alternative to synthetic or other natural polymers already being used in various industries as thickening, suspending, and gelling agents (Ogaji et al. 2012). These functional properties of the microbial polysaccharides are mainly based on their chemical structure and their tendency to interact with other molecules via hydrogen bonding, ionic effect, etc.

2 Physical Properties and Chemical Structure

Applications of microbial polysaccharides in industry are mainly determined by their distinctive physical properties (Kumar et al. 2007). Microbial polysaccharides are long-chain, branched, or unbranched polymers, ionic or nonionic in nature with

different molecular weight and structures. Usually, the microbial exopolysaccharides can be differentiated in two important groups considering their construction units, namely homopolysaccharides and heteropolysaccharides (Donot et al. 2012). Out of these two types, the homopolysaccharides comprise of only one monosaccharide; the heteropolysaccharides are composed of three to ten dissimilar monosaccharides. The monosaccharides making the polysaccharides are most commonly pentoses and hexoses or their derivatives. The monosaccharides mainly present in microbial polysaccharides are D-glucose, D-galactose, and D-mannose; L-fucose and L-rhamnose; and *N*-acetyl-D-glucosamine and N-acetyl-D-galactosamine. Besides, these molecules usually have amino sugars, uronic acids, peptides, etc. (Kumar and Mody 2009; Poli et al. 2010; Caruso et al. 2018, 2019; Jindal and Khattar 2018). Presence of different linkages in polysaccharides, variations in monomer composition, and arrangement along with presence of varied types of linkages result in broad range of shapes and structure of polysaccharides. The complex and entangled structure of any high-molecular weight microbial polysaccharide is mainly considered responsible for its unique and different physical properties. The varied physical properties of polysaccharides are mainly determined by their monosaccharide composition, glycosidic linkage, molecular weight, etc. (Kothari et al. 2015). Presence of the acyl groups either as ester-linked acetate or ketal-linked pyruvate in some microbial exopolysaccharides influences the structures and physical properties of these polymers. Ester-linked O-acetyl group as a representative of organic substituent in microbial polysaccharides structure does not change their overall charge, but the ketal-pyruvate can contribute to charge of these polymers (Sutherland 1990).

3 Polysaccharides Screening Approaches

In order to get the full potential of polysaccharides, fast and reliable screening methods are required to identify EPS producing strains and to isolate novel polysaccharides with unique properties to enhance their applicability. Keeping this in mind, here we describe the most common and publicly available screening approaches that have been used to confirm the presence of EPS.

3.1 Screening Approaches for Solid Media

3.1.1 Detection of EPS-Producing Phenotypes

This technique is the most commonly used method for the last many years to identify bacteria which are able to produce EPS. Generally, EPS producing strains form colonies which may be ropy, mucoid, or slimy. The characteristic feature of "ropy" colony is that it shows high resistance to flow through serological pipette and forms viscous strands during "free fall" from the pipette tip (Vedamuthu and Neville 1986; Van den Berg et al. 1993). These ropy colonies also form long filaments when extended with an inoculation loop (Duenas et al. 1995; Dierksen et al. 1997). The "mucoid" colonies have a glazed and slimy appearance on agar plates and do not form filament-like structure during the above process. Another good approach to

evaluate mucoid and slimy colonies is to compare colony morphology among induced and noninduced EPS production. This method is suitable for those strains which show extracellular sucrase activity, which is inducible in nature and depends on substrate. Thus, when sucrose and raffinose are supplemented in the medium, these induce glucan- and fructan-sucrase in EPS producers (Rühmann et al. 2015a).

3.1.2 Agar-Plates with Dyes

Some dyes interact with polysaccharides with different specificity. These dyes can be used to screen EPS producers on agar plates. For example, Aniline Blue fluorochrome (Sinofluor) shows an intense fluorescence when bound to β-(1-3)-glucans (Evans et al. 1984; Ma and Yin 2011). Calcofluor White dye binds to β-(1-3) and β-(1-4)-glucans as well as succinoglycan and exhibits a blue-green fluorescence under UV light. Congo Red specifically interacts with β-(1-3)- and β-(1-4)-glucans (Wood and Fulcher 1978). The use of dyes to screen EPS-secreting microorganisms can be very useful and economical, but this dye-screening technique is useful for few defined polymers.

3.2 Screening Approaches for Liquid Media

3.2.1 Precipitation

Some screening approaches for EPS identification are suitable for liquid media. Most EPS are highly soluble in aqueous solutions, and their solubility can be decreased by using miscible solvents. Common EPS such as xanthan gum, gellan gum, welan gum, diutan gum, succinoglycan, etc. are precipitated with alcohols or acetone (Phillips and Williams 2000). The efficiency of precipitation of a particular polysaccharide depends on its chemical structure, molecular weight, and final concentration, and the solvent used for precipitation (Swennen et al. 2005). But it needs to be noted that other molecules such as DNA, RNA, and proteins also get precipitated in the same manner (Schmid et al. 2013; Kreyenschulte et al. 2014). Appearance of precipitate can also be taken as a distinguishing feature for polysaccharides as these usually precipitate in the form of fibers, when ethanol or 2-propanol is used as precipitant. But not all polysaccharides are precipitated under the same conditions with alcohols or acetone (Sutherland 1990; Azeredo and Oliveira 1996). This is considered as a limiting factor when the precipitation is taken as the only process to search novel EPS producers. Even then, precipitation process is fast, easy to handle, and purifies the EPS from the cultivation media (Kumar et al. 2007). Dry weight of the EPS precipitate also gives an indication of amount of EPS produced; thus, high EPS producing strains can be screened.

3.2.2 Viscosity

Viscosity of the liquid culture is also taken as one property which has been used for the evaluation of EPS production. All strains that showed “ropy” liquid culture and had a long string formed by the cell deposit are considered to be EPS forming strains (Garai-Ibabe et al. 2010). Other methods to screen strains are based on sensory and

rheological properties (Folkenberg et al. 2006). Ricciardi et al. (1997) developed a rapid and convenient screening method for viscosity of lactic acid bacteria cultures based on the measurement of efflux time in microhematocrit (MH) capillaries. One advantage of this is that only small volumes of samples are required, but manual handling makes it sometimes difficult.

3.2.3 Specific Carbohydrate Screening

Mojica et al. (2007) advocated that specific polysaccharides can be targeted by screening uronic acids in the polymers. Hydrolysis of the EPS sample is combined with development of color by a reaction with uronic acids present in the polymer (Blumenkrantz and Asboe-Hansen 1973). Mannuronic, glucuronic, and galacturonic acids exhibit different calibration curves and therefore can be quantified if any of one of these is present in the EPS. The main advantage of this method is rapid screening for uronic acids in polysaccharides without interference of neutral sugars. Rühmann et al. (2015b) developed an EPS-screening method by combining visual observation of viscosity, and precipitation with a detailed monosaccharide analysis via ultrahigh performance liquid chromatography ultraviolet-electrospray ionization-mass spectrometry (UHPLC-UV-ESI-MS). This technique handles all steps in 96-well microtiter-plate, starting from the strain cultivation up to the carbohydrate fingerprint. First, the cells are separated via centrifugation and filtration. This is followed by purification of polymer by gel filtration. Then gel filtrate aliquot is hydrolyzed, derivatized, and analyzed via an optimized HT-1-phenyl-3-methyl-5-pyrazolone method (Rühmann et al. 2014). Major advantage of this technique is that it is very fast, and other detection modules such as precipitation and visual observation of viscosity are handled in parallel. This helps in screening EPS producers which have maximum potential.

Screening and finding of microbial strains for maximum and novel polysaccharide is the major issue, and it also leads to challenges in the search for new polysaccharide variants from natural or novel strains. This is a fact that all microbes have the capacity to produce the polysaccharides under specific conditions, and available EPS data seems to be still unsatisfactory. Therefore, there is need for high-throughput screening techniques that are capable to identify novel variants of microbial strains producing EPS with unique properties.

After screening and identifying the best microbial strains producing considerable amount of EPS, these are moved further for production, extraction, and purification of polysaccharides. These three steps further determine the cost of recovery of microbial polysaccharide, which plays a significant role in the total production cost.

4 Extraction of Polysaccharides from Microorganisms

Extraction of polysaccharides from microorganisms is challenging due to the variety of EPS with different physicochemical properties and also because polysaccharides are mainly attached tightly to the cell wall. If one is interested in EPS only, then there is a need to detach EPS from microorganisms without destroying the cells. EPS

extraction method is considered to be ideal when it is effective, does not disrupt the EPS structure, and causes minimal cell lysis (Frolund et al. 1996; Morin 1998). The different steps involved in the isolation, extraction, and purification of microbial polysaccharides are shown in Fig. 1. The first step in EPS extraction form the cultures is removal of microbial cells from the medium. EPS produced by the microbial cells may be in bound (capsular polysaccharides, sheath, and loosely bound polymers) or soluble (slimes, colloids, and soluble macromolecules) form. The extraction procedures for these EPS are given below.

4.1 Extraction of Exopolysaccharides Existing as Slime

When exopolysaccharides are present in the form of "slime," these can be isolated from the microorganisms by centrifugation. The speed and duration for centrifugation depend upon the nature and viscosity of polysaccharide to be extracted. Most commonly, ultracentrifugation is used to remove cells or their debris from the culture broth containing polysaccharides (Morin 1998; Mende et al. 2013; Notararigo et al. 2013; Kreyenschulte et al. 2014). However, depending upon the type of microorganism from which the polysaccharide is to be extracted, the technique of extraction may vary. If the exopolysaccharide is not thermo-labile, heat treatment can also be given in order to improve the extraction of EPS from microorganisms, but somehow it reduces the viscosity of polysaccharides. Heat treatment during extraction of xanthan, however, increased the viscosity (Sutherland 1990). At laboratory level, removal of cells by centrifugation or ultracentrifugation is possible, but at industry level involving large volumes these techniques may not be cost-effective (Stredansky et al. 1999). This necessitates the development of alternative methods for the separation of microbial cells. Several mechanical, chemical, and thermal treatments have been established to lyse, deactivate, or remove cells from the culture medium. When chemical treatments are performed at high pH, these generally change thermal properties of the polysaccharides while enzymatic treatments enhance the cost of downstream processes. Commonly, physical treatments such as pasteurization or sterilization are used to kill the microbial cells (Smith and Pace 1982; Garcia-Ochoa et al. 1993).

4.2 Extraction of Exopolysaccharide Existing as a Capsule

For the extraction of exopolysaccharides which are present in capsule form, the first step is to dissociate the capsular exopolysaccharides from the cells. Depending upon the nature of the association of the cells and the polysaccharides, a particular method may be selected. When the exopolysaccharides are weakly associated with the cells, then centrifugation is observed to be helpful for the extraction of capsular

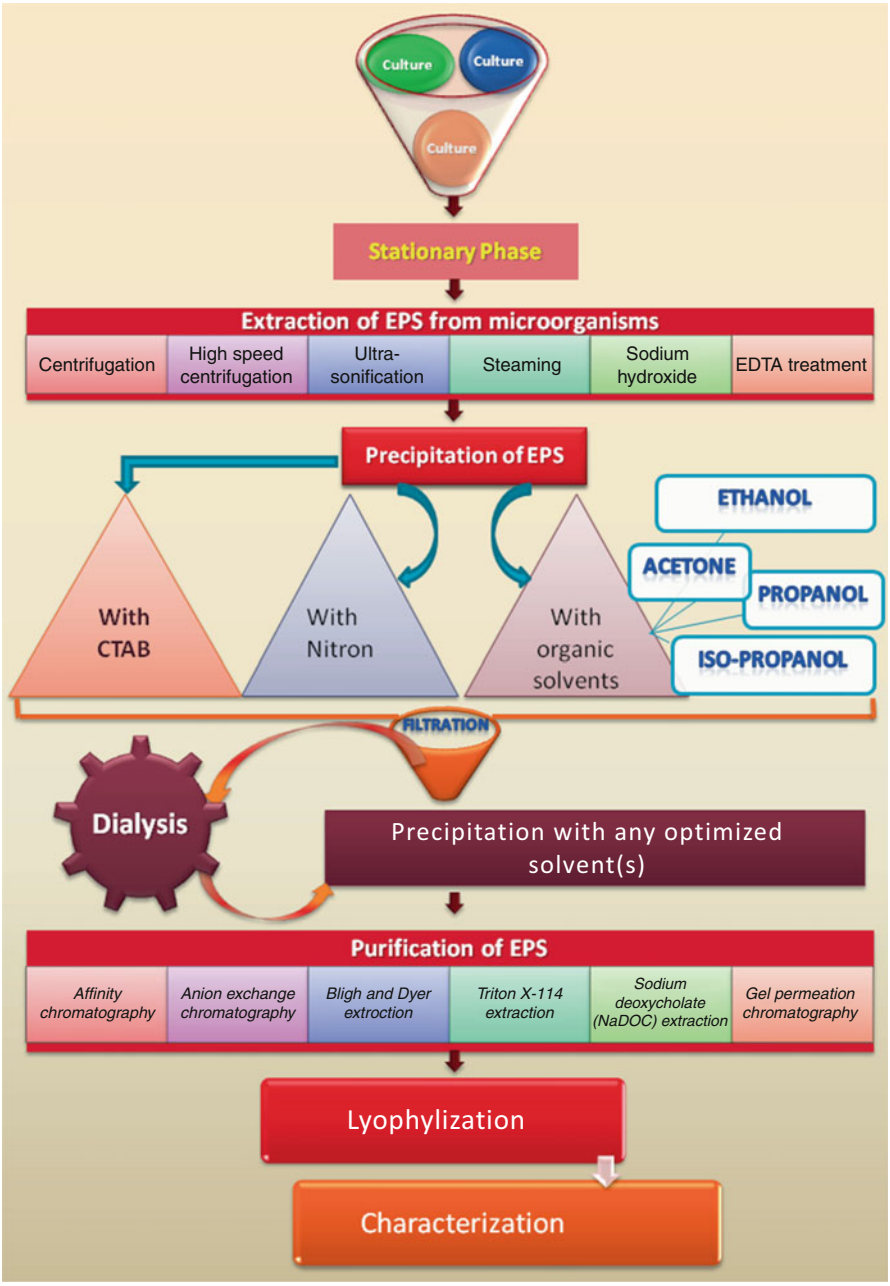


Fig. 1 Diagrammatic representation of steps involved in the isolation and purification of polysaccharides from microbial culture

exopolysaccharides. However, if the capsular exopolysaccharides are strongly associated to the cells, more severe conditions such as alkaline treatment (e.g., with sodium hydroxide), prior to the centrifugation and precipitation with alcohol, are needed (Notararigo et al. 2013). Autoclaving and various alkali treatments may also be used to extract capsular polysaccharides (Freitas et al. 2011; Kreyenschulte et al. 2014; Leroy and De Vuyst 2016). Khattar et al. (2010) extracted the polysaccharides from the cyanobacterial cell wall by treating the cells with a mixture of 0.6 M NaCl and 0.06 M EDTA at 50 ± 2 °C for 3 h. Besides this, some more drastic methods include boiling the cell suspension for 15 min in water, heating at 60 °C in saline solution, heating in a mixture of phenol water at 65 ° C, or sonicating the cell suspension. Autoclaving is also found to be the most frequently used treatment for releasing capsular polysaccharides (Freitas et al. 2011; Kreyenschulte et al. 2014; Leroy and De Vuyst 2016), but these methods cause cell disruption and mixing of cellular polysaccharides, and EPS may occur.

After the extraction of EPS from microorganisms employing any of the above mentioned methods, as may be suitable for a particular type of EPS, the supernatant which is obtained after centrifugation is generally filtered through 0.22μm cellulose acetate filter, to ensure that samples are free of cells, and is saved for precipitation of EPS. Some of the EPS have protein moieties attached to them. Depending upon the application of EPS, these proteins may have to be removed. Proteins from the crude EPS can be removed by treating with trichloroacetic acid (TCA) (Shao et al. 2014; Fontana et al. 2015; Yilmaz et al. 2015; Leroy and De Vuyst 2016) or enzymes such as proteases (Leroy and De Vuyst 2016). TCA treatment often results in the coprecipitation of EPS with culture medium proteins and other impurities. Thus, it is suggested that TCA precipitates should be properly washed to improve EPS recovery (Rimada and Abraham 2003).

5 Precipitation of EPS

Different precipitating agents have been suggested in the literature for precipitation of EPS. The most common precipitating agents are organic solvents: ethanol and methanol (Ahmed et al. 2013; Marcial et al. 2013; Notararigo et al. 2013; Zhang et al. 2013; Ismail and Nampoothiri 2014; Shao et al. 2014; Fontana et al. 2015), acetone (Sutherland 1990; Mende et al. 2013), propane/isopropanol (Kim et al. 1994; Park et al. 2013), combination of acetone and ethanol (Donnarumma et al. 2014), cetyltrimethylammonium bromide (CTAB), and 3, 5, 6-triphenyl-2, 3, 5, 6-tetraaza [2.1.1] bicyclo-1-hexene, commonly known as nitron (Azeredo and Oliveira 1996). Besides these different precipitating agents, the quantity of the solvent is also variable for particular type of EPS precipitation: One, two, or three volumes are normally used. But normally, two volumes seem to be the most usual. The best precipitating agent is one which gets high yield of EPS. For achieving this, different methods for precipitation by using different precipitating agents should be tried (Azeredo and Oliveira 1996).

5.1 Precipitation Methods

(a) *Precipitation with organic solvents*: For solvents such as ethanol, acetone, propanol, and isopropanol, generally 1, 2, and 3 volumes of the solvent are used. The solvents are chilled by keeping them at −18 °C for 24 h before using them for precipitation of EPS. The solvent is added slowly to the EPS-containing solution under gentle magnetic stirring and left for 24 h at 4 °C for polysaccharides to precipitate.
(b) *Precipitation with nitron*: Precipitation of polysaccharides may be achieved by adding 0.1 volumes of 10% solution of nitron in 3% acetic acid to the polysaccharide solution after the addition of 0.1 volumes of 98% sulfuric acid. The precipitation occurs immediately; however, before separating, these precipitates should be allowed to stand in the solvent for at least 24 h.
(c) *Precipitation with CTAB*: This method was used to precipitate alginate (Filali Mouhim et al. 1993) and was followed by Azeredo and Oliveira (1996) for the precipitation of bacterial exopolysaccharides. These authors added 5 ml of sodium sulfate (0.02 M) in 5 ml of the polysaccharide solution. Then, 1 ml of CTAB (3%, w/v) was added to this solution. The precipitates were allowed to stand for 24 h before further analysis.

After precipitation, EPS are separated by centrifugation and are washed with the solvent used for precipitation. Separated crude EPS are dissolved in deionized water and dialyzed against distilled water for 2–4 days at 4 °C employing dialysis membrane with 6–8 kDa cut out (Khattar et al. 2010; Jindal et al. 2011; Górska-Frączek et al. 2013; Marcial et al. 2013; Notararigo et al. 2013; Donnarumma et al. 2014; Ismail and Nampoothiri 2014; Shao et al. 2014; Fontana et al. 2015). Dialysis helps in removing small molecules such as salts of the culture medium and other impurities. After dialysis, EPS may be treated with activated charcoal to decolorize these followed by washing with anhydrous ethanol/acetone/ether. Finally, the dialyzed EPS are lyophilized and may be stored for further characterization (Rimada and Abraham 2003; Khattar et al. 2010). The lyophilized EPS can be treated further for the removal of proteins and other impurities. This can be achieved by treating EPS with 80% (v/v) ethanol solution with 0.1% (v/v) formic acid followed by washing with 96% v/v ethanol (Tuinier et al. 1999; Ruas-Madiedo and de los Reyes-Gavilán 2005). The dissolution of lyophilized EPS in 0.3 M NaOH followed by centrifugation also helps elimination of contaminants (Notararigo et al. 2013).

6 Determination of Precipitation Yield

After precipitation is done, the precipitates may be filtered through glass microfiber membranes or are centrifuged and dried under vacuum or with inert gas. The quantity of EPS yield is determined gravimetrically. The dry exopolysaccharide is milled to the desired mesh size. Depending on the purity of exopolysaccharides, the final preparations of these are off-white to white.

A particular extraction method may be selected depending upon the needs and constraints. The best extraction method will depend on the type of interactions that keep the EPS components together in the matrix. The forces keeping the matrix intact may be different in different EPS; thus, various methods must be tested. As no universal extraction method for all types of EPS exists, it is generally suggested that EPS are extracted only after different techniques are compared and the selected method is optimized and standardized by varying variables such as extraction time, temperature, etc. (Frolund et al. 1996). The extraction efficiency is defined as the total amount of EPS extracted from the total EPS pool, for a given cell sample (Nielsen and Jahn 1999). It is important to note that the EPS extraction efficiency differs significantly according to the extraction method.

7 Purification of EPS

Final purification steps may consist of ion-exchange chromatography (Behare et al. 2013; Ciszek-Lenda et al. 2013; Górska-Frączek et al. 2013; Guo et al. 2013; Li et al. 2013, 2014; Shang et al. 2013; Zhang et al. 2013; Shao et al. 2014; Wang et al. 2014, 2015; Fontana et al. 2015), size exclusion chromatography (Górska-Frączek et al. 2013; Guo et al. 2013; Li et al. 2013, 2014; Notararigo et al. 2013; Shang et al. 2013; Zhang et al. 2013; Miao et al. 2014; Wang et al. 2014, 2015), or preparative SDS-PAGE (Ruas-Madiedo and de los Reyes-Gavilán 2005). If the main aim is to quantify EPS production, then the final purifications steps are less important but are more important when the recovered material is used for polysaccharide characterization (Rimada and Abraham 2003). Purification methods become important when EPS characterization is considered. It is easy to elucidate its structure if the product is pure. It is suggested that whether to use simple or complex medium should be decided when setting up the experiment. EPS produced using simple medium requires only deproteinization while cultivation of microorganisms in complex media requires extensive pretreatment procedures before the centrifugation step (Dave et al. 2020). Purified freeze-dried EPS are ready for physical (viscosity of aqueous solution of EPS at different concentrations, temperatures, and pH) or chemical (presence of sugars, linkages between monomeric units, determination of MW and structure, etc.) characterization. By knowing physical and chemical characteristics and the complete structure of the polymer, one can understand the potential of these varied EPS in a particular application. In this way, these microbial polysaccharides which have many advantages may replace available plant polysaccharides in the near future.

It is reported that the EPS produced by the microorganisms can be contaminated by different bacterial extracellular molecules such as lipopolysaccharides (LPS), which is an integral component of outer cell surface of microorganisms, proteins, etc. These contaminants are also called as endotoxins and can alter the EPS-beneficial effects (Le Du Alicia et al. 2017). Although acid or alkaline treatment can be used to inactivate LPS, these methods also lead to removal/depolymerization of biological molecules such as EPS. Thus, methods which are nondestructive for

LPS removal are required. Few methods, such as Triton X-114 phase separation or affinity chromatography, led to a significant LPS removal from polysaccharides below the nanogram level (Pier et al. 1978).

8 Conclusions

Polysaccharides have gained significant attention from scientists as functional biomolecules for the development of innovative and value-added products in the field of pharmaceutics, food, cosmetics, and the biomedical industry. Production of EPS by microorganisms is of interest not only with respect to its applications in food and health but also to better understand microbial functioning in specific ecosystems. During the last many years, several variations in production, isolation, purification, and quantification protocols have been reported, but there is no generalized method for varied forms of EPS. It is noticed that optimized conditions for isolation, extraction, precipitation, and purification of microbial EPS are different for different microorganisms. The nature of the cultivation medium also plays an important role, as when complex medium is used, then extensive pretreatment and purification steps should be done whereas the use of simple medium is based on simple deproteinization. It is important that different methods of extraction of microbial EPS should be compared, and the best one should be standardized and optimized for a particular type of microorganism/EPS.

References

Ahmed Z, Wang Y, Anjum N, et al. Characterization of exopolysaccharide produced by *Lactobacillus kefiranofaciens* ZW3 isolated from Tibet kefir – part II. Food Hydrocoll. 2013;30:343–50.

Azeredo J, Oliveira R. A new method for precipitating bacterial exopolysaccharides. Biotechnol Tech. 1996;10:341–4.

Behare PV, Singh R, Nagpal R, et al. Exopolysaccharides producing *Lactobacillus fermentum* strain for enhancing rheological and sensory attributes of low-fat dahi. J Food Sci Technol. 2013;50:1228–32.

Blumenkrantz N, Asboe-Hansen G. New method for quantitative determination of uronicacids. Anal Biochem. 1973;54:484–9.

Caruso C, Rizzo C, Mangano S, Poli A, Di Donato P, Nicolaus B, Di Marco G, Michaud L, Giudice AL. Extracellular polymeric substances with metal adsorption capacity produced by *Pseudoalteromonas* sp. MER144 from Antarctic seawater. Environ Sci Pollut Res. 2018;25:4667–77. https://doi.org/10.1007/s11356-017-0851-z.

Caruso C, Rizzo C, Mangano S, Poli A, Di Donato P, Nicolaus B, Finore I, Di Marco G, Michaud L, Giudice AL. Isolation, characterization and optimization of EPSs produced by a cold adapted *Marinobacter* isolate from Antarctic seawater. Antarct Sci. 2019;31:69–79.

Ciszek-Lenda M, Nowak B, Srottek M, et al. Further studies on immunomodulatory effects of exopolysaccharide isolated from *Lactobacillus rhamnosus* KL37C. Cent Eur J Immunol. 2013;38:289–98.

Dave SR, Upadyay KH, Vaishnav, AM, et al. Exopolysaccharides from marine bacteria: production, recovery and applications. Environ Sustain. 2020. https://doi.org/10.1007/s42398-020-00101-5.

Dierksen KP, Sandine WE, Trempy JE. Expression of ropy and mucoid phenotypes in *Lactococcus lactis*. J Dairy Sci. 1997;80:1528–36.
Donnarumma G, Molinaro A, Cimini D, et al. *Lactobacillus rispatus* L1: high cell density cultivation and exopolysaccharide structure characterization to highlight potentially beneficial effects against vaginal pathogens. BMC Microbiol. 2014;14:137.
Donot F, Fontana A, Baccou J, et al. Microbial exopolysaccharides: main examples of synthesis, excretion, genetics and extraction. Carbohydr Polym. 2012;87:951–62.
Duenas M, Irastorza A, Fernandez K, et al. Heterofermentative *Lactobacilli* causing ropiness in Basque country ciders. J Food Prot. 1995;58:76–80.
Evans NA, Hoyne PA, Stone BA. Characteristics and specificity of the interaction of a fluorochrome from aniline blue (Sirofluor) with polysaccharides. Carbohydr Polym. 1984;4:215–30.
Filali Mouhim R, Cornet JF, Fontaine T, et al. Production, isolation and preliminary characterization of the exopolysaccharide of the cyanobacterium *Spirulina platensis*. Biotechnol Lett. 1993;15: 567–72.
Folkenberg DM, Dejmek P, Skriver A. Sensory and rheological screening of exopolysaccharide producing strains of bacterial yogurt cultures. Int Dairy J. 2006;16:111–8.
Fontana C, Li S, Yang Z, et al. Structural studies of the exopolysaccharide from *Lactobacillus plantarum* C88 using NMR spectroscopy and the program CASPER. Carbohydr Res. 2015;402:87–94.
Freitas F, Alves VD, Reis MA. Advances in bacterial exopolysaccharides: from production to biotechnological applications. Trends Biotechnol. 2011;29:388–98.
Frolund B, Palmgren R, Keiding K, et al. Extraction of extracellular polymers from activated sludge using a cation exchange resin. Water Res. 1996;30:1749–58.
Garai-Ibabe G, Areizaga J, Aznar R, et al. Screening and selection of 2-branched (1,3)-β-d-glucan producing lactic acid bacteria and exopolysaccharide characterization. J Agric Food Chem. 2010;58:6149–56.
Garcia-Ochoa F, Casas JA, Mohedano AF. Precipitation of xanthan gum. Sep Sci Technol. 1993;28: 1303–13.
Górska-Frączek S, Sandström C, Kenne L, et al. The structure and immunoreactivity of exopolysaccharide isolated from *Lactobacillus johnsonii* strain 151. Carbohydr Res. 2013;378:148–53.
Guo Y, Pan D, Sun Y, et al. Antioxidant activity of phosphorylated exopolysaccharide produced by *Lactococcus lactis* ssp. lactis. Carbohydr Polym. 2013;97:849–54.
Ismail B, Nampoothiri KM. Molecular characterization of an exopolysaccharide from a probiotic *Lactobacillus plantarum* MTCC 9510 and its efficacy to improve the texture of starchy food. J Food Sci Technol. 2014;51:4012–8.
Jindal N, Khattar JIS. Microbial polysaccharides in food industry. In: Grumezescu AM, Hoban AM, editors. Handbook of food bioengineering, Vol 20: biopolymers for food design. Academic Press; 2018. p. 95–123. https://doi.org/10.1016/B978-0-12-811449-0.00004-9.
Jindal N, Singh DP, Khattar JIS. Kinetics and physico-chemical characterization of exopolysaccharides produced by *Oscillatoria formosa*. World J Microbiol Biotechnol. 2011;27:2139–46.
Khattar JIS, Singh DP, Jindal N, et al. Isolation and characterization of exopolysaccharides produced by the cyanobacterium *Limnothrix redekei* PUPCCC 116. Appl Biochem Biotechnol. 2010;162:1327–38.
Kim S, Kong I, Kwon K, et al. Exopolysaccharides produced by Z. ramigera mutants and analysis of structural change by solution properties. Biotechnol Lett. 1994;16:789–94. https://doi.org/10.1007/BF00133955.
Kothari D, Das D, Patel S, et al. Dextran and food application. In: Ramawat KG, Meérillon JM, editors. Polysaccharides. Springer International Publishing; 2015. p. 1–16.
Kreyenschulte D, Krull R, Margaritis A. Recent advances in microbial biopolymer production and purification. Crit Rev Biotechnol. 2014;34:1–15.
Kumar AS, Mody K. Microbial exopolysaccharides: variety and potential applications. In: Rehm BHM, editor. Microbial production of biopolymers and polymer precursors: applications and perspectives. Norfolk: Caister Academic Press; 2009. p. 229–54.

Kumar AS, Mody K, Jha B. Bacterial exopolysaccharides–a perception. J Basic Microbiol. 2007;47:103–17.
Le Du Alicia G, Agata Z, Corinne S, et al. Purification of the exopolysaccharide produced by *Alteromonas infernus*: identification of endotoxins and effective process to remove them. Appl Microbiol Biotechnol. 2017;101:6597–606.
Leroy F, De Vuyst L. Advances in production and simplified methods for recovery and quantification of exopolysaccharides for applications in food and health. J Dairy Sci. 2016;99:3229–38.
Li JY, Jin MM, Meng J. Exopolysaccharide from *Lactobacillus plantarum* LP6: antioxidation and the effect on oxidative stress. Carbohydr Polym. 2013;98:1147–52.
Li C, Li W, Chen X, et al. Microbiological, physicochemical and rheological properties of fermented soymilk produced with exopolysaccharide (EPS) producing lactic acid bacteria strains. Food Sci Technol (Campinas). 2014;57:477–85.
Ma JJ, Yin RC. Primary study on extracellular polysaccharide producing bacteria in different environments. Anhui Daxue Xuebao Ziran Kexueban. 2011;35:94–100.
Marcial GJ, Messing B, Menchicchi FM, et al. Effects of polysaccharide isolated from *Streptococcus thermophilus* CRL1190 on human gastric epithelial cells. Int J Biol Macromol. 2013;62:217–24.
Mende S, Peter M, Bartels K, et al. Addition of purified exopolysaccharide isolates from *S. thermophilus* to milk and their impact on the rheology of acid gels. Food Hydrocoll. 2013;32:178–85.
Miao M, Ma Y, Jiang B, et al. Structural investigation of a neutral extracellular glucan from *Lactobacillus reuteri* SK24.003. Carbohydr Polym. 2014;106:384–92.
Mojica K, Elsey D, Cooney MJ. Quantitative analysis of biofilm EPS uronic acid content. J Microbiol Methods. 2007;71:61–5.
Morin A. Screening of polysaccharide producing microorganisms, factors influencing the production and recovery of microbial polysaccharides. In: Polysaccharides-structural diversity and functional versatility. New York: Marcel Dekker Inc.; 1998. p. 275–96.
Nielsen PH, Jahn A. Extraction of EPS. In: Wingender J, Neu TR, Flemming HC, editors. Microbial extracellular polymeric substances; characterization, extraction and function. Berlin: Springer; 1999. p. 49–72.
Notararigo S, Nácher-Vázquez M, Ibarburu I, Werning ML, de Palencia PF, Dueñas MT, Aznar R, López P, Prieto A. Comparative analysis of production and purification of homo and heteropolysaccharides produced by lactic acid bacteria. Carbohydr Polym. 2013;93:57–64.
Ogaji IJ, Nep EI, Audu-Peter JD. Advances in natural polymers as pharmaceutical excipients. Pharm Anal Acta. 2012;3:146.
Park JH, Ahn HJ, Kim SG, et al. Dextran-like exopolysaccharide- producing *Leuconostoc* and *Weissell*a from Kimchi andits ingredients. Food Sci Biotechnol. 2013;22:1047–53.
Phillips GO, Williams PA. Preface. In: Phillips GO, Williams PA, editors. Handbook of hydrocolloids. Great Abington/Cambridge: Woodhead Publishing; 2000. p. 1–22.
Pier GB, Sidberry HF, Zolyomi S, et al. Isolation and characterization of a high-molecular-weight polysaccharide from the slime of *Pseudomonas aeruginosa*. Infect Immun. 1978;22:908–18.
Poli A, Anzelmo G, Nicolaus B. Bacterial exopolysaccharides from extreme marine habitats: production, characterization and biological activities. Mar Drugs. 2010;8:1779–802.
Ricciardi A, Parente E, Clementi F. A simple method for the screening of lactic acid bacteria for the production of exopolysaccharides in liquid media. Biotechnol Tech. 1997;11:271–5.
Rimada PS, Abraham AG. Comparative study of different methodologies to determine the exopolysaccharide produced by kefir grains in milk and whey. Lait. 2003;83:79–87.
Ruas-Madiedo P, de los Reyes-Gavilán CG. Methods for the screening, isolation, and characterization of exopolysaccharides produced by lactic acid bacteria. J Dairy Sci. 2005;88:843–56.
Rühmann B, Schmid J, Sieber V. Fast carbohydrate analysis via liquid chromatography coupled with ultra violet and electrospray ionization ion trap detection in 96-well format. J Chromatogr A. 2014;1350:44–50.
Rühmann, B, Schmid J, Sieber V. Methods to identify unexplored diversity of microbial exopolysaccharides. Front Microbiol. 2015a;6. https://doi.org/10.3389/fmicb.2015.00565.

Rühmann B, Schmid J, Sieber V. High throughput exopolysaccharide screening platform: from strain cultivation to monosaccharide composition and carbohydrate fingerprinting in one day. Carbohydr Polym. 2015b;122:212–20.
Schmid J, Mueller-Hagen D, Sieber V, et al. Nucleic and protein extraction methods for fungal exopolysaccharide producers. In: Gupta VK, Tuohy MG, Ayyachamy M, et al., editors. Laboratory protocols in fungal biology: current methods in fungal biology. New York: Springer; 2013. p. 427–34.
Shang N, Xua R, Li P. Structure characterization of an exopolysaccharide produced by *Bifidobacterium animalis* RH. Carbohydr Polym. 2013;91:128–34.
Shao L, Wu Z, Zhang H, et al. Partial characterization and immunostimulatory activity of exopolysaccharides from *Lactobacillus rhamnosus* KF5. Carbohydr Polym. 2014;107:51–6.
Smith IH, Pace GW. Recovery of microbial polysaccharides. J Chem Technol Biotechnol. 1982;32:119–29.
Stredansky M, Conti E, Bertocchi C, et al. Fed-batch production and simple isolation of succinoglycan from *Agrobacterium tumefaciens*. Biotechnol Tech. 1999;13:7–10.
Sutherland IW. Biotechnology of microbial exopolysaccharides. Cambridge: Cambridge University Press; 1990. p. 122–32.
Sutherland IW. Novel and established applications of microbial polysaccharides. Trends Biotechnol. 1998;16:41–6.
Swennen K, Courtin CM, Van Der Bruggen B, et al. Ultrafiltration and ethanol precipitation for isolation of arabinoxylooligosaccharides with different structures. Carbohydr Polym. 2005;62:283–92.
Tuinier R, Zoon P, Olieman C, et al. Isolation and physical characterization of an eocellular polysaccharide. Biopolymers. 1999;49:1–9.
Van den Berg DJC, Smits A, Pot B, et al. Isolation, screening and identification of lactic-acid bacteria from traditional food fermentation processes and culture collections. Food Biotechnol. 1993;7:189–205.
Vedamuthu ER, Neville JM. Involvement of a plasmid in production of ropiness (mucoidness) in milk cultures by *Streptococcus cremoris* MS. Appl Environ Microbiol. 1986;51:677–82.
Vroman I, Tighzert L. Biodegradable polymers. Materials. 2009;2:307–44.
Wang K, Li W, Rui X, et al. Characterization of a novel exopolysaccharide with antitumor activity from *Lactobacillus plantarum* 70810. Int J Biol Macromol. 2014;63:133–9.
Wang J, Zhaob X, Yang Y, et al. Characterization and bioactivities of an exopolysaccharide produced by *Lactobacillus plantarum* YW32. Int J Biol Macromol. 2015;74:119–26.
Wood PJ, Fulcher RG. Interaction of some dyes with cereal β-glucans. Cereal Chem. 1978;55:952–66.
Yilmaz MT, Dertli E, Toker OS, et al. Effect of in situ exopolysaccharide production on physicochemical, rheological, sensory, and microstructural properties of the yogurt drink ayran: an optimization study based on fermentation kinetics. J Dairy Sci. 2015;98:1604–24.
Zhang L, Liu C, Li D, et al. Antioxidant activity of an exopolysaccharide isolated from *Lactobacillus plantarum* C88. Int J Biol Macromol. 2013;54:270–5.
Zhao M, Cui N, Qu F, et al. Novel nano-particulated exopolysaccharide produced by *Klebsiella sp.* PHRC1. 001. Carbohydr Polym. 2017;171:252–8.

Biosynthesis of Bacterial Polysaccharides

21

Yuriy A. Knirel and Johanna J. Kenyon

Contents

Abstract

The present chapter is devoted to biosynthesis pathways of various bacterial surface polysaccharides, including slime exopolysaccharides and capsular polysaccharides, as well as the O-antigen polysaccharide chains of lipopolysaccharides. The polysaccharides are extraordinarily diverse in structure and define the specificity of interaction between bacteria and their environments, including interactions with host organisms, bacteriophages, and natural predators. As such, both homopolysaccharides and heteropolysaccharides produced by bacteria

Y. A. Knirel (✉)
N. D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, Moscow, Russia

J. J. Kenyon
Centre for Immunolgy and Infection Control University of Minho, (CIIC), School of Biomedical Sciences, Queensland University of Technology, Brisbane, QLD, Australia
e-mail: johanna.kenyon@qut.edu.au

J. M. Oliveira et al. (eds.), *Polysaccharides of Microbial Origin*,
https://doi.org/10.1007/978-3-030-42215-8_28

have an important function in bacterial lifestyle, particularly in protection of human pathogens against the immune system response and some antimicrobials. In both gram-negative and gram-positive bacteria, biosynthesis of most cell-surface heteropolysaccharides is accomplished by the Wzy-dependent pathway, whereas some exopolysaccharides, such as a β-(1→6)-linked polymer of D-Glc*p*NAc called PNAG, are synthesized by the synthase-dependent pathway. For synthesis of homopolysaccharides and some heteropolysaccharides with a small (di- or tri-saccharide) repeating unit, gram-negative bacteria use the ATP-binding cassette (ABC) transporter-dependent pathway. A fourth pathway is utilized for the extracellular synthesis of several glucans and fructus by a single sucrase protein. The specific mechanisms of these pathways, including a brief view on the genetics of the polysaccharide biosynthesis, is provided.

Keywords

Exopolysaccharide · O-specific polysaccharide · Capsule · Biosynthesis · Bacterium

1 Introduction

Prokaryotic and eukaryotic microorganisms produce a diverse array of natural carbohydrate macromolecules that play important roles in the structural integrity of the cell, biological processes, adhesion, and adaptability of the organism to stresses in the external environment. They are often highly immunogenic, and for many microorganisms, are required for biofilm formation, evasion of host immune responses, and resistance to bacteriophage, predation, and/or some antimicrobial compounds. Some naturally occurring bacterial glycans are of great interest to the field of bioengineering. For instance, xanthan produced by *Xanthomonas campestris* and gellan produced by *Sphingomonas paucimobilis* have industrial relevance and have been commercialized (Schmid et al. 2015).

Many microbial glycans are excreted into the extracellular milieu as exopolysaccharides (EPSs), which are present as loosely associated slime or a discrete capsular polysaccharide (CPS) forming a tight layer surrounding the cell. In bacteria, CPSs are often associated with the cell surface via a lipid moiety in gram-negative bacteria or the peptidoglycan in gram-positive bacteria. Gram-negative bacteria are also exclusively able to produce O-specific polysaccharides (OPSs), also called O-antigens. They represent the polysaccharide chain of a complex glycolipid known as the lipopolysaccharide (LPS), which is the major component of the outer layer of the outer membrane. The LPS consists of a lipid A anchor joined to a core oligosaccharide, to which the OPS is covalently attached. CPS and OPS structures can resemble each other, and bacterial strains may use the same assembly pathway for both polysaccharide entities (Whitfield 2006).

A great diversity of polysaccharide formats can be produced by bacterial species due to a large number of possible monosaccharide combinations, glycosidic

linkages, sugar sequences, and decoration with various non-sugar constituents, such as phosphate, pyruvyl, lactyl, and acyl (most commonly acetyl) groups. The bacterial glycopolymers can exist as homopolysaccharides that are composed of a single repeating monosaccharide, though the large majority of bacterial polysaccharides are heteropolysaccharides made up of repeating oligosaccharide units that contain 2–8 different sugars joined by specific linkages.

Biosynthesis of bacterial polysaccharides relies on a delicate orchestration of complex pathways requiring multiple enzymes for the synthesis of nucleotide-linked sugar substrates, the linkage of the sugars to each other or to other moieties, polymerization, and export of the polysaccharide to the cell surface. Nonetheless, the assembly and export of bacterial polysaccharides occurs via one of four major pathways: (1) the Wzx/Wzy-dependent pathway; (2) the ATP-binding cassette (ABC) transporter-dependent pathway; (3) the synthase-dependent pathway; and (4) a single sucrase protein for extracellular synthesis. Here, we review the molecular processes involved with these pathways and the genetic control of bacterial polysaccharide biosynthesis.

2 Wzx/Wzy-Dependent Pathway

The Wzx/Wzy-dependent pathway is arguably the most ubiquitous pathway for production of microbial polysaccharides with complex structures. The name of the pathway is derived from early research on gram-negative OPS synthesis, for which a polymerase, known as Wzy (previously Rfc), was found to elongate the polymer chain prior to presentation on the cell surface. This pathway is employed by some commercialized EPSs, such as xanthan, gellan, and welan gums (biosynthesis of these specific polysaccharides is reviewed elsewhere; see Becker 2015; Schmid et al. 2015) but is also widely used for the production of CPSs of important human pathogens, including gram-positive staphylococci and streptococci and gram-negative *escherichia, klebsiella, acinetobacter*, and the majority of OPSs, including those of pathogenic *escherichia, salmonella, yersiniae*, and *pseudomonas*.

Polysaccharide synthesis by the Wzx/Wzy-dependent pathway, as well as other major pathways, begins with the production of activated sugar nucleotide diphosphates or monophosphates in the cytoplasm via enzymatic pathways often starting with glucose-1-phosphate or fructose-6-phosphate (Ståhle and Widmalm 2019). Simple UDP-linked sugars produced by these pathways are also required for other essential cellular metabolic pathways, and therefore the genes that drive their synthesis are commonly located away from loci specifically responsible for polysaccharide biosynthesis. Many complex polysaccharides also include acetamido sugars derived from UDP-*N*-acetyl-D-glucosamine (UDP-D-Glc*p*NAc), and the genes that drive their synthesis are commonly located within genomic loci specific for OPS or EPS synthesis. Following synthesis of activated sugar derivatives, an oligosaccharide unit is assembled on a lipid carrier at the cytoplasmic face of the inner membrane (Fig. 1).

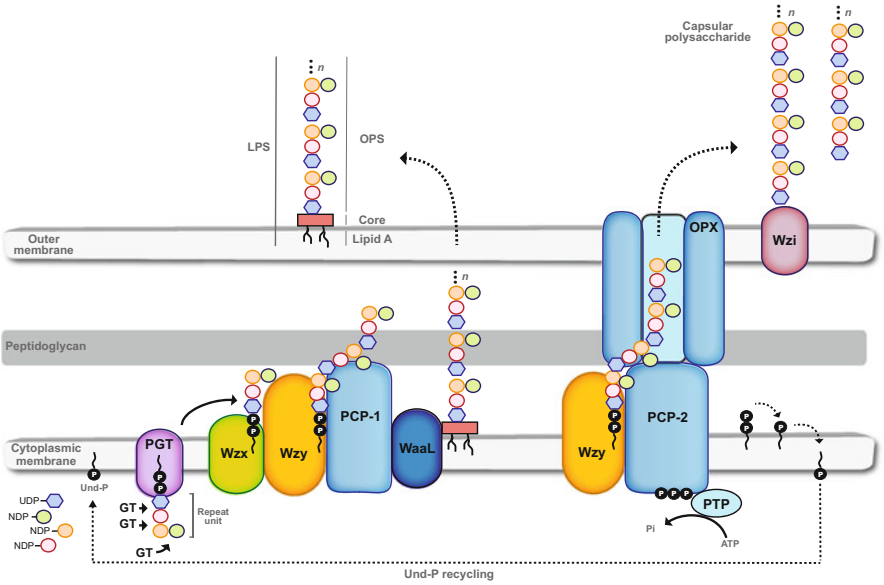


Fig. 1 The Wzx/Wzy-dependent pathway for OPS and CPS biosynthesis in gram-negative bacteria. Activated sugar nucleotide mono- or diphosphates are synthesized in the cytoplasm, and a phosphoglycosyltransferase (PGT) initiates construction of the repeat unit with the addition of the first sugar 1-phosphate to the undecaprenol phosphate (Und-P) lipid carrier creating an Und-PP-sugar moiety. Glycosyltransferases (GTs) add further sugars. Repeat units are flipped into the periplasm by Wzx and polymerized by Wzy. A polysaccharide co-polymerase (PCP) regulates the amount and/or chain length of the polymer. For lipopolysaccharide (LPS) synthesis, the polysaccharide is ligated to the lipid A-core moiety by the O-antigen ligase WaaL, and the LPS is then exported to the cell surface by the Lpt machinery. For CPS synthesis, the PCP is phosphorylated by a cognate phosphotyrosine phosphatase (PTP), and an outer membrane export protein (OPX) facilitates transfer of the polysaccharide to the cell surface. CPS is shed or arranged on the cell surface by Wzi. Und-PP is released upon polymerization and cleaved to give Und-P for recycling

In both gram-positive and gram-negative bacteria, undecaprenol phosphate (Und-P) is used as the lipid carrier and the synthesis of this molecule has been recently reviewed (Caffalette et al. 2020). The assembly of the oligosaccharide unit on Und-P is initiated with the transfer of a hexose 1-phosphate or a *N*-acetylhexosamine 1-phosphate from an activated nucleotide-linked sugar precursor forming a membrane-associated polyprenyl diphosphate sugar moiety (Hug and Feldman 2011). This initiation reaction is catalyzed by a phosphoglycosyltransferase.

Phosphoglycosyltransferases are currently classified into two major superfamilies of enzymes (Lukose et al. 2017). The first is defined by a polytopic membrane topology with ten or more transmembrane helices. This family includes the MraY protein for peptidoglycan synthesis, though the prototype for this family is WecA for synthesis of a polysaccharide known as the enterobacterial common antigen (ECA) as well as OPSs and CPSs beginning with either D-Glc*p*NAc or D-Gal*p*NAc. Members of the second phosphoglycosyltransferase superfamily include a catalytic

domain containing a single predicted transmembrane helix with a conserved proline residue, followed by a C-terminal cytosolic tail. This family is modelled by the WbaP transferase from *Salmonella enterica* that catalyzes the transfer of D-galactose-1-P to Und-P to initiate O-antigen synthesis.

The initiation reaction is followed by the sequential transfer of carbohydrate components to the Und-PP-linked first sugar from individual nucleotide-activated sugar donors onto acceptor sugar substrates in a high energy reaction catalyzed by glycosyltransferases. Glycosyltransferases are considered one of the most functionally diverse groups of enzymes in biological systems. At present, they are classified into more than 110 families in the Carbohydrate Active Enzymes database (CAZy at http://www.cazy.org/) based on amino acid sequence similarities. Despite the immense diversity in sequence, the majority of their structures adopt one of three folds, referred to as GT-A, GT-B, and GT-C (Ståhle and Widmalm 2019). Both GT-A and GT-B enzymes comprise two β/α/β Rossmann-like folds, though GT-A domains are divided by a conserved DXD metal-binding motif at the active site, whereas the GT-B folds are connected by a cleft that binds the nucleotide group of the donor. GT-C transmembrane proteins utilize lipid phosphate-sugar donors.

The activity of a glycosyltransferase can result in either an α- or β-anomeric configuration of the glycosidic linkage formed between sugars. The mechanism resulting in either stereochemical outcome can be classified as inverting or retaining in respect to the anomeric configuration of the donor sugar (Ståhle and Widmalm 2019). Therefore, when different combinations of glycosyltransferases are present, different structural patterns with distinctive physical properties are produced. However, some enzymes are multifunctional and their activity is likely regulated by the presence of specific sugar donors and acceptors. Thus, generation of the polysaccharide will only occur if a specific combination of Gtr, donor sugar, and acceptor sugar are present together in the cytoplasm.

Once a complete oligosaccharide unit has been constructed, it is translocated *en bloc* across the cytoplasmic membrane into the periplasmic space by an integral membrane protein referred to as the Wzx translocase (or Wzx flippase). Wzx enzymes commonly include 12 transmembrane helices, and belong to a family of multidrug-oligosaccharide lipid-polysaccharide exporter proteins (Caffalette et al. 2020). Though they share little similarity in primary sequence, there is some evidence to suggest that Wzx exhibits varying degrees of specificity for repeat unit substrates (Hong et al. 2018).

Following translocation of the oligosaccharide unit to the periplasmic space, it is polymerized into a polysaccharide chain by a Wzy polymerase. This membrane protein continuously transfers an elongating polymer from Und-PP on to the non-reducing terminus of a new Und-PP-linked oligosaccharide by forming a specific linkage between consecutive repeating units (Valvano 2011). Once a completed polysaccharide is removed from Und-PP, the Und-P lipid is released and recycled back to the cytoplasm for further polysaccharide synthesis or use in another essential pathway such as peptidoglycan synthesis (Fig. 1).

Most Wzy include between 10 and 14 transmembrane helices and a periplasmic loop thought to be critical for function (Collins et al. 2017). Though there is currently

no structure available for a Wzy polymerase, the biological function of Wzy has been demonstrated in a *E. coli* O86 O-antigen model (Woodward et al. 2010). Across species, Wzy proteins perform the same basic function. However, they exhibit extensive inter- and intra-species sequence diversity similar to Wzx and, in general, are incapable of polymerizing foreign oligosaccharide units due to the specificity for constituent sugars and/or topology (Valvano 2011).

Wzy polymerization is often performed in conjunction with a polysaccharide co-polymerase (PCP) that drives elongation and regulates the length of the chain. The type of the co-polymerase and export machinery is dictated by the destination of the final polysaccharide product as OPS, CPS or EPS as discussed below.

2.1 O-Specific Polysaccharides

For OPS produced by the Wzx/Wzy-dependent pathway, a PCP-1 co-polymerase known as the Wzz chain-length regulator (previously Rol or Cld) is involved in defining the length of the polysaccharide chain prior to ligation to the lipid A-core by the WaaL O-antigen ligase (Fig. 1). Wzz is an integral membrane protein that exists in an oligomeric state (Collins et al. 2017), and is thought to drive the activity of the Wzy polymerase by promoting extension of the polysaccharide until a specific modal length is achieved. Many bacteria can simultaneously express more than one Wzz protein to achieve multiple and concurrent modal preferences. For example, *S. enterica* sv. Typhimurium produces a second Wzz, known as FepE or Wzz^{FepE}. Wzz^{FepE} drives the polymerization of very long chain O-antigens with more than 100 repeating units such that a bimodal distribution of chain lengths is achieved when both Wzz proteins are present. However, once a mature O antigen is formed, the WaaL O-antigen ligase joins the polysaccharide to the core oligosaccharide component of the LPS (Fig. 1), and the final structure is exported to the cell surface by the Lpt machinery (reviewed in Ståhle and Widmalm 2019).

Though evidence is still lacking on the precise mechanism employed by Wzz to control polysaccharide chain length, several models have been proposed. The first, referred to as the "molecular clock" model, proposes that Wzz determines either OPS polymerization and ligation to the lipid A-core in a time-dependent manner (Bastin et al. 1993). The second model, known as the "molecular ruler" model, suggests that Wzz mediates interaction between Wzy and the WaaL ligase by defining the preferred chain length before allowing ligation of the growing polysaccharide to the lipid A-core (Morona et al. 1995). This model assumes that Wzy, Wzz, and WaaL form a protein complex where Wzz determines a balance between WaaL and Wzy. More recent evidence suggests that Wzz proteins may form a scaffold for Wzy by controlling the size of oligomers, and that the interaction of Wzy with Wzz triggers polymerization until the polysaccharide binding capacity of Wzz is exhausted or until the Wzy and Wzz complex dissociates (Collins et al. 2017).

For many gram-negative bacteria, the majority of genes responsible for the biosynthesis of OPS by the Wzx/Wzy-dependent pathway are found together, referred to as the O-antigen gene cluster, located at a discrete genomic locus.

Occasionally this may be found on a mega-plasmid in some bacteria, though it is common for the cluster to be found at a specific location on the chromosome. The location of the locus may differ between species; for example, in *E. coli* and *S. enterica,* O-antigen gene clusters map to the *galF/gnd* locus adjacent to the *his* operon (Liu et al. 2019), whereas in *Yersinia pseudotuberculosis*, another member of the Enterobacterales order, the O-antigen locus occurs between *hemH* and *gsk* (Kenyon et al. 2013). The clusters can vary greatly in size between different serotypes, and usually include all genes for nucleotide-linked sugar biosynthesis, glycosyl transfer, processing, and modification of the polysaccharide. Therefore, in general, more genes will be present when a more complex polysaccharide consisting of several different sugars and linkages is produced.

2.2 Capsular Polysaccharides

Biosynthesis of CPSs by the Wzx/Wzy-dependent pathway is regulated by tyrosine phosphorylation of polysaccharide co-polymerase proteins belonging to the PCP-2 class. These enzymes are protein tyrosine autokinases (PTK) that consist of two transmembrane domains and a cytosolic tyrosine P loop that includes Walker A and B ATP-binding domains. The level of phosphorylation influences the amount of CPS produced, and a cognate phosphotyrosine phosphatase (PTP) is involved in regulating phosphorylation activity. In some bacterial species, the CPS synthesis is achieved when the PTK is in a phosphorylated state, whereas PTK dephosphorylation is critical for CPS synthesis in other species (Standish and Morona 2014).

In gram-positive bacteria, the PCP-2 protein domains are found separate as two different polypeptides, which are classed as members of the PCP-2a subfamily. In *S. pneumoniae,* for example, CpsC and CpsD are responsible for tyrosine kinase activity and regulate CPS biosynthesis along with the cognate phosphatase CpsB (Standish and Morona 2014). Once exported to the cell surface, the CPS is covalently attached to the peptidoglycan or another surface component, and in *S. pneumoniae*, this is mediated by CpsA. The majority of *S. pneumoniae* CPS are synthesized by the Wzx/Wzy-dependent pathway, and the genes required for synthesis of CPS serotypes via this pathway are found together in the *cps* operon located between *dexB* and *aliA* chromosomal genes (Yother 2011). The 5′ portion of this operon is highly conserved among serotypes and includes the *cpsABCD* processing genes, while the remainder of the locus is considered serotype specific.

In contrast to gram-positive bacteria, the PCP-2 protein domains are incorporated in the same protein in gram-negative bacteria, and these proteins are classed as members of the PCP-2b superfamily. For *E. coli*, structurally variable Group 1 and Group 4 CPSs, as well as the conserved colanic acid EPS, are synthesized by the Wzx/Wzy-dependent pathway (Whitfield 2006). In *E. coli* Group 1 serotype K30 CPS, Wzc is the PCP-2 protein and Wzb is the cognate phosphatase. In order for the mature polysaccharide to be exported through the outer membrane to the cell surface, a third octameric protein, known as Wza, forms an outer-membrane channel through which the CPS passes (Fig. 1). Organization of the CPS on the cell surface is

then mediated by the outer-membrane protein Wzi (Whitfield et al. 2020). Wzi homologues have not been identified in all gram-negative species with CPS produced by the Wzx/Wzy-dependent pathway suggesting alternate mechanisms of CPS organization and attachment. However, both Group 1 and Group 4 CPSs can also be found ligated to the lipid A-core, forming a structure referred to as K_{LPS} that may be co-expressed with a serologically distinct O antigen (Whitfield 2006).

The majority of genes required for the synthesis of *E. coli* Group 1 and Group 4 CPSs map to the same locus on the chromosome as *E. coli* O-antigen gene clusters (*galF/gnd*), where the colanic acid biosynthesis gene cluster is generally located immediately upstream (Whitfield 2006). Though Group 1 and Group 4 CPSs are produced by the same machinery, the organization of their respective gene clusters is a differentiating feature of the two groups. The Group 1 *cps* locus includes *wzi-wza-wzb-wzc* genes in a 5' conserved portion, with serotype specific genes found immediately downstream. For Group 4 CPSs, the serotype-specific genes are found at the *galF/gnd* locus while *wza-wzb-wzc* genes are located elsewhere on the chromosome at the "22-minute locus." Homologous genes for Wzi, Wza, Wzb, and Wzc have also been identified in other gram-negative bacteria, including *Klebsiella* sp. and *Acinetobacter* sp. The *cps* biosynthesis locus in *Klebsiella* sp. resembles that of Group 1 CPS and also maps to the same locus. However, in *A. baumannii* and other *Acinetobacter* sp., the *cps* locus (known as the K locus) maps to *fkpA/lldP* and includes the *wza-wzb-wzc* transcribed in the opposite direction to serotype-specific genes with *wzi* located elsewhere on the chromosome (Kenyon and Hall 2013).

The CPS locus, and indeed loci driving synthesis of other bacterial surface polysaccharides such as the OPS, may be described as hyper-mutable hotspots that enable rapid cell-surface glycodiversification in response to selective pressures, thereby increasing the overall fitness of the organism (Mostowy and Holt 2018). Genetic mechanisms promoting the generation of new or altered serotypes include mutation or the acquisition, loss or exchange of genes by lateral gene transfer. The general architecture of polysaccharide loci and their location at a common locus effectively enables homologous recombination between conserved portions that flank serotype-specific genes, while other exchanges may be mediated by transposable elements.

3 ABC Transporter-Dependent Pathways

The ABC transporter-dependent pathway is employed for biosynthesis of various cell surface glycans and glycoconjugates, such as OPS, CPS, teichoic acids, mycobacterial arabinogalactan, as well as for S-layer protein glycosylation (Greenfield and Whitfield 2012; Caffalette et al. 2020). Particularly, homopolysaccharides and some heteropolysaccharides that have relatively small repeating units (three monosaccharide residues in the main chain at most) are produced by this pathway. In this case, polymerization is accomplished by sequential glycosyl transfer to the non-reducing terminus of a lipid-anchored nascent polymer at the cytosolic face of

the inner membrane and followed by translocation of the mature polysaccharide across the inner membrane mediated by the bacterial ABC transporter MsbA (Fig. 2). Therefore, no polymerization is required after the translocation.

The ABC transporter is composed of two transmembrane domains (Wzm) and two nucleotide-binding domains (Wzt), which are encoded by *wzm* and *wzt* genes, respectively (Greenfield and Whitfield 2012; Hug and Feldman 2011). The Wzt contains the ATPase domain, the hydrolysis of ATP being thought to provide the energy for the passage of a synthesized glycan across the inner membrane. The Wzm has multiple membrane-spanning sequences believed to build a transmembrane channel for the glycan export.

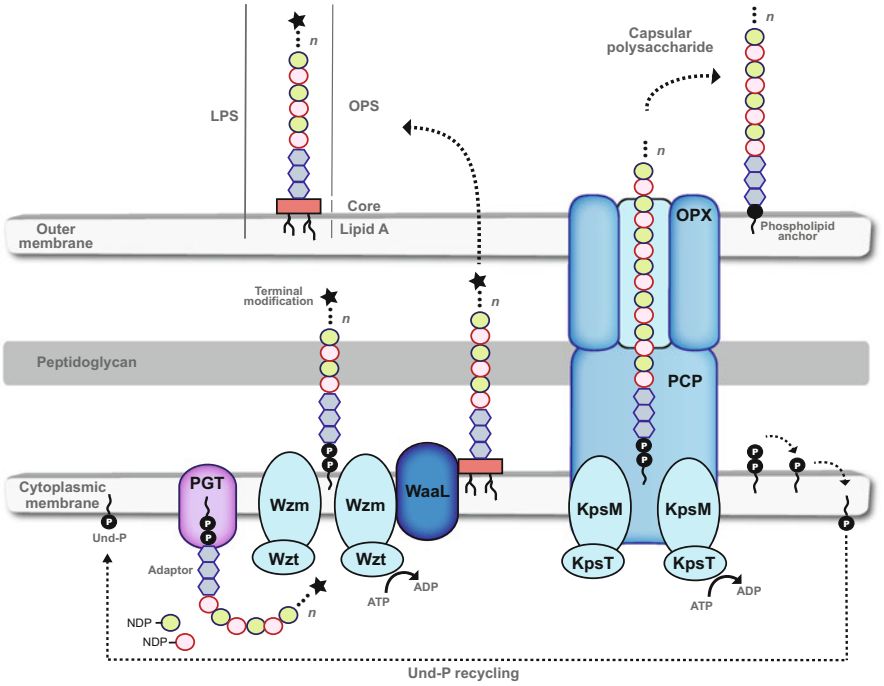


Fig. 2 Representation of the ABC transporter pathway for OPS and CPS biosynthesis in gram-negative bacteria. Nucleotide-linked sugars are synthesized in the cytoplasm, and a phosphoglycosyltransferase (PGT) initiates synthesis with the addition of the first sugar 1-phosphate to the undecaprenol phosphate (Und-P) lipid carrier creating an Und-PP-sugar moiety. Glycosyltransferases (GTs) add an adaptor sequence and further sugars to form a polymer chain, and synthesis may be terminated by a terminal modification (indicated by a star). The polymer is then translocated across the cytoplasmic membrane by the ABC transporter, Wzm/Wzt (referred to as KpsM/KpsT in the *E. coli* CPS synthesis model). For lipopolysaccharide (LPS) synthesis, the polysaccharide is ligated to the lipid A-core moiety by the O-antigen ligase WaaL, and the LPS is then exported to the cell surface by the Lpt machinery. For CPS synthesis, a polysaccharide co-polymerase (PCP) and an outer membrane export protein (OPX) facilitates transfer of the polysaccharide to the cell surface. CPS may be associated with the cell surface via a phospholipid anchor. Und-PP is released upon polymerization and cleaved to give Und-P for recycling

3.1 O-Specific Polysaccharides

As with the Wzx/Wzy-dependent pathway, OPS synthesis begins with construction of the β-D-GlcNAc undecaprenyldiphosphate (Und-PP-D-GlcNAc) primer by transfer of D-GlcNAc-1-P from UDP-α-D-GlcNAc to Und-P, which is accomplished by the activity of a phosphoglycosyltransferase. In Enterobacteriaceae, this step is catalyzed by the inverting GlcNAc-1-P transferase, WecA. In *Pseudomonas aeruginosa,* a bifunctional WbpL transferase initiates biosynthesis of both common polysaccharide antigen (A-band D-rhamnan) by the ABC transporter-dependent pathway and OPS by the Wzy-dependent pathway by transfer of GlcNAc-1-P and N-acetyl-D-fucosamine-1-phosphate (D-FucNAc-1-P), respectively, to Und-P.

Further steps have been studied in detail for synthesis of mannans in *Escherichia coli* O8, O9, O9a, and a galactan in *Klebsiella pneumoniae* O2a (Table 1) (Greenfield and Whitfield 2012). Each of the homopolysaccharides is produced by three enzymes that contain single or multiple putative glycosyltransferase domains. They localize to the cytosolic face of the inner membrane, and none of them are an integral membrane protein.

Mannosyltransferases WbdC, WbdB, and WbdA are involved in the biosynthesis of the *E. coli* mannans. Both WbdC and WbdB are monofunctional (α1→3) mannosyltransferases responsible for synthesis of an adaptor, a short oligosaccharide that separates the Und-PP-D-GlcNAc primer from the repetitive OPS domain. WbdC acts first, transferring a single α-mannose residue to position 3 of the primer monosaccharide, and then WbdB attaches two more α-mannose residues giving rise to a Man-(α1→3)-Man-(α1→3)-Man-(α1→3) trisaccharide adaptor domain. The serotype-specific WbdA multi-domain mannosyltransferase (polymerase) elongates the adaptor by assembling the OPS domain composed of repeating oligosaccharides. WbdA of *E. coli* O9a contains two glycosyltransferase active sites, the N-terminal α(1→2) and C-terminal α(1→3) mannosyltransferase domains, the activity of the former being regulated by the latter. It synthesizes a tetrasaccharide O-unit by cycling turns, by adding two α(1→2) mannose residues followed by two α(1→3) mannose residues (Liston et al. 2014). Remarkably, in WbdA homologues, the number of putative glycosyltransferase domains correlates with the number of different linkage types in the corresponding O-unit. The O8 enzyme contains three mannosyltransferase domains (O8-unit consists of α(1→2), α(1→3), and β(1→3)

Table 1 Structures of repetitive glycan domains of some OPSs synthesized by the ABC transporter-dependent pathway

Bacterium	Polysaccharide structure
Escherichia coli O8 *Klebsiella pneumoniae* O5	→3)-Man-(β1→2)-Man-(α1→2)-Man-(α1→
Escherichia coli O9 *Klebsiella pneumoniae* O3	→2)-Man-(α1→2)-Man-(α1→2)-Man-(α1→3)-Man-(α1→3)-Man-(α1→
E. coli O9a	→2)-Man-(α1→2)-Man-(α1→3)-Man-(α1→3)-Man-(α1→
K. pneumoniae O2a	→3)-D-Gal*f*-(β1→3)-D-Gal*p*-(α1→

linkages), whereas the O9 and O9a versions contain only two (their O-units include α(1→2) and α(1→3) linkages) (Table 1).

Synthesis of the *K. pneumoniae* O2a galactan involves participation of galactosyltransferases WbbO, WbbM, and WbbN. In contrast to the mannosyltransferases, all three show extensive interactions with one another to form a membrane-associated complex. WbbO and WbbN possess α(1→3) galactopyranosyltransferase and β(1→3) galactofuranosyltransferase activities, respectively. The former transfers galactopyranose to the primer, and then WbbN links galactofuranose to the galactopyranose, creating a D-Gal*f*-(β1→3)-D-Gal*p*-(α1→3) disaccharide adaptor. The dual-active polymerase WbbM that possesses C-terminal galactopyranosyltransferase and N-terminal galactofuranosyltransferase domains acts next to synthesize the repetitive domain of the galactan (Table 1) (Clarke et al. 2020). The O2a glycan structure can be diversified by nonreducing terminal extension in the course of cytosolic polymerization with the O1 or O2c antigens or by side-chain glycosylation in the periplasm to create the O2afg or O2aeh antigens, as well as by possible nonstoichiometric O-acetylation (for *K. pneumoniae* OPS structures see Vinogradov et al. 2002).

There are two modes to terminate synthesis of the OPS, which are distinguished by the presence (or absence) of characteristic nonreducing terminal modifications on the polymer. These serve as chain termination and/or export signals.

In one mode, the length of the OPS is controlled by capping the polymer chain at its nonreducing end with a chemical group [e.g., 3-deoxy-β-D-*manno*-oct-2-ulosonic acid (β-Kdo), phosphate, or methylphosphate] to prevent further chain extension]. The modifying cap is transferred by a specific transferase containing a membrane-anchored coiled-coil domain, which serves as a molecular ruler that measures the length of the nascent polysaccharide. For instance, mannan termination in *E. coli* is accomplished by the activity of a WbdD enzyme, which is either a methyltransferase (in *E. coli* O8) or a bifunctional kinase-methyltransferase (in O9 and O9a). Despite the general similarities in the mannan structures, the *E. coli* O8 and O9a WbdD homologues are specific for their cognate OPSs, and not functionally interchangeable. In *E. coli* O9a, WbdD is tethered to the membrane and recruits WbdA into an active enzyme complex by protein-protein interactions. The site of interaction with WbdD is a surface-exposed α-helix in the C-terminal domain of WbdA but the presence of its N-terminal domain is required for the interaction (Liston et al. 2014).

Phosphorylation of the terminal mannose residue is sufficient to terminate chain extension and thus to control the size of the *E. coli* O9a OPS. The following methylation requires prior phosphorylation, but does not itself influence chain length. In contrast, in the O8 system, where the terminal modification consists of a single methyl addition, methylation alone is sufficient to terminate chain extension. Reduction in the length of the O8 and O9a OPSs can be achieved by over-expression of WbdD, thus implying that a correct stoichiometry between WbdD and the other synthesis components is necessary to establish the correct chain length modality. WbdD mutants of *E. coli* O9a lacking kinase-methyltransferase activity are capable of synthesizing the polymer but unable to export OPS across the inner membrane, similar to an ABC-transporter mutant. In both cases, the polymer accumulates inside the cell.

The specificity of the ABC transporter is defined by a discrete carbohydrate-binding domain at the C terminus of the Wzt component. Specific interaction of this domain with the terminal modification is essential for the OPS secretion. That the OPS biosynthesis precedes export suggests that in this pathway the biosynthesis and export machineries function separately. The prerequisite for the non-reducing terminal modification serves as a quality control process that ensures only terminated OPS molecules are moved across the inner membrane. As opposite to Wzt, the Wzm proteins are not specific toward the OPS structure and can translocate foreign polysaccharides, although the efficiency might be reduced.

Terminal capping residues have been identified in a number of other bacterial ABC transporter-dependent OPSs known or predicted to follow the *E. coli* mannan mechanism of chain termination and export (Greenfield and Whitfield 2012).

The other termination mode does not utilize end modification as highlighted for *K. pneumoniae* O2a, which is capable of exporting heterologous OPSs whether they contain a terminal modification or not. This ability implies that the ABC transporter could recognize some conserved feature between various OPSs other than a non-reducing terminal residue, such as Und-PP-D-GlcNAc. Unlike the *E. coli* O9a system, *K. pneumoniae* O2a OPS polymerization may be terminated during or after ABC transporter–mediated export. These processes are obligatorily coupled and must be synchronized through a specific interaction of the glycosyltransferases (or their product) with the ABC transporter. In the *K. pneumoniae* O2a system, the Wzt component of the ABC transporter lacks external C-terminal domain, and the OPS export is independent of the carbohydrate-binding domain but only occurs if the biosynthetic and export components are coexpressed. The polymer chain length is governed by a stoichiometry between the ABC transporter and the synthesis machinery. Over-expressing the ABC transporter results in early chain termination to afford an OPS with a significantly reduced average chain length and a broader modal chain lengths distribution, perhaps owing to premature export of nascent O-antigen chains.

The ABC transporter in nucleotide-free and ATP-bound states forms a continuous solvent-accessible transmembrane channel, which is sufficiently large to accommodate a long polymer, such as OPS (Caffalette et al. 2019). In the ATP-bound conformation, the channel displays a lateral opening toward the periplasmic lipid leaflet. The transport is initiated by interaction of the Und-PP-linked OPS with a small gate helix between the first two β-strands of the Wzm protein, which creates an electropositive pocket that may selectively bind the negatively charged lipid head group of the substrate. Then the head-group inserts into the channel and reorients to the periplasmic side with the polyprenyl chain remaining in the bilayer phase (Caffalette et al. 2020).

Following reorientation by ABC transporter, the OPS is transferred from the Und-PP-linked intermediate to the lipid A-core by the integral membrane protein WaaL to complete a LPS molecule.

One bacterium can employ two pathways for synthesis of different polysaccharides. For instance, in addition to the *E. coli* O8-type mannan produced by the ABC transporter-dependent pathway (Greenfield and Whitfield 2012), *Escherichia*

albertii O9 synthesizes a heteropolysaccharide having a tetrasaccharide O-unit by the Wzx/Wzy-dependent pathway (Naumenko et al. 2020).

3.2 Capsular Polysaccharides

Two mechanistically distinct routes exist for CPS biosynthesis: group 1 and 4 CPSs are produced by the Wzx/Wzy-dependent pathway, whereas group 2 and 3 CPSs require the ABC-transporter export machinery. Apart from differences in transcriptional regulation, whereby group 2 capsules are distinguished by temperature-regulated expression, CPSs of both groups 2 and 3 are produced in a similar fashion. Before export, they are completely synthesized on a Kdo oligosaccharide adaptor linked to a phosphatidylglycerol group, which is believed to anchor the CPS to the cell surface. Group 2 and 3 capsules also have relatively small repeats (K units) consisting of one to three monosaccharide residues in the main chain. Both typically contain acidic monosaccharides, such as Kdo, *N*-acetylneuraminic acid and D-glucuronic acid, as well as a phosphate group that interlinks two monosaccharides or a monosaccharide and glycerol or ribitol. CPSs of this sort occur, e.g., in E. coli, *Neisseria meningitidis,* and Haemophilus influenzae (for CPS structures see Kunduru et al. 2016; Ovodov 2006).

In *E. coli*, CPS biosynthesis starts with the transfer of Kdo from CMP-β-Kdo to lyso-phosphatidylglycerol by glycosyltransferase termed KpsS (Caffalette et al. 2020; Doyle et al. 2019). The Kdo-lipid primer thus produced is then extended by 5–9 Kdo units by single bifunctional retaining glycosyltransferase KpsC having N- and C-terminal catalytic domains with different linkage specificities. The C-terminal domain attaches the second Kdo residue to position 7 of the lipid-linked Kdo residue and subsequently, the N-terminal domain adds multiple Kdo units to position 4 of the Kdo disaccharide, thereby producing a lipid-linked β-Kdo oligosaccharide adaptor. The precise number of β2→4 linked Kdo units is strain specific. In other characterized group 2 and 3 CPS biosynthesis systems KpsS and KpsC function similarly with some modifications, e.g., homologous activities are provided by HcsB/HcsA in Haemophilus influenzae and LipB/LipA in *Neisseria meningitidis*.

A significant exception, however, is represented by the Vi antigen of *S. enterica* sv. Typhi, a linear α1→4-linked polymer of D-GalNAcA, which is usually non-stoichiometrically O-acetylated at position 3. The Vi antigen is linked to a unique glycolipid terminus composed of a reducing N-acetylhexosamine residue carrying two β-hydroxylated acyl groups (β-hydroxymyristoyl or β-hydroxymyristoyl), thereby resembling half of a lipid A moiety of LPS (Hu et al. 2017). Accordingly, no KpsS and KpsC homologs are present in this species.

For CPS produced by the ABC transporter-dependent mechanism, the Kdo adaptor is extended by addition of sugar residues to the non-reducing terminus of the growing polysaccharide chain. This process is catalyzed by a processive glycosyltransferase, which remains associated with the polymer during synthesis. Based on sequence similarity and catalytic activities, the enzymes involved can be classified into two structural families: those containing a single Rossmann-like

domain or two domains with the active site located at the interface between the two. Homopolysaccharides are synthesized by a single glycosyltransferase-containing polypeptide, whereas heteropolymers can be synthesized by polypeptides comprising one or multiple glycosyltransferase domains or by multiple polypeptides each containing a single glycosyltransferase domain (Willis and Whitfield 2013).

In a coupled biosynthesis and export process, the transporter could export the substrate either before elongation is complete or following the glycan assembly (Whitfield 2006). The polymers are transported across the inner membrane to the periplasm in a single step by an ABC transporter–containing secretion system at the expense of ATP hydrolysis. Similar to OPS transporters, the functional CPS transporters are usually heterodimers that consist of two integral inner-membrane domains with six transmembrane helices and two cytoplasmic nucleotide-binding domains, referred to in *E. coli* as KpsM and KpsT, respectively (Fig. 2). Mutations in *kpsT* result in an inability to complete the export process and accumulation of an intracellular polymer at the periphery of the cytoplasm in proximity to the inner membrane.

KpsMT proteins have no specificity for polymer structure and provide another example of the ability of the transporter from one serotype to export glycans with other structures. This data suggest that the ABC transporter recognize a conserved element, such as lipid modification, but lipidation of the glycan, by itself, is not sufficient for the export.

ABC transporters of CPSs share several conserved features with transporters of OPSs, such as *E. coli* mannans, including a similar transmembrane topology and an N-terminal amphipathic helix. However, the cytosolic gate helix that likely recruits the Und-PP–linked OPS to initiate transport, is missing in the CPS transporters, perhaps reflecting the utilization of a different glycolipid anchor.

Completion of transport from the periplasm to the cell surface requires two other components, namely, multimeric periplasmic and outer membrane proteins, referred to as KpsE and KpsD in *E. coli*, which are thought to form a protein complex enabling synthesis and translocation from the cytoplasm to the cell surface in a coordinated process. The presence of homologs of *kpsTMED* genes serves as hallmark of genetic loci directing the production of ABC transporter-dependent CPSs (Willis and Whitfield 2013).

A peculiar feature of *Campylobacter jejuni* CPSs is the presence of various isomeric heptoses, 6-deoxyheptoses, and O-methylheptoses, as well as uncommon phase-variable non-sugar components, including 2-aminoethanol, 2-amino-1,3-propanediol, and *O*-methyl phosphoramidate. A similarity of the *cps* gene clusters of this species and those of *E. coli* group 2 and 3 CPSs suggests that the CPSs of *C. jejuni* are synthesized by the ABC-dependent pathway also. Comparison of the *cps* clusters of various strains revealed multiple mechanisms of CPS structural variation, including exchange of *cps* genes and entire clusters by horizontal transfer, gene duplication, deletion, fusion, and contingency gene variation (Karlyshev et al. 2005).

Sinorhizobium meliloti 1021 synthesizes succinoglycan called EPS I by the Wzx/Wzy-dependent pathway but under phosphate starvation or upon mutation in a

regulatory gene, it switches to production by the ABC transporter-dependent pathway of a galactoglucan (EPS II) decorated with pyruvic acid acetal and *O*-acetyl groups (Becker 2015).

3.3 Other Glycopolymers

Classical wall teichoic acids of gram-positive bacteria are polymers of polyol (most commonly glycerol or ribitol) repeat units linked via phosphodiester bonds and occasionally glycosylated. Export of these polymers follows steps similar to those for OPS biosynthesis by the ABC transporter-dependent pathway (Brown et al. 2013). Teichoic acid of *Staphylococcus epidermidis* is assembled by TagF, which catalyzes the transfer of a phospho-lycerol moiety from CDP-glycerol to the growing polymer chain. Wall teichoic acids are generated intracellularly on an Und-PP anchor and transported to the cell surface by the TarGH, an ABC transporter that resembles the Wzm/Wzt transporter, including a similar transmembrane topology and the presence of a cytosolic gate helix. TarGH likely does not discriminate between different teichoic acid structures yet may recognize the conserved Und-PP-GlcNAc moiety. After translocation across the membrane, the teichoic acid is attached to peptidoglycan, possibly following a modification, such as D-alanylation.

Two closely related ABC transporters participate in protein N-glycosylation in *Campylobacter jejuni* and LPS biosynthesis in *Helicobacter pylori* (Hug and Feldman 2011). In these bacteria, single polypeptides (half transporters), named PglK and Wzk, respectively, translocate the Und-PP-linked glycans across the cytoplasmic membrane. They possess relaxed specificity having no apparent structural requirements toward the glycan moiety of the substrates. These transporters can accomplish the export of diverse glycans and complement each other's role in glycoprotein or LPS biosynthesis. PglK or Wzk are closely related to a flippase responsible for translocation of the lipid A-core in the LPS biosynthesis pathway.

Classical two-component ABC transporters appear to be involved in S-layer protein glycosylation in *Paenibacillus alvei* (Hug and Feldman 2011).

4 Synthase-Dependent Pathway

The synthase-dependent pathway employs an inner membrane-embedded glycosyltransferase (synthase) and a co-polymerase or a multi-protein complex consisting of two from them. Synthase catalyzes a vectorial polymerization reaction usually by a processive mechanism, resulting in extension of the polysaccharide chain with simultaneous extrusion of the nascent polymer across the plasma membrane (Fig. 3). This pathway and the genetic loci involved are less complex than those of Wzy-dependent polymers.

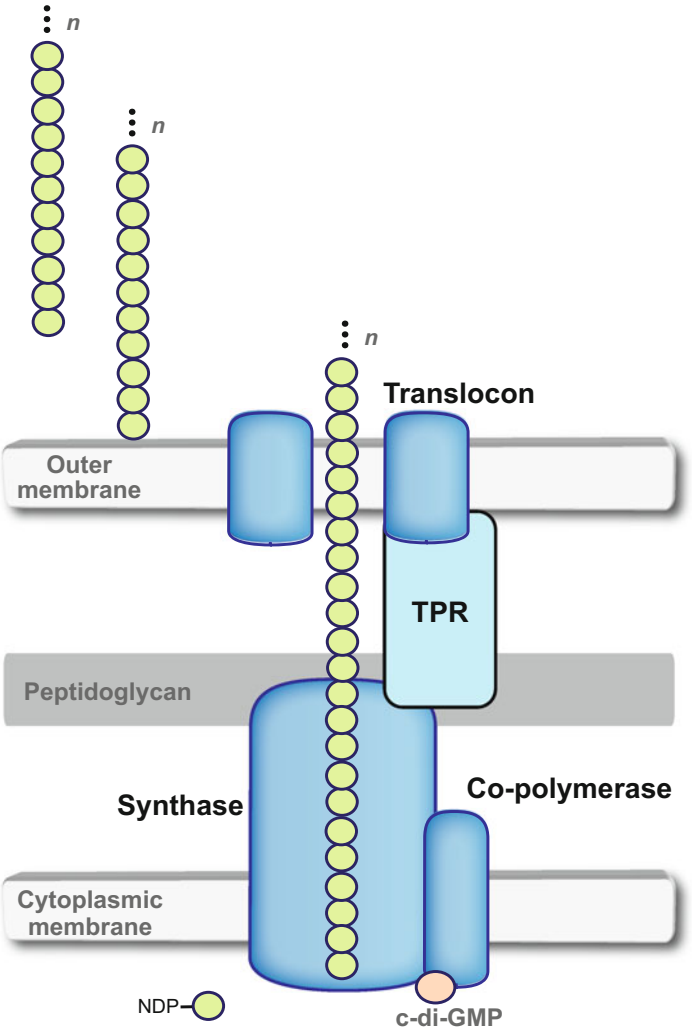


Fig. 3 Representation of the synthase polysaccharide synthesis pathway in gram-negative bacteria. Polysaccharide is produced by processive polymerization using a glycosyltransferase (synthase) and a co-polymerase, which facilitate the translocation of the polymer across the cytoplasmic membrane. A tetratricopeptide repeat (TPR) may direct the polymer through the translocon for export to the cell surface

The glycosyltransferase modules of synthases are typically assigned to the GT2 family of inverting glycosyltransferases and share sequence motifs which correlate with the processivity of these enzymes. Polymer secretion is dependent on tetratricopeptide repeat domains coupled to a β-barrel porin (translocon). The process is post-translationally regulated by binding the bacterial secondary messenger bis-(3-5')-cyclic dimeric guanosine monophosphate (c-di-GMP) to the synthase complex (Low and Howell 2018; Steiner et al. 2013).

4.1 Exopolysaccharides

A number of exopolysaccharides (EPSs), both homopolysaccharides and heteropolysaccharides having relatively simple structures, such as glucans, alginate, and some hexosaminoglycans, are produced using the synthase-dependent mechanism. Many from them are known as components of biofilm, a slimy extracellular matrix in which bacterial cells stick to each other and often also to a biotic or abiotic surface. The polymer length and export of the biofilm-forming EPSs are regulated by hydrolases or (in case of alginate) a lyase, which can disrupt the preformed biofilms and prevent biofilm formation. In gram-negative bacteria, EPSs frequently (but not always) carry a lipid moiety at the reducing end.

Rhizobacteria employ the synthase-dependent pathway for production of various glucans, including cellulose, sophorans, curdlan, and (1→3,1→6)-β-glucans.

Biosynthesis of cellulose, a major component of biofilms formed by rhizobia and several other bacteria, occurs by polymerization using the UDP-D-Glc nucleotide sugar precursor, the polymer chain being elongated at the non-reducing end. Bacterial cytoplasmic membrane cellulose synthase proteins are encoded in the *bcs* operon (Low and Howell 2018; Schmid et al. 2015).

The large central domain of the integral membrane protein BcsA contains a single UDP-glucose binding site and the catalytic site, and cellulose synthesis occurs within the cytosolic domain of BcsA. Activation of the synthesis begins with binding of c-di-GMP to the PilZ domain of BcsA. Another component of the synthase complex, BcsB, possesses a C-terminal transmembrane helix that anchors the protein to the inner membrane. The carbohydrate-binding domains and BscB–BcsA interaction interface are responsible for binding and translocation of the cellulose polymer as it moves through the transmembrane domain of the BcsA/B complex, thus allowing for processive polymerization to occur. In some bacterial species, BcsA and BcsB are fused as a single polypeptide.

Located downstream from the cellulose synthase operon is a gene *bcsZ* encoding an endo-acting β-glycosidase, which hydrolyzes glucans consisting of more than three glucose monomers and has an essential role in the cellulose production. From additional proteins included in the synthase complex, BcsC is proposed to function as a pore formation protein to enable cellulose secretion, whereas BcsD seems to be involved in control of crystallization process of cellulose nanofibrils. The accessory proteins BcsE/F/G/R/Q interact with the BcsA/B synthase complex and accounted for the extra volume on the cytoplasmic face. BcsQ is proposed to regulate stability of the BcsA/B complex, and BcsE/F/G are responsible for production of modified cellulose at the inner membrane with BcsG specifically functioning to add the phosphoethanolamine modification. The formation of cellulose microfibrils is proposed to be consistent with multimerization of the synthase complex.

Cyclic (1→2)-β-glucans (cyclosophorans) that contain 15–28 glucose residues may occur either as extracellular material or in periplasm, where they play a role in osmoregulation. Some of the glucose residues may be substituted at position 6 with glycerol 1-phosphate. The sophorans are synthesized through protein-linked oligosaccharide intermediates (ChvB or NdvB encoded in the *chv* and *ndv* chromosomal regions in *Agrobacterium tumefaciens* or *Rhizobium meliloti*, respectively). The

protein component of the intermediates is a unique cyclic (1→2)-β-glucan synthase, which catalyzes the overall cyclosophoran synthesis, including initiation (protein glycosylation), chain elongation, and cyclization. The *chv* and *ndv* chromosomal regions also code for the ChvA/NdvA protein, which is probably involved in the transport of cyclosophorans to the periplasmic space (Castro et al. 1996).

Curdlan, a water insoluble (1→3)-β-glucan without any substituents, is produced by, e.g., *Agrobacterium.* Four genes *crdA, crdS, scrdR*, and *crdB* are involved in curdlan biosynthesis, as well as pss_{AG}, which enhances curdlan production. The c*rdASC* genes form a cluster and are likely to be co-transcribed, whereas *crdR* and pss_{AG} occur elsewhere in the genome.

The key enzyme in curdlan biosynthesis is β-glucosyltransferase (curdlan synthase) CrdS, an integral membrane protein with seven transmembrane helices and a large intracellular hydrophilic region, which has high sequence similarity to the bacterial cellulose synthase BcsA. However, as opposite to the latter, CrdS lacks any c-di-GMP binding domain. Polymerization is believed to occur by the repetitive addition of glucosyl residues from UDP-D-Glc using an inverting mechanism that is mediated through a single displacement reaction via a glycosyl-enzyme intermediate. It presumably occurs within the catalytic domain on the large cytoplasmic loop of CrdS followed by extrusion of the polysaccharide from the cell. Two other genes in the cluster are predicted to encode a membrane-anchored protein CrdA that may play a role in transfer of curdlan across the cytoplasmic membrane, and a periplasmic protein CrdC that may function in passage of the polymer through the periplasm. The helix-turn-helix protein CrdR transcriptionally activates curdlan biosynthesis, and a membrane protein Pss_{AG} plays a role in membrane phospholipid synthesis and functions to enhance curdlan production (McIntosh et al. 2005; Thompson et al. 2018; Schmid et al. 2015).

Cyclic (1→3,1→6)-β-glucans are found in the periplasm of some rhizobacteria. They are typically composed of two blocks of three (1→3)-linked glucose residues, each separated by a block of three (1→6)-linked glucose units. In *Bradyrhizobium japonicum* and *Rhizobium loti*, the glucan has a single-glucose branch at position 6, and some molecules are substituted at position 6 with phosphocholine. In *B. japonicum*, the cyclic glucan is synthesized with the aid of (1→3)- and (1→6)-β-glycosyltransferases. The mechanism of cyclization remains unknown, but on the analogy of cyclosophorans, it has been proposed that the terminal non-reducing glucose residue of a produced oligoglucan is able to accept the protein-linked "reducing" glucose residue to release the cyclic molecule (McIntosh et al. 2005).

Poly-β-(1→6)-N-acetyl-D-glucosamine (PNAG) is synthesized by, and is an important virulence factor of, numerous pathogenic bacteria, and is also produced by some fungal and eukaryotic organisms. In gram-negative bacteria, PNAG production, modification, and export are dependent on the *pgaABCD* operon. The process is initiated by the synthase complex of the inner-membrane proteins PgaC and PgaD, which is responsible for the synthesis and transport of PNAG across the cytoplasmic membrane. PgaC contains a glycosyltransferase domain and interacts with PgaD in a c-di-GMP-dependent manner. PgaA is a two-domain protein that contains an N-terminal periplasmic tetratricopeptide repeat interaction domain and a

C-terminal outer-membrane porin that facilitates PNAG export across the outer membrane (Low and Howell 2018; Steiner et al. 2013; Little et al. 2018).

PgaB, a two-domain periplasmic protein with an N-terminal carbohydrate esterase domain, displays metal-dependent deacetylation activity on PNAG oligomers. N-Deacetylation of PNAG is a critical step in polymer maturation and is required for the formation of robust biofilms. The C-terminal domain of PgaB possesses a glycoside hydrolase activity and can cleave partially deacetylated PNAG, disrupt pre-formed PNAG-dependent biofilms, and potentiate killing by antibiotics. Only deacetylated PNAG could be cleaved, suggesting that PgaB deacetylates and hydrolyses the polymer in sequential order. A GlcN-GlcNAc-GlcNAc motif in the deacetylated PNAG substrate has been found to be required for cleavage of the polymer.

Listeria monocytogenes produces an insoluble EPS composed of a backbone of β-(1→4)-linked D-ManNAc with α-D-Gal branching on every other main-chain residue. The *pssA-E* operon is responsible for the EPS biosynthesis, which is activated by binding c-di-GMP by the PssE receptor. Polymerization is performed by inverting glycosyltransferase PssC, which is similar to cellulose and PNAG synthases but utilizes UDP-α-D-ManNAc as substrate. PssD assists the movement of the growing polysaccharide chain onto the cell surface, similarly to the BcsB subunit of cellulose synthases. The α-galactosyltransferase that decorates the ManNAc chain has not been identified (Köseoğlu et al. 2015).

The pellicle polysaccharide (PEL) occurs in biofilms formed by *Pseudomonas aeruginosa* and *Ralstonia solanacearum*. It is composed predominantly of (1→4)-linked D-Gal*p*NAc and D-Glc*p*NAc and their *N*-deacetylated counterparts that are randomly distributed along the linear polymer chain. The positive charge enables interaction of the Pel polysaccharide with other key biofilm matrix components. The *pelABCDEFG* operon is responsible for the PEL phenotype.

The cytoplasmically located single glycosyltransferase PelF accomplishes polymerization, whereas PelE and PelG are involved in transportation across the inner membrane. The cytoplasmic domain of PelD is a c-di-GMP receptor responsible for the c-di-GMP mediated regulation of PEL production. Translocation of PEL across the outer membrane occurs via a membrane-embedded porin PelC, which is unique to the PEL system. PelC is proposed to function as an electronegative funnel to guide cationic PEL toward the export channel formed by PelB. As PgaB of the PNAG biosynthesis pathway, a multi-domain PEL-modifying protein PelA exhibits both hydrolase and N-deacetylase activities (Franklin et al. 2011).

Alginates are synthesized by brown algae and bacteria belonging to the genera *Pseudomonas* and *Azotobacter*. They are made of variable amounts of (1→4)-linked β-D-mannuronic acid and its C5-epimer α-L-guluronic acid. The comonomers are arranged in blocks of continuous mannuronic acid residues (M-blocks), guluronic acid residues (G-blocks), or mixed MG-blocks. The arrangement and sequence of the sugar residues and in particular the formation of G-blocks is similar in algal and *A. vinelandii* alginates, whereas the polymers derived from *Pseudomonas* lack G-blocks.

Initially, alginate is synthesized as a poly-β-(1→4)-D-mannuronic acid. The *alg* operon responsible for the biosynthesis of alginate includes, among others, the genes

required for synthesis of the nucleotide-sugar precursor GDP-D-ManA. The activated D-ManA is polymerized presumably by membrane-anchored Alg8, a glycosyltransferase, which is a component of a multiprotein complex spanning the cytoplasmic membrane (Alg8, Alg44), the periplasm (AlgX, AlgK, AlgG, AlgL), and the outer membrane (AlgE). A scaffold of the periplasmic proteins has been proposed to guide the nascent alginate chain through the periplasm for secretion by the alginate-specific channel protein AlgE in the outer membrane. AlgE is linked to the periplasmic scaffold via an interaction with the lipoprotein AlgK, which interacts with AlgX. The latter links the periplasmic scaffold to the polymerase subunits via an interaction with Alg44, a co-polymerase which also binds c-di-GMP required for activation of alginate synthesis. A membrane-anchored sensor protein MucR is required to specifically activate alginate formation by generating a localized c-di-GMP pool likely in proximity to Alg44.

After coupled polymerization and transport across the inner membrane, alginate is modified in the periplasm by C5-epimerization by AlgG and O-acetylation with the help of four proteins AlgIJFX or partially degraded by alginate lyase AlgL, which is an exo-enzyme, cleaving sugars at the polysaccharide end by the mechanism of β-elimination (Schmid et al. 2015). These modifications are critical for the formation of biofilm, bacterial virulence, and persistence.

Given the predicted structural similarity of the transmembrane and glycosyltransferase domains of PgaC and Alg8 to BcsA of the cellulose biosynthesis pathway, one could predict that these systems use a similar mechanism for polymerization and export, whereby the transmembrane domains of these proteins and the corresponding co-polymerase proteins Alg_{44} and PgaD form a conduit for passage of alginate and PNAG, respectively, through the inner membrane.

4.2 Capsular Polysaccharides

Structures of the CPSs that are produced by the synthase-dependent pathway are shown in Table 2. With a disaccharide repeating unit composed of β-Glc and β-GlcA (Table 2), the CPS of the gram-positive pathogen, *S. pneumoniae* type 3, is the simplest of the pneumococcal heteropolysaccharides. As for the CPS of *S. pneumoniae* type 37 (see below), this CPS is synthesized by the synthase-dependent pathway rather than Wzy-dependent pathway characteristic for all other *S. pneumoniae* serotypes. The molecular basis for this phenomenon is mutation of most sequences in the genetic locus between *dexB* and *aliA* genes for synthesis of *S. pneumoniae* CPS by the Wzx/Wzy-dependent pathway, and biosynthesis of the type 3 CPS being driven by two other genes in the locus: *cps3D* for UDP-Glc dehydrogenase and *cps3S* or *wchE* for bifunctional synthase (Yother 2011; Llull et al. 2001).

The Cps3S synthase is similar to those that catalyze syntheses of cellulose, chitin, and hyaluronan in both prokaryotes and eukaryotes (Yother 2011; Geno et al. 2015). It initiates biosynthesis of the type 3 CPS by transfer of D-Glc from UDP-D-Glc to phosphatidylglycerol, an abundant lipid in *S. pneumoniae* membranes, then transfers D-GlcA from UDP-D-GlcA to the phosphatidylglycerol-linked glucose, and extends

Table 2 Structures of CPSs synthesized by the synthase-dependent pathway

Bacterium	Polysaccharide structure
Streptococcus pneumoniae type 3	→3)-β-D-Glc*p*A-(1→4)-β-D-Glc*p*-(1→
S. pneumoniae type 37 *Propionibacterium freudenreichii*	→3)-β-D-Glc*p*-(1→ 2 ↑ 1 β-D-Glc*p*
Streptococcus pyogenes *Streptococcus equisimilis* *Streptococcus uberis* *Streptococcus zooepidemicus* *Pasteurella multocida* type A	→4)-β-D-Glc*p*A-(1→4)-β-D-Glc*p*NAc-(1→ hyaluronan
P. multocida type F	→4)-β-D-Glc*p*A-(1→3)-β-D-Gal*p*NAc-(1→ chondroitin
Escherichia coli K4	→4)-β-D-Glc*p*A-(1→3)-β-D-Gal*p*NAc-(1→ 3 ↑ 1 β-D-Fru*f*
P. multocida type D *E. coli* K5	→4)-β-D-Glc*p*A-(1→4)-α-D-Glc*p*NAc-(1→ heparosan
Comamonas testosteroni	→4)-α-D-GlcpA-(1→4)-α-D-GlcpNAc-(1→ testosteronan

the chain by alternating additions of D-Glc and D-GlcA to its nonreducing end. When the oligosaccharide chain reaches a length of eight sugars, the lipid-linked octasaccharide is translocated to the external face of the cytoplasmic membrane. At this point, the substrate binds tightly to the carbohydrate binding site of the synthase, resulting in rapid, processive synthesis of high-molecular-weight polymer and a reorientation of the synthase-lipid-saccharide complex that permits the extrusion of the polymer across the membrane as synthesis continues.

Regulation of the transition from oligosaccharide to polysaccharide synthesis, as well as control of the final chain length of the polymer, is dependent on the ratio of UDP-D-Glc to UDP-D-GlcA. If the concentration of UDP-D-GlcA is insufficient, the lipid-linked oligosaccharide is unable to attain the critical octasaccharide length, and chain synthesis terminates with the lipid-linked oligosaccharide being ejected from the synthase in an abortive translocation reaction and unable to serve as a substrate for further elongation. Unlike Wzx/Wzy-dependent CPSs, the type 3 capsule is not linked to peptidoglycan but remains membrane bound via linkage to phosphatidylglycerol or interactions with the Cps3S synthase.

As opposite to other polysaccharides that are synthesized by the synthase-dependent pathway, the CPS of *S. pneumoniae* type 37 is branched with β1→3-linkage between the sugar residues in the main chain and β1→2-linkage by which the side-chain glucose residue is attached to each glucose residue of the main chain (Table 2). A cryptic CPS locus occurs between *dexB* and *aliA*, and *tts*, the only gene required for serotype 37 synthesis, is located elsewhere on the chromosome (Llull et al. 2001).

The type 37 CPS is synthesized from UDP-D-Glc by a single transmembrane enzyme Tts, which is a unique inverting β-glycosyltransferase (synthase) having a dual biochemical activity for making both (1→3)- and (1→2)-linkages to yield the branched type 37 polysaccharide. It remains to be determined whether the formation of the glycosidic bonds of both types occur simultaneously or synthesis of a curdlan-like β1→3-glucan precedes that of the sophorose unit. No lipid-linked intermediate is required for biosynthesis of the branched *S. pneumoniae* type 37 CPS, and the nascent type 37 CPS chain does not use specific transporters to cross the membrane.

A β-glucan having the same structure as the CPS of *S. pneumoniae* type 37 is produced by a gram-positive food-grade bacterium *Propionibacterium freudenreichii*. The capsular phenotype of this bacterium correlates with expression of a unique chromosomal gene *gtf* having strong homology with the *tts* synthase gene of *S. pneumoniae* (Deutsch et al. 2010).

Some pathogenic bacteria form a capsule composed of glycosaminoglycans (GAGs) or, more exact, glycosaminoglucuronans. Members of this group: hyaluronan, chondroitin, heparosan, and testosteronan are linear heteropolysaccharides composed of disaccharide repeats containing an *N*-acetylhexosamine (D-GlcNAc or D-GalNAc) and D-GlcA. This strategy forms the basis of molecular camouflage since vertebrates possess naturally occurring GAGs that are essential for life. All bacterial GAGs are produced by the synthase-dependent pathway, the enzymes involved are dual-action glycosyltransferases that utilize the corresponding UDP-sugar precursors and a metal cofactor. They are relatively small integral membrane proteins that share a similar topology with generally four transmembrane domains and a large cytoplasmic loop (Yother 2011; DeAngelis 2012). The majority of GAG biosynthetic genes are clustered together on chromosomal operons.

Hyaluronan or hyaluronic acid (HA) is composed of disaccharide repeats of β-D-GlcA and β-D-GlcNAc (Table 2). It is found in some gram-positive streptococci and a gram-negative pathogen *Pasteurella multocida* type A. HA is made as a free glycan, not attached to protein or lipid.

HA synthases are bifunctional, inverting glycosyltransferases, which do not require a lipid or another primer for polymer biosynthesis, rather initiate it de novo with only the UDP-sugars and a divalent cation to coordinate the UDP-sugar donor molecule for nucleophilic attack by the acceptor molecule. Mechanisms for regulating chain length are unresolved, but relative substrate concentrations seem to be important (Yother 2011).

Streptococcal HA synthases (Class I) have a single glycosyltransferase module with similarity to chitin synthase. They possess both domain A and domain B candidates but the number and the nature of substrate-binding sites are unknown. Being 70% identical to each other, Class I HA synthases mediate the same overall processive reaction distinguished by adding new sugars to the reducing end of the growing polymer. They also appear to facilitate HA transfer across the membrane.

The HA synthase of *P. multocida* type A (Class II) is quite different. It polymerizes the chain from the non-reducing end and has two interacting glycosyltransferase domains (one in a peripheral membrane protein and one in a cytoplasmic protein) with no B domain. There are two UDP-sugar binding sites, and the reaction mediated

by this enzyme is non-processive, the nascent polymer product releasing after each elongation step to reposition the new terminal sugar unit for the next transfer.

The CPS of *P. multocida* type F represents unsulfated chondroitin containing β-D-GlcA and β-D-GalNAc (Table 2). Chondroitin synthase of type F is 90% identical to the HA synthase of *P. multocida* type A and possesses the same structural organization. Both enzymes have two similar relatively independent selective glycosyltransferase sites and appear to utilize the same general catalytic mechanism for non-processive polymerization by non-reducing end elongation (DeAngelis 2012).

The *E. coli* K4 CPS has an unsulfated chondroitin backbone with fructose residues β-linked at position 3 of the D-GlcA residues. KfoC, a bifunctional chondroitin glycosyltransferase necessary for elongation of the chondroitin chain, is about 60% identical to the *P. multocida* types A and F synthases and thus probably utilizes similar motifs and domains (DeAngelis 2012). It has been suggested that the fructose branch is added to the K4 polymer chain after the chondroitin repeat is formed.

The CPS of *P. multocida* type D and *E. coli* K5 is heparosan, a non-sulfated unepimerized heparin consisting of β-D-GlcA and α-D-GlcNAc (Table 2). As both UDP-sugar precursors are α-linked, biosynthesis of this polymer involves two mechanism types: retaining to produce the α-linkages and inverting to form the β-linkage.

In *E. coli* K5, there are two distinct proteins, α-D-GlcNAc-transferase KfiA and β-D-GlcA-transferase KfiC, which work in concert to form heparosan. In *P. multocida* type D, there are two homologous dual-action two-domain heparosan synthases (PmHS1 or HssA and PmHS2 or HssB). One region of PmHS1 is similar to KfiA of *E. coli* K5 and the other to KfiC. PmHS2 is better able to generate polysaccharide in the absence of exogenous acceptor (de novo synthesis) and yields smaller molecular-weight-product size distributions than PmHS1. As opposite to *hssA*, the *hssB* gene is located outside the capsule gene cluster (Otto et al. 2012; DeAngelis and White 2004). As HA synthases, heparosan synthases of *P. multocida* prefer Mg^{2+} rather than Mn^{2+} required by *Streptococcus* for the GAG biosynthesis. The difference in metal preference may be an indication of differences in coordination geometry at the active site structures and/or in the reaction mechanisms.

The overall organization of the CPS biosynthesis loci of *P. multocida* types A, D, and F are quite similar, with highly homologous UDP-glucose dehydrogenase genes following the virtually identical synthase genes found in most *P. multocida* type A, D, and F isolates. The occurrence of multiple polysaccharide synthases in a single strain invokes the potential for capsular variation (DeAngelis and White 2004).

In *Avibacterium paragallinarum*, there are two genotypes (I and II) of the capsular locus. The genotype I genes encode proteins that are most similar to proteins from *P. multocida* CPS types A and F, and those of genotype II to proteins from *P. multocida* CPS type D and *E. coli* K5. Accordingly, genotype I strains contain a hyaluronan or chondroitin capsule, whereas heparosan is present in genotype II strains (Wu et al. 2010).

A recently described GAG of *Comamonas testosteroni*, testosteronan, consists of the same sugars, D-GlcA and D-GlcNAc, as the biomedically relevant GAGs heparosan and hyaluronan but has distinct glycosidic linkages (Table 2). Testosteronan

is synthesized by a bifunctional synthase CtTS that exhibits similarity with *P. multocida* synthase PmHS1 responsible for the synthesis of a heparosan capsule. A divalent cation, Mg^{2+} or Mn^{2+}, is required for the CtTS activity (Otto et al. 2011).

4.3 O-Specific Polysaccharides

The only known example of an O-specific polysaccharide that is assembled by the synthase-dependent mechanism is that of *S. enterica* sv. Borreze O:54, which represents a homopolymer of *N*-acetyl-D-mannosamine (D-ManNAc) with alternating β-(1→3) and β-(1→4) linkages. The enzymes involved in its production are encoded by genes *wbbE, wbbF*, and *mnaA* carried on a naturally occurring 6.9-kb plasmid, which is unique in possessing a functional O-antigen gene cluster (Keenleyside and Whitfield 1996).

Assembly of the O:54 polysaccharide on the cytoplasmic face of the inner membrane begins with the transfer of D-GlcNAc-1-P to Und-P by the phosphoglycosyltransferase WecA encoded in the ECA gene locus. This locus also contains a functional homolog of *mnaA*, the gene coding for UDP-D-GlcNAc 2-epimerase that converts UDP-D-GlcNAc to UDP-D-ManNAc. Following the precursor synthesis, a monofunctional glycosyltransferase WbbE transfers the first D-ManNAc residue to Und-PP-D-GlcNAc, committing the intermediate to O:54 biosynthesis and creating an adaptor on which chain extension occurs. Next, bifunctional glycosyltransferase (synthase) WbbF effects polymerization through stepwise addition of sugar residues to afford the repeat domain of the OPS, with growth occurring at the non-reducing end. The absence of a dedicated transporter for the O:54 antigen led to the proposal that WbbF also is responsible for the export of the Und-P-linked glycan across the inner membrane. Therefore, the O:54 synthesis involves a unique mechanism for delivering nascent OPS to the O-antigen ligase, which transfers the translocated product to lipid A-core at the periplasmic face of the inner membrane.

That biosynthesis of the O:54 polysaccharide involves a lipid-linked intermediate suggests fundamental differences between the activity of WbbF and that of other characterized synthases, which are responsible for the synthesis and export of a variety of important bacterial extracellular biopolymers, such as cellulose and GAGs. However, molecular modelling of the glycosyltransferase domain of WbbF indicates a similar architecture, and site-directed mutagenesis confirmed that functionally important sequence motifs shared by WbbF and classical synthases are likely conserved despite the use by WbbF of a lipid acceptor for chain extension (Wear et al. 2020).

5 Single Sucrase Pathway

Several glucans and fructans are synthesized extracellularly by a single transglycosidase (sucrase), a protein classified as glycoside hydrolase (Xu et al. 2019). Dextrans mainly consist of poly-α-(1→6)-D-glucose with α-(1→3)-linked branches, and there are also dextrans containing other linkages. Levan is a fructan including

mainly α-(2→6)-linkages with occasional α-(2→1)-branches, and inulin type fructan possesses the opposite α-(2→1)-chain with α-(2→6)-branches. Dextransucrases are produced by lactic acid bacteria, primarily *Leuconostoc mesenteroides* but also some *Lactobacillus* species. Levansucrases are widely distributed among gram-positive bacteria, and several plant pathogens carry more than one enzyme. Inulinsucrases are only produced by lactic acid bacteria. When fed with maltose as a sole priming substrate, fructansucrases can also produce maltosylfructosides.

All sucrases catalyze the direct transfer of glucose or fructose obtained by cleavage of sucrose onto a growing oligo- or polysaccharide chain outside the cell. Dextransucrases are secreted and anchored to the cell wall. The enzymes are built of multiple domains but only the C-domain consists of one contiguous polypeptide and is involved in glucan production and/or binding. The exact enzymatic mechanisms of chain initiation and elongation of the polymers are not well understood and the existing evidences are discussed in a recent review (Schmid et al. 2015).

6 Conclusions

Microbial surface polysaccharides play crucial roles in the interaction of microorganisms with the environment, including other biological systems. Multi-enzyme pathways for their biosynthesis are complex, and the precise mechanisms of protein function and protein interaction have long been the focus of research over several decades. Though further work is still needed to generate specific detail on the complexities of these pathways, the general sequence of each synthesis pathway is well understood. While only four common pathways are known for the processing and export of bacterial surface polysaccharides, the format of these glycan structures are extraordinary diverse, which presents a varied panel of natural substrates that could be harnessed and utilized for human application.

References

Bastin DA, Stevenson G, Brown PK, et al. Repeat unit polysaccharides of bacteria: a model for polymerization resembling that of ribosomes and fatty acid synthetase, with a novel mechanism for determining chain length. Mol Microbiol. 1993;7:725–34.

Becker A. Challenges and perspectives in combinatorial assembly of novel exopolysaccharide biosynthesis pathways. Front Microbiol. 2015;6:687.

Brown S, Santa Maria JP Jr, Walker S. Wall teichoic acids of gram-positive bacteria. Annu Rev Microbiol. 2013;67:313–36.

Caffalette CA, Corey RA, Sansom MSP, et al. A lipid gating mechanism for the channel-forming O antigen ABC transporter. Nat Commun. 2019;10:824.

Caffalette CA, Kuklewicz J, Spellmon N, Zimmer J. Biosynthesis and export of bacterial glycolipids. Annu Rev Biochem. 2020;89:741–68.

Castro OA, Zorreguieta A, Ielmini V, Vega G, et al. Cyclic β-(1,2)-glucan synthesis in rhizobiaceae: roles of the 319-kilodalton protein intermediate. J Bacteriol. 1996;178:6043–8.

Clarke BR, Ovchinnikova OG, Sweeney RP, et al. A bifunctional O-antigen polymerase structure reveals a new glycosyltransferase family. Nat Chem Biol. 2020;16:450–7.

Collins RF, Kargas V, Clarke BR, et al. Full-length, oligomeric structure of Wzz determined by cryoelectron microscopy reveals insights into membrane-bound states. Structure. 2017;25:806–15.
DeAngelis PL. Glycosaminoglycan polysaccharide biosynthesis and production: today and tomorrow. Appl Microbiol Biotechnol. 2012;94:295–305.
DeAngelis PL, White CL. Identification of a distinct, cryptic heparosan synthase from *Pasteurella multocida* types A, D, and F. J Bacteriol. 2004;186:8529–32.
Deutsch SM, Pierre Le Bivic P, Hervé C, et al. Correlation of the capsular phenotype in *Propionibacterium freudenreichii* with the level of expression of *gtf*, a unique polysaccharide synthase-encoding gene. Appl Environ Microbiol. 2010;76:2740–6.
Doyle L, Ovchinnikova OG, Myler K, Mallette E. Biosynthesis of a conserved glycolipid anchor for gram-negative bacterial capsules. Nat Chem Biol. 2019;15:632–49.
Franklin MJ, Nivens DE, Weadge JT, Howell PL. Biosynthesis of the *Pseudomonas aeruginosa* extracellular polysaccharides, alginate, Pel, and Psl. Front Microbiol. 2011;2:167.
Geno KA, Gwendolyn L, Gilbert GL, Song JS, et al. Pneumococcal capsules and their types: past, present, and future. Clin Microbiol Rev. 2015;28:871–99.
Greenfield LK, Whitfield C. Synthesis of lipopolysaccharide O-antigens by ABC transporter-dependent pathways. Carbohydr Res. 2012;356:12–24.
Hong Y, Liu MA, Reeves PR. Progress in our understanding of Wzx flippase for translocation of bacterial membrane lipid-linked oligosaccharide. J Bacteriol. 2018;200:e00154–17.
Hu X, Chen Z, Xiong K, et al. Vi capsular polysaccharide: synthesis, virulence, and application. Crit Rev Microbiol. 2017;43:440–52.
Hug I, Feldman MF. Analogies and homologies in lipopolysaccharide and glycoprotein biosynthesis in bacteria. Glycobiology. 2011;21:138–51.
Karlyshev AV, Champion OL, Churcher C, Brisson JR, Jarrell HC, Gilbert M, … Szymanski CM. Analysis of Campylobacter jejuni capsular loci reveals multiple mechanisms for the generation of structural diversity and the ability to form complex heptoses. Mol Microbiol. 2005;55(1):90–103.
Keenleyside WJ, Whitfield C. A novel pathway for O-polysaccharide biosynthesis in *Salmonella enterica* serovar Borreze. J Biol Chem. 1996;271:28581–92.
Kenyon JJ, Hall RM. Variation in the complex carbohydrate biosynthesis loci of *Acinetobacter baumanni*i genomes. PLoS One. 2013;8:e62160.
Kenyon JJ, Cunneen MM, Reeves PR. Genetics and evolution of *Yersinia pseudotuberculosis* O-specific polysaccharides: a novel pattern of O-antigen diversity. FEMS Microbiol Rev. 2013;41:200–17.
Köseoğlu VK, Heiss C, Azadi P, et al. *Listeria monocytogenes* exopolysaccharide: origin, structure, biosynthetic machinery and c-di-GMP-dependent regulation. Mol Microbiol. 2015;96:728–43.
Kunduru BR, Nair SA, Rathinavelan T. EK3D: an *E. coli* K antigen 3-dimensional structure database. Nucleic Acids Res. 2016;44:D675–81.
Liston SD, Clarke BR, Greenfield LK, et al. Domain interactions control complex formation and polymerase specificity in the biosynthesis of the *Escherichia coli* O9a. J Biol Chem. 2014;290:1075–85.
Little DJ, Pfoh R, Le Mauff F. PgaB orthologues contain a glycoside hydrolase domain that cleaves deacetylated poly-β(1,6)-N-acetylglucosamine and can disrupt bacterial biofilms. PLoS Pathog. 2018;14:e1006998.
Liu B, Furevi A, Perepelov AV, et al. Structure and genetics of *Escherichia coli* O antigens. FEMS Microbiol Rev. 2019. https://doi.org/10.1093/femsre/fuz028.
Llull D, Garcıá E, López R. Tts, a processive β-glucosyltransferase of *Streptococcus pneumoniae*, directs the synthesis of the branched type 37 capsular polysaccharide in pneumococcus and other gram-positive species. J Biol Chem. 2001;276:21053–61.
Low KE, Howell PL. Gram-negative synthase-dependent exopolysaccharide biosynthetic machines. Curr Opin Struct Biol. 2018;53:32–44.
Lukose V, Walvoort MTC, Imperiali B. Bacterial phosphogylcosyl transferases: initiators of glycan biosynthesis at the membrane interface. Glycobiology. 2017;27:820–33.

McIntosh M, Stone BA, Stanisich VA. Curdlan and other bacterial (1→3)-β-D-glucans. Appl Microbiol Biotechnol. 2005;68:163–73.

Morona R, van den Bosch L, Manning PA. Molecular, genetic, and topological characterization of O-antigen chain length regulation in *Shigella flexneri*. J Bacteriol. 1995;177:1059–68.

Mostowy RJ, Holt KE. Diversity-generating machines: genetics of bacterial sugar-coating. Trends Microbiol. 2018;26:1008–21.

Naumenko OI, Zheng H, Shashkov AS, et al. *Escherichia albertii* EA046 (O9) harbors two polysaccharide gene clusters for synthesis of the O-antigen by the Wzx/Wzy-dependent pathway and a mannan shared by *Escherichia coli* O8 by the Wzm/Wzt-dependent pathway. Int J Biol Macromol. 2020;142:609–14.

Otto NJ, Solakyildirim K, Linhardt RJ, DeAngelis PL. *Comamonas testosteronan* synthase, a bifunctional glycosyltransferase that produces a unique heparosan polysaccharide analog. Glycobiology. 2011;21:1331–40.

Otto NJ, Green DE, Masuko S, et al. Structure/function analysis of *Pasteurella multocida* heparosan synthases. Toward defining enzyme specificity and engineering novel catalysts. J Biol Chem. 2012;287:7203–721.

Ovodov YS. Bacterial capsular antigens. Structural patterns of capsular antigens. Biochem Mosc. 2006;71:937–54.

Schmid J, Sieber V, Rehm B. Bacterial exopolysaccharides: biosynthesis pathways and engineering strategies. Front Microbiol. 2015;6:496.

Ståhle J, Widmalm G. Lipopolysaccharides of gram-negative bacteria: biosynthesis and structural aspects. Trends Glycosci Glycotechnol. 2019;31:E159–71.

Standish AJ, Morona R. The role of bacterial protein tyrosine phosphatases in the regulation of the biosynthesis of secreted polysaccharides. Antioxid Redox Signal. 2014;20:2274–89.

Steiner S, Lori C, Boehm A, Jenal U. Allosteric activation of exopolysaccharide synthesis through cyclic di-GMP-stimulated protein–protein interaction. EMBO J. 2013;32:354–68.

Thompson MA, Onyezir MC, Fuqua C. Function and regulation of *Agrobacterium tumefaciens* cell surface structures that promote attachment. Curr Top Microbiol Immunol. 2018;418:143–84.

Valvano MA. Common themes in glycoconjugate assembly using the biogenesis of O-antigen lipopolysaccharide as a model system. Biochem Mosc. 2011;76:729–35.

Vinogradov E, Frirdich E, MacLean LL, et al. Structures of lipopolysaccharides from *Klebsiella pneumoniae*. Eluicidation of the structure of the linkage region between core and polysaccharide O chain and identification of the residues at the non-reducing termini of the O chains. J Biol Chem. 2002;277:25070–81.

Wear SS, Hunt BA, Clarke BR, Whitfield C. Analysis of the topology and active-site residues of WbbF, a putative O-polysaccharide synthase from *Salmonella enterica* serovar Borreze. J Bacteriol. 2020;202:e00625–19.

Whitfield C. Biosynthesis and assembly of capsular polysaccharides in *Escherichia coli*. Annu Rev Biochem. 2006;75:39–68.

Whitfield C, Wear SS, Sande C. Assembly of bacterial capsular polysaccharide and exopolysaccharides. Annu Rev Microbiol. 2020;74:521–43.

Willis LM, Whitfield C. Structure, biosynthesis, and function of bacterial capsular polysaccharides synthesized by ABC transporter-dependent pathways. Carbohydr Res. 2013;378:35–44.

Woodward R, Yi W, Li L, et al. *In vitro* bacterial polysaccharide biosynthesis: defining the functions of Wzy and Wzz. Nat Chem Biol. 2010;6:418–23.

Wu JR, Chen PY, Shien JH, et al. Analysis of the biosynthesis genes and chemical components of the capsule of *Avibacterium paragallinarum*. Vet Microbiol. 2010;145:90–9.

Xu Y, Cui Y, Yue F, et al. Exopolysaccharides produced by lactic acid bacteria and bifidobacteria: structures, physiochemical functions and applications in the food industry. Food Hydrocoll. 2019;94:475–99.

Yother J. Capsules of *Streptococcus pneumoniae* and other bacteria: paradigms for polysaccharide biosynthesis and regulation. Annu Rev Microbiol. 2011;65:563–81.

Part IV

Polysaccharide properties, Functionalizations, and Modifications

Polysaccharides of Fungal Origin

22

Focus on the Capsule of the Pathogen *Cryptococcus neoformans*

Maggie P. Wear and Arturo Casadevall

Contents

Abstract

Cryptococcus neoformans was first isolated as a human pathogen in the 1890s. Subsequent study has shown that the polysaccharide capsule of *C. neoformans* is a key virulence factor. Unlike in bacteria, the pathways for fungal polysaccharide synthesis are not well characterized with only a single glycosytransferase – β-(1,2)-xylosyltranferase Cxt1 – having been confirmed. While there is evidence that polysaccharide synthesis occurs in the cytosol and is transported to the extracellular space in extracellular vesicles, how the capsule attaches to the cell and why some polymers are secreted instead of incorporated into the capsular architecture remains a mystery. In this chapter we explore this along with our current biophysical, structural, and immunological understanding of cryptococcal polysaccharides. From these studies we outline

M. P. Wear (✉) · A. Casadevall
Department of Molecular Microbiology and Immunology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA
e-mail: mwear1@jhu.edu; acasadevall@jhu.edu

J. M. Oliveira et al. (eds.), *Polysaccharides of Microbial Origin*,
https://doi.org/10.1007/978-3-030-42215-8_29

the outstanding questions in the field and identify a bevy of techniques and applications open for future research.

Keywords

Cryptococcus · Fungal polysaccharides · Glucuronoxylomannan (GXM) · Virulence factor · Structure

1 Introduction: A History of *C. neoformans*

The only encapsulated fungal pathogen *Cryptococcus neoformans* was first isolated from a lesion on a human female tibia and described by Otto Busse in publications in 1894 and 1895, far before bacterial polysaccharides were described (Barnett 2010) (Fig. 1a). It was independently identified by two other researchers in that timeframe. The naming convention for *C. neoformans*, however, was not settled until 1935, 5 years after capsular shedding was first described. Rhoda Benham was responsible for the final naming along with the first examination of immune responses to *C. neoformans* showing that the capsule was antigenic (Benham 1935). *C. neoformans* remains, to this day, the only known pathogenic encapsulated yeast, making it unique among fungal pathogens. Through the 1940s and 1950s research on *C. neoformans* continued, much of it focused on the immune response to the pathogen. During this time the four serotypes of *C. neoformans* – A, B, C, and D – were defined and the idea of polysaccharides as antibody epitopes was first proposed. But it was not until 1950 that the first characterization of the capsular polysaccharides (PSs) of *C. neoformans* came from Drouhet et al. (1950). He showed that the capsular polymers were made of xylose, mannose, and uronic acid (Drouhet et al. 1950). Following the identification of the component monosaccharides of the capsule, the first PS structures were proposed in the 1960s. This work was updated in the 1980s and 1990s using Nuclear Magnetic Resonance (NMR) to define the PS structures as we know them today (Fig. 1B). In the midst of this structural work, Bulmer & Sans showed that the capsule itself was essential for the virulence of *C. neoformans* (Bulmer and Sans 1968). This work was re-visited and confirmed using complementation by Chang & Kwon-Chung in 1994 (Chang and Kwon-Chung 1994). While we know that the polysaccharide capsule is critical to fungal virulence, our understanding of the structure of this complex capsule – primary, secondary, and tertiary – and how it contributes to fungal virulence, has not advanced as far. In this chapter we will focus more on what is known, and unknown, when it comes to the capsule of *C. neoformans*. In addition, we hope that our brief comparison of bacterial versus fungal polysaccharide synthesis, transport, and structural complexity will help readers gain a better understanding of the similarities and differences in microbial polysaccharide pathways. Finally, we will delve into the technological advancements that have been made utilizing these fungal polymers to develop better diagnostic tools and vaccines against cryptococcosis.

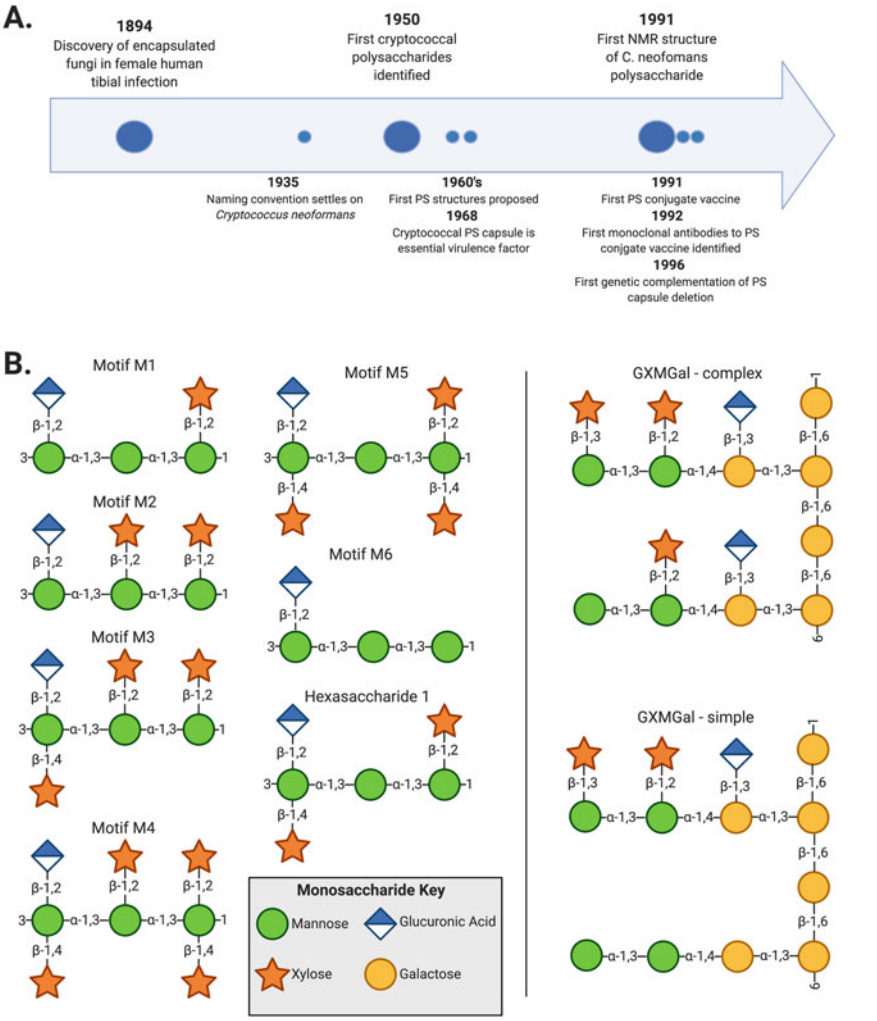


Fig. 1 History and structure of cryptococcal polysaccharides. (**a**) History of the identification of *Cryptococcus neoformans* and the polysaccharide capsule. (**b**) Structural motifs of the major (left) polysaccharide GXM and the minor (right) secreted polysaccharide GXMgal

2 Comparison of Bacterial and Fungal Polysaccharide Synthesis, Transport, and Attachment

While bacteria and cryptococcal fungi are known to produce polysaccharide capsules essential for their virulence, much more is known about bacterial polysaccharides. There are two reasons for this, first bacterial polysaccharides have been the subject of years of broad and intense study and, second, they are simpler in nature

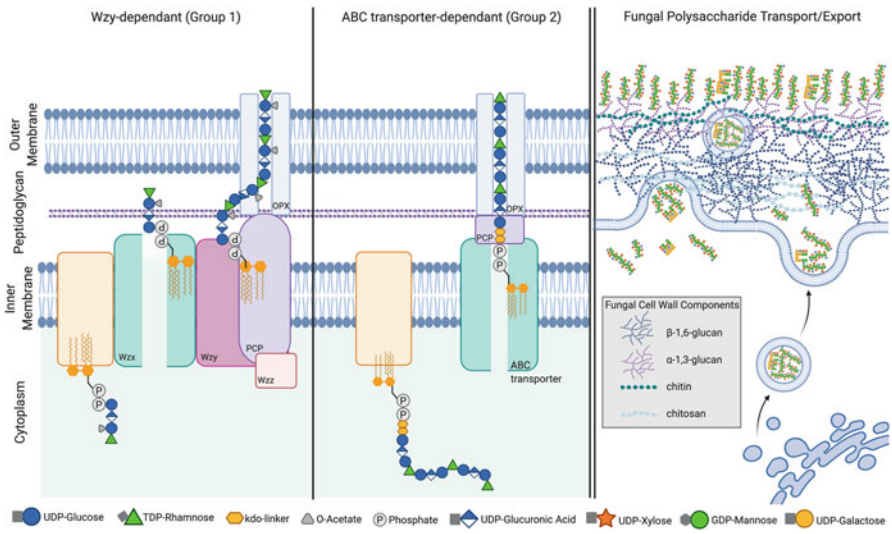


Fig. 2 Microbial polysaccharide transport and export. (left) Overview of Group 1 transport/export in bacteria. (center) Overview of Group 2 transport/export in bacteria. (right) Overview of current theory of fungal polysaccharide transport/export

often with a single monosaccharide repeat varying in linkage and a linear structure. While bacterial capsular polysaccharides (CPS) display substantial structural diversity, they are synthesized and assembled by one of two pathways (Whitfield 2006) (Fig. 2). The initiation of CPS synthesis occurs at the cytosolic face of the inner membrane where nucleotide activated sugar donors are used to build polymers on lipid-anchored acceptors (Sande et al. 2019). At this point, bacterial polysaccharide synthesis and transport diverges along two paths – Wzy-dependent group 1 or ABC transporter-dependent group 2 assembly (Fig. 2). In the Wzy-dependent pathway, individual units of CPS are built on undecaprenol diphosphate and exported across the inner membrane by Wzx, a MurJ flipase. Individual subunits are assembled into full length polymers in the periplasmic space by the Wzy polymerase, namesake of the pathway (Fig. 2) (Sande et al. 2019). By comparison the polymers in the ABC transporter-dependent pathway are entirely synthesized in the cytoplasm. Repeat units are added to a terminal phosphatidyl glycerol lipid linked short ß-linked 3-deoxy-D-manno-octulosonic acid oligosaccharide (Fig. 2) (Willis and Whitfield 2013). Transport of polysaccharides from the periplasmic space to the cell surface occurs by a single mechanism and involves two families of proteins – Outer membrane polysaccharide export (OPX) protein, and a periplasmic adaptor protein. Export occurs via the adaptor protein linking the inner membrane transport machinery to the outer membrane OPX protein, forming a channel resembling drug efflux pumps (Cuthbertson et al. 2009). Together, decades of work have led to a coherent picture of bacterial polysaccharide synthesis, transport, and assembly. While some parts of the pathway are better understood than others, the rough mechanism of action has been described.

In comparison to the bacterial pathways, our understanding of fungal polysaccharide synthesis, transport, and attachment remains in its infancy. Our lack of understanding arises from a number of factors. First, it is important to understand that the relative complexity of bacterial polysaccharides is determined by the type of linkage in the polymer repeat. In comparison, fungal polysaccharide complexity resides on a number of factors (i) the variation of mannose O-acetylation, (ii) the branching of xylose and glucuronic acid, which also contains, (iii) variability in xylose linkage. In addition, (iv) *C. neoformans* produces two types of CPS, glucuronoxylomannan (GXM) and glucuronoxylomanogalactan (GXMgal). GXM and GXMgal vary both in structure and constituent sugar residues. Finally, while GXMgal seems to have a consistent repeat unit, (v) a polymer of GXM can be made of a mixture of seven known repeat units. Although GXM makes up 90% of the CPS, mutations exist wherein *C. neoformans* produces only GXMgal, accelerating access to, and as a result understanding of, this minor polymer. Therefore, our current understanding indicates fungal polysaccharides are at least an order of magnitude more complex than their bacterial counterparts. Similar to bacterial polymer synthesis, fungal polymer synthesis proceeds from nucleotide activated sugar donors. However, currently there is evidence for fungal polymer synthesis both inside the cytosol (Rodrigues et al. 2007) as well as extracellularly (Freeze and Elbein 2009) (Fig. 2). While we know that at least three glycosyltransferases are necessary for the synthesis of chains of polysaccharide, seventy genes have been identified, three putative mannosyltransferases proposed, and only a single transferase – Cxt1 β-1,2-xylosyltranferase – has been confirmed (Chang and Kwon-Chung 1994, 1998; Lombard et al. 2014; Klutts and Doering 2008; Klutts et al. 2007). Thus, our understanding of cryptococcal polysaccharide synthesis has made great progress but researchers have yet to identify the glycosyltransferases necessary to put together the α-1,3-mannose backbone or for the majority of the branchpoints including β-1,4-xylose and β-1,2-glucuronic acid substitutions.

Though our understanding of *C. neoformans* CPS synthesis remains early, investigations of fungal CPS attachment have yielded a better understanding. *C. neoformans* CPS is anchored to the cell wall. A number of studies have examined genetic mutations which disrupt this connection, all result in reduced cell viability and often yield avirulent mutants. This reinforces the link between CPS and virulence in *C. neoformans*. The cryptococcal cell wall is complex, made up of at least a half-dozen glycans and can be divided into two layers – an alkali-insoluble matrix of β-glucan and chitin on the inner layer where melanin is deposited, and a less organized alkali-soluble predominantly α- and β-glucan outer layer (Wang et al. 2018). In an elegant set of experiments Doering and colleagues were able to show that the deletion of Ags1, an α-1,3-glucan synthase and transporter, results in loss of capsule formation (Wang et al. 2018). However, secreted polysaccharide is still produced. While this secreted polysaccharide – referred to as exopolysaccharide (EPS) – has been observed since 1935, its correlation to CPS remains a controversy. The clear involvement of α-1,3-glucan is supported further by recent observations of α-1,3-glucan linked to oligosaccharides of cryptococcal origin by our group (unpublished observations). However, the story is not as simple as a connection

between CPS and α-1,3-glucan, two putative glycohydrolases – Pdx1 and Pdx2 – are required for normal CPS association with the cell wall (Kumar et al. 2014). Observations of cryptococcal growth in different nutrient environments shows changes in capsular radius from virtually undetectable to greater than 30 μm in diameter. Arguably the Pdx proteins would be required during capsular growth as they function in the cell wall remodeling process, but their direct role in CPS attachment to the cell wall is not well characterized.

Our understanding of CPS synthesis, transport, and attachment has advanced significantly in the past two decades, but it is clear that we have much yet to discover. Two recent reviews on the cryptococcal capsule, one by our group and another by Doering and colleagues, ended with a series of questions probing the areas where our understanding has not advanced as quickly (Wang et al. 2018; Casadevall et al. 2019). These open areas encompass the mechanism of PS binding to the cell wall, roles for the myriad of proteins shown to affect capsule synthesis and architecture, and the identification of glycosyltransferases necessary to produce the CPS polymers. Finally, we have yet to establish where polysaccharide synthesis occurs, there is evidence in the literature to support cytosolic and extracellular synthesis.

3 Current Understanding of Cryptococcal Polysaccharides

The capsule is the most important virulence factor of the human pathogenic fungi *Cryptococcus neoformans* and *gattii* (McClelland et al. 2006). The capsule protects the fungus against phagocytosis and phagocytic cell oxidative bursts and shed polysaccharide has been associated with protean effects on the immune system that could contribute to virulence by undermining the effectiveness of the immune response. The capsular polysaccharide is also a mainstay of diagnosis since polysaccharide shed into blood and cerebrospinal fluid constitutes the cryptococcal antigen that, when detected, establishes the diagnosis of cryptococcosis (McClelland et al. 2006). Finally, capsular polysaccharide is the key immunogenic component of protein-polysaccharide conjugate vaccines, which have been shown to elicit protective antibody responses in mice and are immunogenic in humans (Devi et al. 1991; Devi 1996; Casadevall et al. 1992).

Despite more than a century of study we know very little about the capsule, because it poses a formidable architectural and structural problem. The capsule is composed mostly of water, which means that it is easily disrupted by any technique that requires dehydration, such as electron microscopy (Maxson et al. 2007a). The polysaccharides composing the capsule are macromolecules with masses exceeding 1 million Da that are heterodisperse. The sheer size of these molecules means that a full-scale structural solution by NMR is not possible and their heterodisperse nature precludes crystallization. Analysis of the capsule by NMR has provided critical information on its composition but required shearing of PS molecules and processing that could have altered its structure. Hence, much of what we know about the capsule is indirect knowledge from diverse techniques.

We know the capsule of *C. neoformans* to be made up of three predominant components – glucuronoxylomannan (GXM) accounts for ~90% of the capsule content, but two other components glucuronoxylomannogalactan (GXMGal) and mannoprotein have been shown to play roles in host microbe interaction and protection of the yeast cell. The dominant polymer, GXM is made up of short, tri-mannose repeat units, referred to as motifs, of which there are seven reported (Fig. 1b). With an α-(1,3)-mannose backbone modified with a single β-(1,2)-glucuronic acid and varied β-(1,2)- and β-(1,4)-xylose, these motifs also reportedly contain 6-Oacteylation of the mannose, though the location and density of these modifications in each motif remains unknown. While NMR has allowed for the characterization of these four to seven residue polymers, no study has been able to determine how these motifs fit together to yield the megadalton sized polymers reported to extend the entire radius of the *C. neoformans* capsule. In this section we will explore what is known about cryptococcal polysaccharides through the lens of the techniques used and the information they can yield.

3.1 Biophysical Characterization

Historically the carbohydrates produced by *C. neoformans* have been assumed to be very large – 10 kDa–13 mDa in size (Maxson et al. 2007a; Frases et al. 2008, 2009). Further observations have been limited by a number of factors directly affecting the polysaccharides, the most consequential of which is dehydration, but also the lack of technology to examine whole, intact polymers. In the past 50 years, efforts to characterize the capsule of cryptococcal species has often utilized indirect biophysical techniques. These techniques are varied and provide information about the size, shape, and properties of polymers, even in a mixture, often in a nondestructive manner. Bacterial polysaccharides, being mostly linear, can benefit from rheological analysis, however fungal polysaccharides being branched require other techniques (Burchard 2008). For cryptococcal polysaccharides optical tweezers (OT) and light scattering (LS) have been used most broadly. While both of these techniques yield similar information about their targets, they examine different materials. OT is used on whole cells, therefore examines the intact capsular polysaccharide, while LS is used on relatively dilute solutions and examines secreted EPS.

Optical Tweezers (OT) utilizes infrared laser light to interact with the micrometric dielectric non-absorbent particles within the water in a sample (Frases et al. 2011). This results in light reflection within the optics (radiation pressure), and light refraction within the optics (gradient force). When the refraction is greater than the reflection, the polysaccharide particles are trapped and can be imaged. OT measurements yield information on viscosity, Young's modulus, and adhesion time for the particles trapped and imaged in the system. OT analysis of cryptococcal polysaccharides using polystyrene bead penetration shows that the outer regions of the capsule are less dense than the inner regions suggesting capsule enlargement occurs by axial lengthening of polysaccharides (Frases et al. 2011). Modifications to the original OT techniques have yielded a microrheology technique which allows for

measurement of the relative viscosity of a sample (η_r) as well as the viscoelasticity (η_c). These are important biophysical measurements because of the relationship between viscosity, size, mass, shape, and intermolecular interactions of molecules in solution. Araujo and colleagues examined the η_c of intact CPS using OT at a range of frequencies (~6.3–220 rad/sec) by comparing the reactions of a bead attached to the capsule to that of a reference bead (de S Araújo et al. 2019). These results show the dramatic loss is viscoelasticity with mAb 18B7 $\eta_c = 113 \pm 7$ as opposed to alone $\eta_c = 15 \pm 2$ (de S Araújo et al. 2019). Tasseri and colleagues measured the η_r of *C. neoformans* EPS from a number of clinical isolates in water from 0.002 to 10 g/L concentrations (Tassieri et al. 2015). The viscosity measurements from OT allow for the characterization of shape using two regimes of measurement, the low concentration or semi-dilute and the high concentration or entangled regime. The result of these measurements for EPS is a rod-like shape (Tassieri et al. 2015). While this suggests a linearity to the polysaccharides that is also seen with electron microscopy, these observations are limited by the necessity of sample dehydration prior to measurement. These observations also run counter to observations by light scattering which suggest EPS contains branched polymers (Cordero et al. 2011). Further OT experiments with stimulus angle frequencies between 10 and 10^6 rad/s performed with serotype A EPS at 0.25, 0.5, and 1 mg/ml reveal that complex viscosity decreases with increasing frequency independent of concentration (de S Araújo et al. 2019). There are generally two types of fluids with regards to viscosity, those that follow Newtonian laws of viscosity and therefore viscosity is constant regardless of the stressor, and non-Newtonian fluids where viscosity changes with the application of a stressor, like a change in frequency resulting in shear pressure. These experiments with EPS show that it is a non-Newtonian fluid with changing viscosity similar to that observed for plant polysaccharides (de S Araújo et al. 2019). It can be argued that EPS contains roughly linear polymers while the capsular architecture utilizes divalent cation bridging between polymers yielding the complex branched polymers also reported (de S Araújo et al. 2019; Nimrichter et al. 2007). Studies using OT indicate that the capsular polymers are linear, rod-like shapes that create viscous, non-Newtonian fluids and are therefore sensitive to changes in their environment. This suggests that the shedding of polysaccharides into the environment may be a reaction to changes the fungi encounter, like the impact of immune cells during infection.

Light scattering (LS) exploits the fact that when light, or any radiation, hits an object it causes changes in the light frequency, angular distribution, polarization, and intensity to define the size, shape, and molecular interactions of the object (Frases et al. 2011). Three types of LS have been used in the characterization of cryptococcal polysaccharides – Static light scattering (SLS), Dynamic light scattering (DLS), and Multi-angle light scattering (MALS) – each with its own advantages and limitations. The use of a single light beam in SLS, but at multiple angles, allows for the measurement of scatter intensity at those different angles yielding information about average molecular weight, radius of gyration, and second virial coefficient (Frases et al. 2011; Pontes and Frases 2015). The molecular weight of the sample is characterized by the hydrodynamic radius and the radius of gyration. The

hydrodynamic radius is the radius of the molecule under inspection assuming that it is round and is measured in both static and dynamic light scattering. As the size of the molecule being studied increases the isotropic (single point refraction/reflection) and anisotropic (colloidal refraction/reflection) scattering must be considered. The use of different angles allows for the dissection of isotropic from anisotropic scatter by plotting the scatter intensity. This slope of the plot yields both the radius of gyration and the related average molecular weight. While SLS can measure the interaction of individual molecules or complexes in a dilute solution where the solute is known (second virial coefficient), it cannot tell us much about these interactions. DLS, which measures light scattering over time, factors in Brownian motion and allows for measurement of polydispersity in addition to hydrodynamic radius (Pontes and Frases 2015). Polydispersity, referring to the variation in average molecular weight in a solution, again requires one to know the solvent as well as the temperature of the sample.

From the application of SLS and DLS to the polysaccharides of *C. neoformans* we have learned that EPS and CPS show differences in molecular mass, size, and viscosity (Frases et al. 2008). While this suggests that EPS and CPS are structurally different the relationship between them remains elusive. When we examine the variety of studies and their shared results just for CPS analysis by light scattering, we can see there is great variability (Table 1). Two studies – Cordero and colleagues and Frases and colleagues – utilized the same DMSO extraction method for CPS isolation, yet their results are quite varied. Some of this may be due to the methods used to culture the fungal cells prior to isolation, but this does not explain the seeming differences in radius of hydration (R_h) which would not be expected to vary within cultures of the same strain (Table 1). In fact, this extends to analysis of EPS. Two studies examined the same strain 24,067, and utilized the same extraction technique, yet their molecular weight and radius of gyration measurements are far outside the standard deviation of measurement (Table 2). As noted above, each of these techniques has limitations and while light scattering techniques are useful for defining the listed biophysical parameters, they cannot determine if the method of isolation has changed the structure of the polymers from their native state. To determine if the method of isolation alters EPS and CPS from their native state, we require structural analyses.

3.2 Structural Characterization

The polysaccharides composing the capsule are macromolecules with masses exceeding 1 million Da that are heterodisperse. The sheer size of these molecules means that a full-scale structural solution by NMR is not possible. However, analysis of the capsule by NMR and EM has provided critical information on its composition, even though it requires shearing of PS molecules and processing that could have altered its structure. Structural studies have utilized a series of steps to isolate the carbohydrates that also break them down into smaller pieces. This has allowed for analysis, albeit of altered polysaccharides. During the development of these isolation

Table 1 Biophysical properties of CPS

Strain	M_w ($\times 10^6$)	Avg size (nm)	Rg (nm)	Rh (nm)	PD	Capsule size (μm)	References
H99 (A)[a]	174.0 ± 12	N/D	207.1 ± 8.2	178.7 ± 11.1	0.453 ± 0.004	3.8 ± 0.5	Cordero et al. (2011)
H99	N/D	570 & 4241	N/D	3300 ± 500	0.5 ± 0.1	3.3 ± 1.2	Frases et al. (2009)
NIH191 (B)	164.0 ± 6.4	N/D	204.8 ± 4.2	1380.9 ± 9.6	0.439 ± 0.005	2.2 ± 0.2	Cordero et al. (2011)
NIH191	N/D	412 & 4217	N/D	1440 ± 60	0.386 ± 0.009	4.1 ± 1.3	Frases et al. (2009)
24,067 (D)	215.0 ± 32.0	N/D	215.4 ± 20	1754.8 ± 37.3	0.414 ± 0.005	5.8 ± 0.5	Cordero et al. (2011)
24,067	N/D	393 & 4397	N/D	3800 ± 700	0.50.2	4.5 ± 0.6	Frases et al. (2009)
B-3501 (D)	248 ± 39.0	N/D	191 ± 8.1	1033.9 ± 22.4	0.365 ± 0.009	2.3 ± 0.2	Cordero et al. (2011)
B-3501	N/D	585 & 4038	N/D	1500 ± 100	0.4 ± 0.1	3.8 ± 0.9	Frases et al. (2009)

N/D not determined

[a]Parentheses denoted serotype

Table 2 Biophysical properties of EPS

Strain	M_w ($\times 10^6$)	Avg size (nm)	Rg (nm)	Rh (nm)	PD	Capsule size (μm)	References
H99	1.7 ± 0.06	N/D	78 ± 2.8	N/D	N/D	0.6 ± 0.2	McFadden (2006)
B-3501	2.6 ± 0.52		151 ± 20			0.5 ± 0.1	McFadden (2006)
24,067	2.2	N/D	88.6	N/D	N/D	N/D	Nimrichter et al. (2007)
24,067	1.21 ± 0.12	N/D	55.8 ± 9.5	N/D	N/D	N/D	Frases et al. (2008)

N/D not determined

techniques carbohydrates were fractionated by molecular weight. While we now know that these molecular masses were not accurate for the size of the polymers the fraction would contain, they are effective for obtaining samples convenient for further examination. Analyses carried out on isolated EPS fractions have, until now, focused on the larger size fractions or unfractionated EPS and have yielded a wealth of information.

Electron Microscopy (EM) has the advantage of allowing for viewing of the entire cell, or in the case of *C. neoformans* – the capsule. When coupled with other technologies, this can be quite powerful. The use of different EM techniques has yielded different images of the capsule itself from short radiating fibrils (Takeo et al. 1973) to long thin coiled microfibrils (Edwards et al. 1967) to a fibrillar network (Cleare and Casadevall 1999; Sakaguchi et al. 1993; Sakaguchi 1993). Coupling EM with high resolution microscopy has further allowed for a qualitative estimation of width (~4 nm) and size (~10.63 kDa) of the fibers making up the polysaccharide network (McFadden et al. 2006a). Coupling scanning EM with gamma-irradiation (γ-IR) yields yet different information about the capsule. The free radicals resulting from the radiolysis of water by γ-IR have been shown to be able to break glycosidic bonds and in the *C. neofomans* capsule this results in loss of the less dense outer region of the capsule, though this is not uniform but can be seen as the loss of chunks of the fibrillar network (Maxson et al. 2007b). In addition, an EM analysis of polyphosphates, which are known to sequester cations like the calcium shown to form divalent cation bridges between GXM polymers, shows that these polyphosphates are localized to the cell wall with calcium (Ramos et al. 2017). When examining polyphosphate mutants (phoΔΔΔ and vtc4Δ) EM shows that sequestration of polyphosphates at the cell wall does not occur and the capsular fibers are thicker than in wild-type (Ramos et al. 2017). In addition, the external addition of polyphosphates increases the efficiency of building a capsule on acapsular mutants (cap67Δ) (Ramos et al. 2017). These results are enlightening and indicate that GXM polymers can be broken by γ-IR, polysaccharide-containing vesicles reside near lipid bilayers (Oliveira et al. 2009), and polyphosphates at the cell wall help regulate divalent cations necessary for polymer bridging and likely tertiary capsular

architecture. However, we have to keep in mind that EM requires dehydration and dehydration is known to alter capsular structure. In fact, when Cleare and Casadevall performed extended alcohol dehydration, they saw that the fibrillar structure collapsed entirely and no fibrillar network was observed (Cleare and Casadevall 1999). So, while we have learned a lot about the capsule from the application of EM, it is important to realize that the images of these structures in their dehydrated state may be an artificial representation of capsular architecture.

A series of NMR studies spanning the 1990s represents a tour-de-force of work to characterize the polysaccharides of *C. neoformans* by Robert Cherniak and colleagues (Skelton et al. 1991; Turner and Cherniak 1991; Turner et al. 1992; Bacon and Cherniak 1995; Sheng and Cherniak 1997; Vaishnav et al. 1998; Cherniak et al. 1998). This work culminated with the characterization of the EPS of 106 cryptococcal strains utilizing 1D [^{1}H] NMR and an artificial neural network applied to the structural reporter group section (SRG 5.0–5.4 ppm) and resulted in the identification of six of the seven GXM structural motifs still utilized today (Fig. 1b) (Cherniak et al. 1998). Further work expanded the known number of GXM structural motifs from six, adding hexasaccharide 1, to seven (Nimrichter et al. 2007; Bacon et al. 1996), and showed that strains of different serotypes contain different motifs. While each serotype has predominant motifs – serotype A, M2 motif; serotype D, M1 motif; serotype B, M3 motif; and serotype C, M4 motif – but that there are two additional motifs without predominance in a serotype. Beyond this, simply because a cryptococcal strain is serotype A does not mean that it will contain the M2 motif and only the M2 motif. In fact cryptococcal strains of serotype A express a range of motifs from 100% M2 (strain Mu-1) to 45% M2 and 55% M3 (strain 150) to 57% M2, 12.7% M1, 15.7% M3, 2.3% M5, and 12% M6 (H99), which lead Cherniak to develop Chemotypes for the different strains of *C. neoformans* (Cherniak et al. 1988; McFadden et al. 2006b). Unfortunately regular use of the chemotypes was never adopted such that we often do not know the motif makeup of newer strains, resulting in the reader not knowing if a serotype A strain expresses exclusively the M2 GXM motif or is more heterogeneous like H99, which expresses five different GXM motifs of which M2 is the dominant.

This bevy of NMR structural characterization extended beyond that of GXM and resulted in the structural characterization of GXMGal (previously referred to as GalXM) as well (Fig. 1b) (Vaishnav et al. 1998). While GXMGal only makes up between 1% and 10% of the polysaccharide capsule of *C. neoformans*, a genetic mutant *Δcap67* was discovered which results in the production of only GXMGal and no GXM (Vaishnav et al. 1998). As a result, our understanding of GXMGal greatly exceeds that of GXM. GXMGal is not directly associated with the cell wall, is smaller than GXM, and has a charge density favorable for anion-exchange chromatographic isolation. While smaller GXMGal is still a branched complex polymer and full length GXMGal has at least 15 anomeric peaks in the SRG region of a 1D [^{1}H] spectra, indicating that, at the time, it was too complex for full length characterization (Vaishnav et al. 1998). After partial depolymerization by smith degradation the structure of GXMGal was reconstituted from five chromatographic fractions (Fig. 1b).

Since the 1990s little has changed in our understanding of the structure of GXM and GXMGal. We have added an additional motif to GXM, a hexasaccharide with a set of xylose branches that were not previously characterized (Nimrichter et al. 2007). However, further investigations have found that the dehydration necessary for the storage of non-sterile samples may have an effect on the structure of the polymers (Maxson et al. 2007a; Cleare and Casadevall 1999) bringing to the foreground the need to update our understanding of cryptococcal polysaccharides using the more sophisticated technologies in NMR that are now available.

3.3 Immunological Characterization

Our understanding of the cryptococcal capsule as an immunogen follows its history and preceded our structural knowledge of the capsular polysaccharides. The serotypes of cryptococcus were first proposed as a division into two groups by Rhoda Benham, who was also responsible for finalizing the naming convention in 1935 (Walter and Coffee 1968). This was then refined into three serotypes – A, B, and C – by Evans and colleagues based on reciprocal agglutination studies in 1949 and 1950. While these three serotypes were known for quite some time, the discovery of serotype D did not occur until 1968 with a study aimed at utilizing serotype as an epidemiological tool (Wilson et al. 1968). While serotype A is known to be the most prevalent in mammalian infections and cryptococcosis, an updated cryptococcal serology denotes that serotypes A and D, along with the hybrid A/D are *C. neoformans* while serotypes B and C belong to *C. gattii* in the cryptococcal clade. But how does serotype correlate with the polysaccharide capsule? We know from the structural studies by Cherniak and colleagues that each of the first four GXM SRG motifs correlate with a serotype (see above structural characterization), but this is not the end of the story because there is vast variation within each serotype. Within a single serotype one strain may express a single GXM motif while another may express four or more different GXM motifs, though the predominant GXM motif will be shared among strains of the same serotype. Consequently, it was proposed by Cherniak that microvariation within serotypes, as explained by the heterogeneity of GXM motifs expressed, may account for differences in virulence (Cherniak and Sundstrom 1994).

For many years polyclonal sera from rabbits was used both for diagnosis of cryptococcosis and serotyping the cryptococcal strain responsible for infection. In 1987, the first monoclonal mouse antibodies to *C. neoformans* serotype A PS were identified by two groups – Eckert & Kozel and Dromer and colleagues (Eckert and Kozel 1987; Dromer et al. 1987a). From this work, the IgG E1 monoclonal antibody (mAb) was described (Dromer et al. 1987b). Following this work, Casadevall and Scharff attempted to derive mAbs from *C. neoformans* chronic infected mice (Casadevall and Scharff 1991). They observed an antibody peak between 11- and 18-days post infection with a decline thereafter even though the mice were chronically infected, suggesting that infection was not the most efficient method of eliciting an antibody response. Through this work only 4 of 60 mice showed

antibody titers >1:200 and fusions yielded 9 antibodies none of which were the desirable IgG1 isotype. IgG1 is the most desirable isotype because of its ability to promote phagocytosis, complement fixation, and bind the Fc receptor in macrophages, neutrophils, and lymphocytes. Then the first vaccine for *C. neoformans* was developed utilizing tetanus toxoid (TT) to boost the immune response to GXM when they were conjugated together (Devi et al. 1991). Immunizing mice with the GXM-TT conjugate, unlike utilizing whole *C. neoformans* infection, yielded an antibody response in all of the immunized mice (Casadevall et al. 1992). From these mice, which had antibody titers >1:1000, 33 mAb cell lines were isolated and characterized (Casadevall et al. 1992; Mukherjee et al. 1993). A number of these antibodies represent some of the most robust molecular tools utilized in cryptococcal research to this day. They have been utilized to develop ELISAs (Casadevall et al. 1992), in immunofluorescence to identify the different layers of the capsule (Maxson et al. 2007b), and one mAb 18B7 went through phase 1 clinical trials as a possible therapeutic for cryptococcosis (Larsen et al. 2005).

Enzyme-Linked Immunosorbent Assays (ELISAs) have been used as diagnostics and for biochemical analysis since their development in the 1970s. ELISAs exploit the interaction between antibodies and their substrates by binding the substrate – often a protein – to a polystyrene plate and probing with polyclonal sera or mAb. When *C. neoformans* mAbs were being identified the interaction between purified secreted polysaccharides and polystyrene plates was reported to be weak. To get around this, the first cryptococcal polysaccharide-specific ELISAs were indirect or sandwich ELISAs using sets of anti-GXM mAbs (Casadevall et al. 1992). Here the plate would be coated with a secondary antibody of one isotype – often IgM – then the primary mAb, followed by the polysaccharide, then another primary mAb or a different isotype – often IgG – followed by a tagged secondary to that isotype conjugated with an indicator like horseradish peroxidase (HRP) or alkaline phosphatase (AP). In fact, the ELISA for the quantification of polysaccharides currently uses is this double sandwich with two mAbs identified in the 1992 Casadevall study – 2D10 (IgM) and 18B7 (IgG1). Since this time the interaction between polysaccharides and polystyrene plates has been further investigated and found to be stable. More recently direct ELISAs with purified exopolysaccharides or capsular polysaccharide have been used to assay the sera of mice in response to polysaccharide-protein conjugate vaccines (unpublished data). In addition to these direct and indirect ELISAs, competition ELISAs are used to determine if two antibodies bind to the same epitope of GXM. Additionally, this method can be used to determine the epitope(s) bound by a sera from an immunized animal.

Beyond the quantification of polysaccharide and specificity of sera, these mAbs have been used to further characterize the *C. neoformans* polysaccharide capsule. Work by Maxson and colleagues utilized γ-IR in conjunction with immunofluorescence to identify different regions of the capsule and further our understanding of its architecture (Maxson et al. 2007b). Immunofluorescence allows imaging of an entire cell while fluorescently labeling just the epitope bound by an antibody. In this technique secondary antibodies conjugated with fluorophores, or directly conjugate primary antibodies efficiently label their epitope. When two antibodies with different

epitopes, and different fluorophores are utilized, the regions occupied by the different epitopes can be elucidated. In their work Maxson and colleagues observed that two mAbs 12A1 and 18B7 occupy different locations within the capsule, 12A1 remaining at the outer edge while 18B7 occupied a region inside of 12A1 but still a distance from the cell wall (Maxson et al. 2007b). This suggests that there are at least 3 regions of the capsule – the outer edge which is labeled by 12A1, a middle region which is labeled by 18B7, and an inner region which is not bound by either antibody. In the future there are a number of further promising experiments using immunofluorescence to characterize architectural features of the capsule like examining other strains of *C. neoformans*, as all of these experiments were performed with H99 which is known to express a mixture of GXM motifs.

In a bottom-up approach, these mAbs can be used to further define the polymers that make up the capsule of *C. neoformans*. Recently the Casadevall and Oscarson labs have collaborated to produce synthetic oligosaccharide arrays based upon the motifs of GXM (Guazzelli et al. 2020). These glycan arrays are printed with each synthesized oligosaccharide at a variety of concentrations, and in triplicate allowing for more precise analysis of antibody binding. While the GXM motifs were described some time ago, our understanding of how these 4–9 residue oligosaccharides fit together to form megadaltons-in-size polymers has been elusive. Utilizing these synthetic glycan arrays has shown that a decasaccharide containing two M2 motif repeats is bound by many of the anti-GXM mAbs that have been identified (Guazzelli et al. 2020). As this technology, and our abilities to manipulate it, improves it shows great promise in revealing larger and larger epitopes. In the future perhaps even showing how the motifs come together in GXM capsular polymers.

4 Future Application and Technologies for the Study of Cryptococcal Polysaccharides

Though we have known about microbial polysaccharides since the early 1900s, the molecular toolbox for their analysis has remained small. This is not terribly surprising when one considers the architecture of fungal polysaccharides in particular. Cryptococcal polysaccharides are complex with as many as seven repeating units or motifs and polymers containing a mixture of these motifs, their hydrated structure and the number of branch points only add to this complexity. In the capsule, GXM is relatively neutral, despite the charged carboxylic acid moieties, or perhaps because of calcium bridges between them holding polymers together (Nimrichter et al. 2007). This precludes the use of charge to aid in isolation. As noted earlier, though size fractions have been used for rough isolation, analysis shows that cryptococcal polysaccharides do not segregate according to the molecular weight cut offs (MWCO) of filters. While size exclusion chromatography (SEC) has been used for preliminary analysis, we must assume that the same would be true for SEC as for MWCO. So how can we characterize these polymers?

Recent work by Crawford and colleagues utilized a hydroxylamine-armed fluorescent probe to label the reducing end of cryptococcal polysaccharides (Crawford et al.

2019). This probe, referred to as the oxime probe, showed carbohydrate reducing ends exclusively at the cell wall-capsule interface of whole *C. neoformans* cells (Crawford et al. 2019). Additionally, the oxime probe labeled both EPS and CPS from *C. neoformans* suggesting that isolated polysaccharides contain reducing-ends as well (Crawford et al. 2019). The oxime probe, along with a recently developed reversible fluorenylmethyloxycarbonate 3-(methoxyamino)-propylamine linker (F-MAPA) (Wei et al. 2019), present the cryptococcal polysaccharide field with new molecular tools. Both the oxime probe and F-MAPA reversible linker are fluorescent and UV reactive, allowing for the isolation of single polymers from mixed samples using High Performances Liquid Chromatography (HPLC) or their visualization through other methods like gel electrophoresis. In the future the application of these molecular tools, and hopefully the development of others, have the potential to propel our understanding of cryptococcal polysaccharides forward.

Early studies to develop a vaccine against *C. neoformans* using killed whole cells or crude polysaccharide preparations showed no protection, while purified polysaccharide showed an increase in survival, all immunized mice eventually succumbed to the infection (Gladebusch 1958). In the 1990s a new polysaccharide conjugate vaccine against cryptococcosis was developed with the GXM-TT conjugate mentioned above which was developed from the serotype A strain NIH 371 (Devi et al. 1991; Devi 1996). Two methods were used to develop conjugates, both of which yielded antibodies in BALB/c mice (Devi et al. 1991) and were shown to protect against *C. neoformans* infection (Devi 1996). While the authors note that the GXM-TT conjugate vaccine shows promise and that human immunization seems feasible, there are no further publications to show that human immunization was attempted. A synthetic heptasaccharide based on the M2 motif on GXM (see Fig. 1b) was conjugated to human serum albumin (HSA) to yield the conjugate vaccine M2-HSA (Oscarson et al. 2005). Unfortunately, this vaccine did not elicit protective antibodies or improve the survival of immunized mice challenged with *C. neoformans* (Nakouzi et al. 2009). However, the antibodies produced in response to M2-HSA were predominantly IgG and mAbs were isolated for further use in characterization of the capsule (Nakouzi et al. 2009). The authors note that antibody binding to GXM seems to be conformational and therefore may require larger synthetic oligosaccharides. One of the major hurdles to overcome in the development of a vaccine to *C. neoformans*, or any pathogen, is the fact that cryptococcal polysaccharides elicit both protective and non-protective antibodies. Studies to develop a cryptococcal vaccine have shown that any potential vaccine must push the balance toward the production of protective antibodies and limit the production of non-protective antibodies. In the future, characterization of the immune response to any potential polysaccharide for vaccine development – either synthetic or native purified – is essential prior to conjugation and further development as a vaccine.

The current COVID-19 pandemic has revealed the utility of passive immunity for the treatment of disease once again. Currently, convalescent plasma, serum antibodies from an individual who has recovered from the disease, is one of the leading treatments for patients suffering from COVID-19 (Sheridan 2020). With the number of anti-GXM mAbs identified it is no wonder that antibodies have also been explored

as therapeutics for cryptococcosis (Casadevall 1998). A phase 1 clinical trial for passive immunotherapy with the mAb 18B7 in patients suffering from cryptococcosis was the first such trial for a fungal disease in humans (Larsen et al. 2005). Since most patients suffering from cryptococcosis are immunocompromised, enhancing the immune response with a directed mAb is a logical method to investigate. The study, which examined an array of doses from 0.01 to 1 mg/kg in HIV-infected patients showed that mAb 18B7 was well tolerated (Larsen et al. 2005). Although the study suggested these results were promising and warranted further development of 18B7 as a therapeutic, it was not pursued further. While 18B7 was not pursued as a therapeutic, there are further anti-GXM mAbs and the continued development for further mAbs during the course of conjugate vaccine testing that may undergo this same analysis and be pursued further. It is clear that passive immunotherapy with mAbs has great potential for the treatment of cryptococcosis in the future.

5 Conclusion

Expounding the history of *C. neoformans* lays bare the dramatic advancements that have been made in the last century plus of work. Though, it also clarifies how vast our lack of understanding remains. It is our hope that advancements in glycosciences will soon allow for the identification of the remaining glycosyltransferases necessary for the synthesis of both GXM and GXMgal along with the other necessary enzymes for the elongation and size regulation of these polymers. With the new molecular tools, like the reducing end-linked probes (described above) allowing for HPLC analysis of polysaccharides, it is now possible to define how the identified GXM motifs fit together into the longer polymers observed. Further, new isolation techniques to preserve the GXM structure and prevent dehydration while maintaining sterility (unpublished data) will shortly allow for direct comparison between EPS and CPS. These comparisons will in turn allow for the characterization of similarities and differences between the two and resolve the variability in biophysical measurements (see Tables 1 and 2). Additionally, advances in both solution and solid-state NMR now allow us to examine how dehydration affects cryptococcal polysaccharide structure and determine if these effects are reversible. Further, utilization of the vast collection of anti-GXM mAbs to clarify their specific polysaccharide epitopes promises to advance our understanding of the polymers which elicit protective mAbs. Finally, the combination of reducing-end probes, HPLC purification, and NMR will allow for the mapping of 6-O-acetylation (6-OAc) adducts on cryptococcal polysaccharides. The 6-OAc mark is critical for antibody binding, though molecular modeling studies show very little alteration in structure, so the mapping of 6-OAc is of great interest. Together the utilization of these molecular techniques shows promise for the development of new antibody therapeutics as well as more targeted cryptococcal conjugate vaccines.

With new tools to interrogate capsular architecture the need for a method of categorizing strains beyond serotype becomes clear. Defining the connection between GXM motifs to build capsular polymers necessitates codifying the

difference between a serotype A strain expressing a single motif and one, like H99, with mixed motif expression. The use of chemotypes, previously developed from NMR analysis of a cadre of *C. neoformans* strains, seems the best course of action. The use of chemotype reference for *C. neoformans* strains will additionally allow for the better identification of antibody epitopes as well as the development of conjugate vaccines.

While it is clear that the study of complex, branched polymers like those expressed by *C. neoformans* are exceedingly challenging, a review of the recent developments are quite inspiring. This bevy of recent advancement makes it clear that cryptococcal polysaccharides sit on the precipice of another great expansion in understanding and hopefully new therapeutic developments.

References

Bacon BE, Cherniak R. Structure of the O-deacetylated glucuronoxylomannan from *Cryptococcus neoformans* serotype C as determined by 2D 1H NMR spectroscopy. Carbohydr Res. 1995;276(2):365–86.

Bacon BE, Cherniak R, Kwon-Chung KJ, Jacobson ES. Structure of the O-deacetylated glucuronoxylomannan from *Cryptococcus neoformans* Cap70 as determined by 2D NMR spectroscopy. Carbohydr Res. 1996;283:95–110.

Barnett JA. A history of research on yeasts 14: medical yeasts part 2, *Cryptococcus neoformans*. Yeast. 2010;27(11):875–904.

Benham RW. The terminology of the Cryptococci with a note on Cryptococcus Mollis. Mycologia. 1935;27(5):496–502.

Bulmer GS, Sans MD. *Cryptococcus neoformans*. 3. Inhibition of phagocytosis. J Bacteriol. 1968;95(1):5–8.

Burchard W. Light scattering from polysaccharides as soft materials. In: Borsali R, Pecora R, editors. Soft matter characterization. Dordrecht: Springer Netherlands; 2008. p. 463–603.

Casadevall A. Antibody-based therapies as anti-infective agents. Expert Opin Investig Drugs. 1998;7(3):307–21.

Casadevall A, Scharff MD. The mouse antibody response to infection with *Cryptococcus neoformans*: VH and VL usage in polysaccharide binding antibodies. J Exp Med. 1991;174(1):151–60.

Casadevall A, Mukherjee J, Devi SJ, Schneerson R, Robbins JB, Scharff MD. Antibodies elicited by a *Cryptococcus neoformans*-tetanus toxoid conjugate vaccine have the same specificity as those elicited in infection. J Infect Dis. 1992;165(6):1086–93.

Casadevall A, Coelho C, Cordero RJB, Dragotakes Q, Jung E, Vij R, et al. The capsule of *Cryptococcus neoformans*. Virulence. 2019;10(1):822–31.

Chang YC, Kwon-Chung KJ. Complementation of a capsule-deficient mutation of *Cryptococcus neoformans* restores its virulence. Mol Cell Biol. 1994;14(7):4912–9.

Chang YC, Kwon-Chung KJ. Isolation of the third capsule-associated gene, CAP60, required for virulence in *Cryptococcus neoformans*. Infect Immun. 1998;66(5):2230–6.

Cherniak R, Sundstrom JB. Polysaccharide antigens of the capsule of *Cryptococcus neoformans*. Infect Immun. 1994;62(5):1507–12.

Cherniak R, Jones RG, Reiss E. Structure determination of *Cryptococcus neoformans* serotype A-variant glucuronoxylomannan by 13C-n.m.r. spectroscopy. Carbohydr Res. 1988;172(1):113–38.

Cherniak R, Valafar H, Morris LC, Valafar F. *Cryptococcus neoformans* chemotyping by quantitative analysis of 1H nuclear magnetic resonance spectra of glucuronoxylomannans with a computer-simulated artificial neural network. Clin Diagn Lab Immunol. 1998;5(2):146–59.

Cleare W, Casadevall A. Scanning electron microscopy of encapsulated and non-encapsulated *Cryptococcus neoformans* and the effect of glucose on capsular polysaccharide release. Med Mycol. 1999;37(4):235–43.

Cordero RJB, Frases S, Guimaräes AJ, Rivera J, Casadevall A. Evidence for branching in cryptococcal capsular polysaccharides and consequences on its biological activity. Mol Microbiol. 2011;79(4):1101–17.

Crawford C, Cordero RJB, Guazzelli L, Wear MP, Oscarson S, Casadevall A. Exploring *Cryptococcus neoformans* capsule structure and assembly with a hydroxylamine-armed fluorescent probe. BioRxiv. 2019.

Cuthbertson L, Mainprize IL, Naismith JH, Whitfield C. Pivotal roles of the outer membrane polysaccharide export and polysaccharide copolymerase protein families in export of extracellular polysaccharides in gram-negative bacteria. Microbiol Mol Biol Rev. 2009;73(1):155–77.

de S Araújo GR, Viana NB, Pontes B, Frases S. Rheological properties of cryptococcal polysaccharide change with fiber size, antibody binding and temperature. Future Microbiol. 2019;14:867–84.

Devi SJ. Preclinical efficacy of a glucuronoxylomannan-tetanus toxoid conjugate vaccine of *Cryptococcus neoformans* in a murine model. Vaccine. 1996;14(9):841–4.

Devi SJ, Schneerson R, Egan W, Ulrich TJ, Bryla D, Robbins JB, et al. *Cryptococcus neoformans* serotype A glucuronoxylomannan-protein conjugate vaccines: synthesis, characterization, and immunogenicity. Infect Immun. 1991;59(10):3700–7.

Dromer F, Salamero J, Contrepois A, Carbon C, Yeni P. Production, characterization, and antibody specificity of a mouse monoclonal antibody reactive with *Cryptococcus neoformans* capsular polysaccharide. Infect Immun. 1987a;55(3):742–8.

Dromer F, Charreire J, Contrepois A, Carbon C, Yeni P. Protection of mice against experimental cryptococcosis by anti-*Cryptococcus neoformans* monoclonal antibody. Infect Immun. 1987b;55(3):749–52.

Drouhet E, Segretain G, Aubert JP. Capsular polyoxide of a pathogenic fungus, *Torulopsis neoformans*. Relation with virulence. Ann Inst Pasteur. 1950.

Eckert TF, Kozel TR. Production and characterization of monoclonal antibodies specific for *Cryptococcus neoformans* capsular polysaccharide. Infect Immun. 1987;55(8):1895–9.

Edwards MR, Gordon MA, Lapa EW, Ghiorse WC. Micromorphology of *Cryptococcus neoformans*. J Bacteriol. 1967;94(3):766–77.

Frases S, Nimrichter L, Viana NB, Nakouzi A, Casadevall A. *Cryptococcus neoformans* capsular polysaccharide and exopolysaccharide fractions manifest physical, chemical, and antigenic differences. Eukaryot Cell. 2008;7(2):319–27.

Frases S, Pontes B, Nimrichter L, Viana NB, Rodrigues ML, Casadevall A. Capsule of *Cryptococcus neoformans* grows by enlargement of polysaccharide molecules. Proc Natl Acad Sci U S A. 2009;106(4):1228–33.

Frases S, Viana NB, Casadevall A. Biophysical methods for the study of microbial surfaces. Front Microbiol. 2011;2:207.

Freeze HH, Elbein AD. Glycosylation precursors. In: Varki A, Cummings RD, Esko JD, Freeze HH, Stanley P, Bertozzi CR, et al., editors. Essentials of glycobiology. 2nd ed. Cold Spring Harbor: Cold Spring Harbor Laboratory Press; 2009.

Gladebusch H. Active immunization against *Cryptococcus neoformans* on JSTOR [Internet]. 1958 [cited 2020 Jun 29]. Available from: https://www-jstor-org.proxy1.library.jhu.edu/stable/30098404#metadata_info_tab_contents.

Guazzelli L, Ulc R, Bowen A, Crawford C, McCabe O, Jedlicka AJ, et al. A synthetic glycan array containing *Cryptococcus neoformans* glucuronoxylomannan capsular polysaccharide fragments allows the mapping of protective epitopes. 2020.

Klutts JS, Doering TL. Cryptococcal xylosyltransferase 1 (Cxt1p) from *Cryptococcus neoformans* plays a direct role in the synthesis of capsule polysaccharides. J Biol Chem. 2008;283(21):14327–34.

Klutts JS, Levery SB, Doering TL. A beta-1,2-xylosyltransferase from *Cryptococcus neoformans* defines a new family of glycosyltransferases. J Biol Chem. 2007;282(24):17890–9.

Kumar P, Heiss C, Santiago-Tirado FH, Black I, Azadi P, Doering TL. Pbx proteins in *Cryptococcus neoformans* cell wall remodeling and capsule assembly. Eukaryot Cell. 2014;13(5):560–71.

Larsen RA, Pappas PG, Perfect J, Aberg JA, Casadevall A, Cloud GA, et al. Phase I evaluation of the safety and pharmacokinetics of murine-derived anticryptococcal antibody 18B7 in subjects with treated cryptococcal meningitis. Antimicrob Agents Chemother. 2005;49(3):952–8.

Lombard V, Golaconda Ramulu H, Drula E, Coutinho PM, Henrissat B. The carbohydrate-active enzymes database (CAZy) in 2013. Nucleic Acids Res. 2014;42(Database issue):D490–5.

Maxson ME, Cook E, Casadevall A, Zaragoza O. The volume and hydration of the *Cryptococcus neoformans* polysaccharide capsule. Fungal Genet Biol. 2007a;44(3):180–6.

Maxson ME, Dadachova E, Casadevall A, Zaragoza O. Radial mass density, charge, and epitope distribution in the *Cryptococcus neoformans* capsule. Eukaryot Cell. 2007b;6(1):95–109.

McClelland EE, Bernhardt P, Casadevall A. Estimating the relative contributions of virulence factors for pathogenic microbes. Infect Immun. 2006;74(3):1500–4.

McFadden D, Zaragoza O, Casadevall A. The capsular dynamics of *Cryptococcus neoformans*. Trends Microbiol. 2006a;14(11):497–505.

McFadden DC, De Jesus M, Casadevall A. The physical properties of the capsular polysaccharides from *Cryptococcus neoformans* suggest features for capsule construction. J Biol Chem. 2006b;281(4):1868–75.

Mukherjee J, Casadevall A, Scharff MD. Molecular characterization of the humoral responses to *Cryptococcus neoformans* infection and glucuronoxylomannan-tetanus toxoid conjugate immunization. J Exp Med. 1993;177(4):1105–16.

Nakouzi A, Zhang T, Oscarson S, Casadevall A. The common *Cryptococcus neoformans* glucuronoxylomannan M2 motif elicits non-protective antibodies. Vaccine. 2009;27(27):3513–8.

Nimrichter L, Frases S, Cinelli LP, Viana NB, Nakouzi A, Travassos LR, et al. Self-aggregation of *Cryptococcus neoformans* capsular glucuronoxylomannan is dependent on divalent cations. Eukaryot Cell. 2007;6(8):1400–10.

Oliveira DL, Nimrichter L, Miranda K, Frases S, Faull KF, Casadevall A, et al. *Cryptococcus neoformans* cryoultramicrotomy and vesicle fractionation reveals an intimate association between membrane lipids and glucuronoxylomannan. Fungal Genet Biol. 2009;46(12):956–63.

Oscarson S, Alpe M, Svahnberg P, Nakouzi A, Casadevall A. Synthesis and immunological studies of glycoconjugates of *Cryptococcus neoformans* capsular glucuronoxylomannan oligosaccharide structures. Vaccine. 2005;23(30):3961–72.

Pontes B, Frases S. The *Cryptococcus neoformans* capsule: lessons from the use of optical tweezers and other biophysical tools. Front Microbiol. 2015;6:640.

Ramos CL, Gomes FM, Girard-Dias W, Almeida FP, Albuquerque PC, Kretschmer M, et al. Phosphorus-rich structures and capsular architecture in *Cryptococcus neoformans*. Future Microbiol. 2017;12:227–38.

Rodrigues ML, Nimrichter L, Oliveira DL, Frases S, Miranda K, Zaragoza O, et al. Vesicular polysaccharide export in *Cryptococcus neoformans* is a eukaryotic solution to the problem of fungal trans-cell wall transport. Eukaryot Cell. 2007;6(1):48–59.

Sakaguchi N. Ultrastructural study of hepatic granulomas induced by *Cryptococcus neoformans* by quick-freezing and deep-etching method. Virchows Archiv B Cell Pathol. 1993;64(1):57–66.

Sakaguchi N, Baba T, Fukuzawa M, Ohno S. Ultrastructural study of *Cryptococcus neoformans* by quick-freezing and deep-etching method. Mycopathologia. 1993;121(3):133–41.

Sande C, Bouwman C, Kell E, Nickerson NN, Kapadia SB, Whitfield C. Structural and functional variation in outer membrane polysaccharide export (OPX) proteins from the two major capsule assembly pathways present in *Escherichia coli*. J Bacteriol. 2019;201(14):e00213–9.

Sheng S, Cherniak R. Structure of the 13C-enriched O-deacetylated glucuronoxylomannan of *Cryptococcus neoformans* serotype A determined by NMR spectroscopy. Carbohydr Res. 1997;301(1–2):33–40.

Sheridan C. Convalescent serum lines up as first-choice treatment for coronavirus. Nat Biotechnol. 2020;38(6):655–8.

Skelton MA, Cherniak R, Poppe L, van Halbeek H. Structure of the De-O-acetylated glucuronoxylomannan from *Cryptococcus neoformans* serotype D, as determined by 2D NMR spectroscopy. Magn Reson Chem. 1991;29(8):786–93.

Takeo K, Uesaka I, Uehira K, Nishiura M. Fine structure of *Cryptococcus neoformans* grown in vitro as observed by freeze-etching. J Bacteriol. 1973;113(3):1442–8.

Tassieri M, Del Giudice F, Robertson EJ, Jain N, Fries B, Wilson R, et al. Microrheology with optical tweezers: measuring the relative viscosity of solutions "at a glance". Sci Rep. 2015;5:8831.

Turner SH, Cherniak R. Glucuronoxylomannan of *Cryptococcus neoformans* serotype B: structural analysis by gas-liquid chromatography-mass spectrometry and 13C-nuclear magnetic resonance spectroscopy. Carbohydr Res. 1991;211(1):103–16.

Turner SH, Cherniak R, Reiss E, Kwon-Chung KJ. Structural variability in the glucuronoxylomannan of *Cryptococcus neoformans* serotype A isolates determined by 13C NMR spectroscopy. Carbohydr Res. 1992;233:205–18.

Vaishnav VV, Bacon BE, O'Neill M, Cherniak R. Structural characterization of the galactoxylomannan of *Cryptococcus neoformans* Cap67. Carbohydr Res. 1998;306(1–2):315–30.

Walter JE, Coffee EG. Distribution and epidemiologic significance of the serotypes of *Cryptococcus neoformans*. Am J Epidemiol. 1968;87(1):167–72.

Wang ZA, Li LX, Doering TL. Unraveling synthesis of the cryptococcal cell wall and capsule. Glycobiology. 2018;28(10):719–30.

Wei M, McKitrick TR, Mehta AY, Gao C, Jia N, McQuillan AM, et al. Novel reversible fluorescent glycan linker for functional glycomics. Bioconjug Chem. 2019;30(11):2897–908.

Whitfield C. Biosynthesis and assembly of capsular polysaccharides in *Escherichia coli*. Annu Rev Biochem. 2006;75:39–68.

Willis LM, Whitfield C. Structure, biosynthesis, and function of bacterial capsular polysaccharides synthesized by ABC transporter-dependent pathways. Carbohydr Res. 2013;378:35–44.

Wilson DE, Bennett JE, Bailey JW. Serologic grouping of *Cryptococcus neoformans*. Proc Soc Exp Biol Med. 1968;127(3):820–3.

Bioactivities of Bacterial Polysaccharides

23

Karina Cruz-Aldaco, Mayela Govea-Salas, Rafael Gomes-Araújo, Miriam Desiree Dávila-Medina, and Araceli Loredo-Treviño

Contents

Abstract

Microorganisms produce a wide variety of molecules that they use to communicate with the environment around them. In the case of bacteria, this interaction is mainly through membrane proteins and polysaccharides, especially through extracellular polysaccharides and these molecules, additionally, provide

K. Cruz-Aldaco
Department of Food Research (DIPA), School of Chemistry, Universidad Autónoma de Querétaro, Querétaro, Mexico

M. Govea-Salas · M. D. Dávila-Medina · A. Loredo-Treviño (✉)
School of Chemistry, Universidad Autónoma de Coahuila, Saltillo, Mexico
e-mail: araceliloredo@uadec.edu.mx

R. Gomes-Araújo
School of Chemistry, Universidad Autónoma de Coahuila, Saltillo, Mexico

Tecnologico de Monterrey, School of Engineering and Sciences, Monterrey, Mexico

J. M. Oliveira et al. (eds.), *Polysaccharides of Microbial Origin*,
https://doi.org/10.1007/978-3-030-42215-8_30

protective functions for bacteria. Of importance to humans, it is that these polysaccharides have various beneficial actions such as antitumor, antiviral, antibacterial, antioxidant, and even immunostimulatory activities. This last activity is of utmost importance because through it, the optimal development of the human immune system is favored. In exchange, the host provides conditions for the microorganism to thrive. Likewise, these polysaccharides have properties that make them of interest not only for their specific biological properties, but they can also be used in various areas such as food, biomedical, and pharmacological areas to improve texture properties or as a means of drug delivery. Research about these properties as well as their production could be of advantage to humans since microbial culture, if well performed, can be a safe and cheap alternative to obtain these molecules.

Keywords

Biological activities · Lactic acid bacteria · Immunostimulant · Antiviral · Antitumor

1 Introduction

Each human is an ecosystem that maintains 10^{13}–10^{14} microorganisms with which we cohabit. One of the ways that microorganisms have for this is the action on our immune systems since we have coevolved with commensal bacteria and it seems that they have an important role in physiological development and health functions (Mazmanian and Kasper 2006). Commensal bacteria have been proposed to evolve to improve host health; this may be through the polysaccharides of the microorganisms. In this chapter, we will see the influence that bacterial polysaccharides have on various biological activities, as well as the influence of their structure on these activities. First, the structure will be reviewed, then the various biological activities, and finally, techniques for production, purification, and extraction of these polysaccharides will be explored.

2 Polysaccharide Structure

Carbohydrates are abundant on the bacterial surface and they are the means for the interaction between bacteria and host. They include lipopolysaccharides, lipid and teichoic acids, peptidoglycans, glycoproteins, as well as capsular polysaccharides. Variations in the composition of sugars, ring shapes, bond positions, isomers, and the different conformations will define the action of these epitopes. For example, purified polysaccharides induce specific IgM responses, with no detectable IgG response. Protein-conjugated polysaccharides activate T-cell responses and this is used in vaccine development (Nikaido 1988; Upreti et al. 2003).

Microbial polysaccharides are divided into homopolysaccharides (HoPS) and heteropolysaccharides (HePS). Examples of the former are cellulose, dextran, mutate, alternate, pullulan, levan, and curdlan. Of the latter are gellan and xanthan. In LAB, HoPS repeating units consist of a single type of sugar, such as D-glucose or D-fructose, and form glucans and fructans. Conversely, HePS are composed of glucose, galactose, rhamnose and in some cases N-acetyl-glucosamine and N-acetyl-D-galactosamine but they can also have phosphate or some other units in their structure. These heteropolysaccharides, their yield, composition, and structure, seem to be influenced by the type of culture and fermentation conditions (Caggianiello et al. 2016).

Bacterial polysaccharides can be divided into capsule polysaccharides, lipopolysaccharide (LPS), and exopolysaccharide (EPS) depending on where they are located. The exopolysaccharide is the first molecule that has contact with the host organism and can be divided into HoPs or HePs; therefore, there could be many differences between them (Zhou et al. 2019).

The size and composition of polysaccharides influence penetration capacity and molecular binding. For example, in studies it was found that a fraction of lipopolysaccharide rich in galactose has greater anti-inflammatory activity toward macrophages than others without as much of this carbohydrate. This is because monosaccharides appear to be associated with the affinity of receptors on immune cells. Furthermore, the sulfate groups, their position, and quantity are responsible for the activity. This is seen in trials where the antioxidant activity of chemically sulfated and "native" polysaccharides was compared and it was found that the latter had greater activity, as well as the addition of Se in immuno-stimulating activities resulted in increased stimulation in comparison with native structures (Chaisuwan et al. 2020). These differences in the structure of bacterial polysaccharides vary according to the environment in which the microorganism is cultured or where they thrive, that is, by the pH, temperature, water content, and concentration of mono and divalent cations (Brandenburg et al. 2003). This is just one example of the influence of structure on the biological activities that different polysaccharides can have, especially exopolysaccharides (EPS) (Zhou et al. 2019).

Bacterial polysaccharides can also associate with other molecules and form glycoconjugates, glycolipids, and glycoproteins. For example, glycolipids have different functions such as acting as receptors in cell membranes, for cell aggregation, etc. These also modulate the immune response and can be toxins. Modulating the immune response includes things like mononuclear cell activation. For their part, glycoproteins contribute to the stability of the structures; in addition, in these the carbohydrate content varies much more (Brandenburg et al. 2003).

An example of these carbohydrates is an immunomodulatory polysaccharide, expressed by commensal bacteria, which is involved in directing the proper development of the mammalian immune system. This is produced by *Bacteroides fragilis* and is a zwitterionic polysaccharide (ZPS) that activates CD4 + T cells and with it you can correct some immune defects (Mazmanian and Kasper 2006). Unlike virulent polysaccharides, this symbiotic bacteria ZPS is a member of a family of health-promoting molecules (Mcpherson and Harris 2004; Hooper et al. 2003). This

polysaccharide has a positive and a negative charge in each unit that is repeated. This polysaccharide was initially thought to consist of A and B polysaccharide (LPS), but was found to have more diversity. Among the important features is that each LPSA and LPSB molecule is positively and negatively charged in each unit that is repeated. This is unusual in bacterial polysaccharides since most are negatively charged or neutral. This feature is suggested to be crucial for T cell activation (Mazmanian and Kasper 2006), since when the bacterium is endophaged by an antigen presenting cell, this polysaccharide is lysed into smaller sections and the negative "side" is binded to the presenter molecules and the positive "side" of the ZPS is the part that is presented to the CD4 + T cell.

As for LPSA, it consists of several hundred repeating units of a tetrasaccharide. It is a right-handed helix with two repeating units per turn. The molecule is covered with positive and negative charges, which alternate along the sides of the helical backbone and are exposed on the outer surface. This favors the interaction with other molecules such as with the major histocompatibility complex (MHC) in the peptide-binding groove of MHC molecules as described briefly above. The sugars that conform to the PSA vary depending on the strains studied (Mazmanian and Kasper 2006).

2.1 Polysaccharide Synthesis

These EPS are produced in four ways. (1) the extracellular synthesis pathway; (2) the ATP-binding cassette (ABC) transporter-dependent pathway; (3) the synthase-dependent pathway, and (4) the Wzx/Wzy-dependent pathway, which is the "universal" pathway for the synthesis of EPS in lactic acid bacteria. For the purpose of explanation, these microorganisms will be taken as an example. They use the extracellular synthesis pathway to synthesize homopolysaccharides. In summary, this is accomplished with the action of various enzymes such as fructansucrase and glucansucrase that will bind the specific monomers. Once the polysaccharide is synthesized, the chains are released into the extracellular environment (Zhou et al. 2019).

For the synthesis of heteropolysaccharides, various enzymes are involved, such as glycosyltransferases, polysaccharide length regulation proteins, polymerization and export proteins, while homopolysaccharides are synthesized by a single enzyme (Zhou et al. 2019). EPS production in LABs has been correlated with clusters of *eps* or *cps* genes, located in *Streptococcus thermophilus* or in *Lactobacillus plantarum* on the bacterial chromosome or in plasmids in species such as *Lactococcus lactis* and *Pediococcus damnosus*. These clusters include genes that encode regulatory and enzyme factors involved in EPS biosynthesis, polymerization, and secretion. These enzymes include glycosyltransferases, which are responsible for the characteristics of the assembly of the repetitive units of EPS (Caggianiello et al. 2016).

Regarding the Wzx/Wzy-dependent pathway, it is almost exclusive for lactic acid bacteria. It is more complicated than the HoPS route and involves more enzymes and sites to synthesize the heteropolysaccharides that will have a greater variability in terms of their structure. The next description is adapted from Mazmanian and Kasper

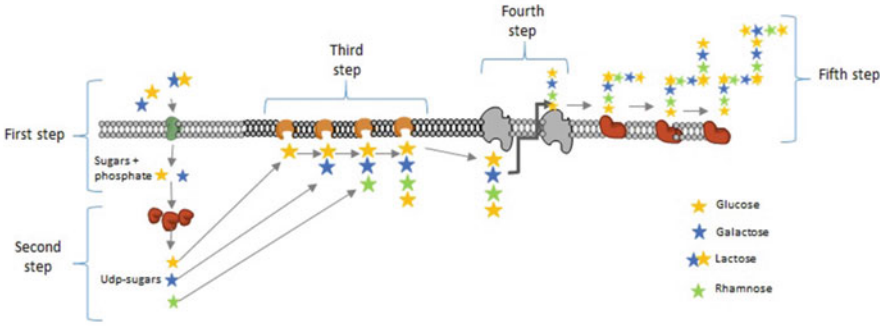


Fig. 1 Exopolysaccharide synthesis via Wzx/Wzy. (Adapted from Mazmanian and Kasper 2006)

(2006) and Caggianiello et al. (2016). The route consists of five steps. (1) Transport and phosphorylation of mono and disaccharides (in the example of Fig. 1, glucose and galactose), which can be carried out either by the phosphotransferase system (PST) assisted, which can also do phosphorylation or permease assisted with which a specific kinase must act. In case there is lactose or phospho-lactose, there must be a galactosidase to obtain glucose and galactose/phospholactose. All this in order to obtain monosaccharides that will be used to form the extracellular heteropolysaccharide. (2) Formation of sugar nucleotides. Phosphoglucomutase and galactose-1-phosphate uridyltransferase mediate the transition from glucose-6-phosphate and from galactose-1-phosphate to monosaccharides with the phosphate group at carbon 1 and subsequently to UDP-glucose, dTDP-rhamnose, UDP-galactose with the action of other enzymes. Once these units are "ready," the polymerization begins. (3) The synthesis of repetitive units. The individual units, attached to an undecaprenyl diphosphate anchor located on the inner surface of the membrane, are assembled by several glycosyltransferases in sequence. (4) Translocation of the repeating units from the intracellular to the extracellular surface. This is done by means of a flipase (WzX). (5) Polymerization of repeat units and release of the long chain. Repeated units are catalyzed by an outer membrane protein (Wzy) to form a long chain, and the length of the chain is regulated by a regulatory protein in Gram-positive bacteria or by a protein complex formed by the polysaccharide co-polymerase and the outer membrane families in Gram-negative bacteria; eventually EPS is released into the extracellular space. This process is shown in Fig. 1.

The Synthesis of sugars occurs in the cytoplasm; they are assembled in the lipid carrier molecule undecaprenyl phosphate through monosaccharides transfer from nucleotide sugars by specific glycosyltransferases; then a flipase (Wzx) moves the repeating units from the inside of the membrane to the outside, where they will be polymerized by Wzy (Caggianiello et al. 2016). It is important to note that the enzymes that act in this process are many more than those shown in the illustration. For example, the system for incorporating lactose is different from that of glucose or galactose, as well as there must be an enzyme that breaks down lactose into its

monosaccharides. In the same way, there is an enzyme that is responsible for converting sugars into rhamnose (steps 1 and 2). In step 3 the sugars are polymerized and then in step 4, with the Wzx/Wzy complex, the "base" polysaccharide is transferred to the outside and, finally, there the polymerization is carried out to form the final EPS.

2.2 EPS Activities and Uses

EPS is used in various areas such as cell protection, adhesion, and biofilm formation, but it does not serve as a carbon source. Examples of monopolysaccharides are cellulose, curdlan, dextran, levan, and pullulan, while for heteropolysaccharides they are alginate, haloglycan, mauran, and succinoglycan (Chaisuwan et al. 2020).

Polysaccharides are associated with various mechanisms such as prebiosis, probiosis, tolerance to stress associated with food processing, and technological properties in food as well as biological activities. In lactic acid bacteria (LAB), the exopolysaccharides are homo- or heteropolysaccharides, which are consistent with repeating units of sugars or derivatives thereof and can be branched. In some cases, EPS increases the viscosity of your environment (Caggianiello et al. 2016).

EPS is thought to protect bacterial cells against extreme conditions such as abiotic or biotic stress, including temperature, light intensity, pH, or osmotic stress. Also, EPS can influence cell adhesion and recognition mechanisms (Caggianiello et al. 2016). It has been found that the structure of different exopolysaccharides affects their prebiotic properties and this same structure can confer probiotic and technological characteristics to the strains that produce them (Caggianiello et al. 2016).

EPS contributes to viscosity and rheology, which can be used for industrial applications. Likewise, EPS has been shown to have physiological and biological functions such as antioxidant, antibacterial, immunoregulatory function, anti-tumor, antiviral, among others (Zhou et al. 2019).

Within EPS there are hyperbranched polymers (HBP). These, obviously, have different characteristics in terms of rheology, low viscosity, high solubility, high density compared to unbranched polysaccharides, and a greater number of functional groups. For example, *Leuconostoc citreum* b-2 produces a highly branched EPS consisting of a backbone of α- (1–6) -D-Glcp (75%) with 19% α- (1–3) branched and only with some α- (1–2) branching. This HBP has a water solubility index of 80%, which is high, and a water absorption capacity of 450%; therefore it has a potential application in the food, cosmetic, and pharmaceutical industries (Chen et al. 2019).

The modification of these HBPs has been explored, so that they have properties that can form rubber films and are flexible at room temperature to have applications as biomaterials and as biodegradable films. This has been achieved, for example, in *Leuconostoc mesenteroides* NRRL B-512 F where the HBP contained up to 20% α- (1–3) -D-Glcp and long branches, which had non-Newtonian and non-thixotropic behaviors in solution (Chen et al. 2019).

One of the most popular and most widely used HBPs is xantham gum, which is produced by various species of *Xanthomonas*, such as *X. arboreal, X. axonopodis,*

X. campestris, X. fragaria, etc. This gum has good solubility in cold water and the aqueous solutions have highly pseudoplastic behavior and have synergistic action with galactomannans. It has been used in biomedical areas as a material for drug administration and tissue engineering (Chen et al. 2019).

3 Antibacterial Activity of Bacterial Polysaccharides

Bacterial infections and their complications are extremely important worldwide, resulting from bacteria adaptations and multidrug resistance by abuse of antibiotics. The development of antibiotic substitutes is an urgent need to explore new sources of natural compounds with antibacterial activity. Food poisoning and nosocomial infections are the main way to bacterial infection by *Staphylococcus aureus, Bacillus cereus*, *Listeria monocytogenes*, *Escherichia coli*, *Candida* spp., *Salmonella* spp., P*seudomonas* spp., *Clostridium difficile*, *Acinetobacter baumannii*, Vancomycin-resistant *Enterococcus*, among others. Naturally, bacterial polysaccharides (BPS) are produced to provide various benefits to bacteria, such as protection against toxic or antimicrobial agents, predation by protozoa, attack by phages, and evasion of the immune system by other pathogenic bacteria (Zeidan et al. 2017; Lynch et al. 2018). Several studies have shown the antibacterial activity of lactic acid bacteria-derived EPS against pathogenic bacteria. Li et al. (2014a), after studying antibacterial activity, by agar diffusion, of exopolysaccharide (EPS) from 23 LAB found that the EPS of *Bifidobacterium bifidum* WBIN03 and *Lactobacillus plantarum* R315 at 300 μg/mL had a great activity against *C. albicans* Z1, *Cronobacter sakazakii* ATCC29544, *E. coli* O157: H7, *L. monocytogenes* CMCC54007, *S. aureus* CMCC26003, *B. cereus* ATCC14579, *S. typhimurium* ATCC13311, and *Shigella sonnei* ATCC25931. Jeong et al. (2017) showed bactericidal activity against *L. monocytogenes* and *S. enteritidis using* 1% (v/v) of EPS from *Lactobacillus kefiranofaciens* DN1 isolated from kefir. An EPS isolated from Lactobacillus sp. Ca6 at 10 mg/mL exhibited antibacterial activity against pathogenic bacteria such as *S. enterica* ATCC 43972 and *Micrococcus luteus*, in an agar-well diffusion assay (Trabelsi et al. 2017). In a recent report, four LAB were isolated from different Tunisian spontaneously fermented foods and beverages, bovine and turkey meat sausages, date palm sap and cow milk, and molecularly identified as *Leuconostoc citreum, Leuconostoc mesenteroides, Pediococcus pentosaceus, and Leuconostoc pseudomesenteroides*, respectively. EPS extracted from these bacteria showed high antibacterial activity at 1 mg/mL with an anti-biofilm activity against *E. coli* of 90%, 88% to *Enterococcus faecalis,* and 86.9% to *S. aureus* (Abid et al. 2018). Shahid et al. (2018) showed the antibacterial activity of EPS isolated from *Lactobacillus reuteri* SHA101 and *Lactobacillus vaginalis* SHA110 isolated from gut samples of healthy poultry (hen) against *E. coli* ATCC 25922, *S. typhimurium* CMCC (B) 50115, and *S. petrasii* subsp. *pragensis* KY196531 by agar diffusion with 5 mg/mL of EPS. Nehal et al. (2019) showed bactericidal activity with a new EPS from *Lactococcus lactis* F-mou against *B. cereus* ATCC 10702, *E. coli* ATCC 8739, *Acinetobacter baumannii* ATCC BAA-1710, *Proteus mirabilis* ATCC 7002,

P. aeruginosa ATCC 49189, *S. aureus* ATCC 6538, *Enterobacter cloacae* ATCC13047, and *L. monocytogenes* ATCC 19115 with concentrations of EPS between 6.75 and 31.9 mg/mL, and fungicidal activity against *C. albicans* ATCC 10231 with 24 mg/mL. With the same emphasis, Aullybux et al. (2019) isolated bacteria from Mauritius seawater, identified as *Bacillus*, *Halomonas*, *Psychrobacter,* and *Alcaligenes* species and showed antibacterial activity with EPS extracted from these bacteria against *E. cloacae, S. typhimurium, E. coli, S. aureus, S. saprophyticus,* and *P. mirabilis.*

Conclusively, LAB-derived EPS show an excellent and irrefutable antimicrobial activity against Gram-negative and Gram-positive bacteria pathogens. This activity could help probiotics in colonization of the surface of the gastrointestinal tract due the hydrophilic surface and reduction of auto-aggregation, inhibiting pathogenic bacteria growth by competitive inhibition in the host (Zhou et al. 2019). Probiotic bacteria produce self-inducing factors, generally acyl-homoserine lactone in Gram-negative and oligopeptide in Gram-positive bacteria, which sensing the density or size of surrounding pathogenic bacteria allows to regulate the biofilm formation that plays an important role in the range of infections, virulence, and cell communications (Chen et al. 2016; Alayande et al. 2018). Moreover, LAB-derived EPS are classified as biomolecules foreign to pathogenic bacteria, yet are not permeated or transported intracellularly, so a possible in vitro antibacterial activity mechanism is that they combine with the biofilm-related signal molecules or glycocalyx receptors in the surface of pathogenic bacteria, disrupting cell communication that interferes in the biofilm formation and eventually generating the antimicrobial effect (Spano et al. 2016). The mechanism of antimicrobial activity of EPS was elucidated in vitro; however, the functional mechanism in vivo is more complex and involves more processes from probiotic host. Nerveless, antibiotics are the main antimicrobial medicine and the EPS represent a resource with a high potential antimicrobial medicine against pathogens with increasing antibiotics resistance in biomedical area.

4 Antiviral Activity of Bacterial Polysaccharides

There are many diseases caused by infection with various viruses; it occurs in humans and animals, in which various treatment alternatives have been sought to those already determined as antiviral drugs or medical therapies. However, in recent years much attention has been paid to the effects of bacterial polysaccharides (BPS) on different viral diseases and infections in vitro. The main reported mechanisms of antiviral activity caused by metabolites of lactic acid bacteria such as BPS are the inhibition of late stages of viral replication, causing reduced infectivity; inhibition of adsorption/penetration of the virus into cells due to the interaction of the bacterial-metabolite with the virus; and stimulating an immune system response (Yang et al. 2017a). Such is the case of the exopolysaccharides of *Lactobacillus casei* that it has been reported they can stimulate the synthesis and accumulation of interleukin 12, promoting the activity of

natural killer cells and the synthesis of immunoglobulins in the spleen, allowing the host to have a better immune response against the Influenza A virus infection (Jung et al. 2017).

Biliavska et al. (2019) evaluated the antiviral activity of the exopolysaccharides of lactic acid Bacteria (EPS) against Human Adenovirus Type 5 (HAdV-5), the cause of respiratory, intestinal, and urogenital tract infection diseases. EPS were obtained and purified from the Genera *Pediococcus*, *Leuconostoc,* and *Lactobacillus* (from pickled apple, sauerkraut, and pickled tomato juice). They infected Madin-Darby bovine kidney cells with HAdV-5 (infection titer 7.0×10^7 PFU/mL) and evaluated the viability and antiviral activity with the EPS (375, 750 and 1500 μg/mL). They found that cell viability reduces only up to 17% and show antiviral activity due to the degradation of the viral particles, blocking viral DNA replication, observing through the analysis of the cell cycle by flow cytometry causing decrease of the titer of viruses.

Moreover, there are reports that EPS obtained of *Lactobacillus plantarum* LRCC5310 (isolated and identified from traditional Korean fermented vegetable food kimchi) showed antiviral activity against Human Rotavirus (HRV) Wa strain in vitro. MA104 cells were infected with HRV (0.01 MOI) and incubated with de EPS to continue the cell culture. The virus was quantified by qPCR and a decrease of viral RNA copy numbers reducing to 7.56 log compared with the untreated control was observed with 1.95 mg of EPS. Also, they observed that EPS generated a high percentage of adhesion, interfering with the binding of rotavirus to MA104 cells in vitro. Likewise, antiviral activity in vivo was evaluated in BALB/c mouse model by oral infected dose (10 μL of 2×10^4 FFU as infective dose 50%/mL and 1 mg/mice) of EPS by 5 days. Then they were euthanized and histopathological analyzes of the intestine were performed. They observed that EPS treatment in vivo decreased epithelial lesions of the intestine, decreased diarrhea symptoms while it reduced virus replication in the intestine (Kim et al. 2018). Similarly, other studies related to *L. plantarum*, indicating its antiviral effects against herpes simplex virus 1 and 2, and influenza virus due to a stimulation of the immune response by the induction of interleukins and immunoglobulins in the host (Yang et al. 2017b). In addition, EPS produced by microorganisms present in the microbiota of the human body have been shown to have antiviral activity, as in the case of *Lactobacillus gasseri* CMUL57 isolated from vaginal microbiota that showed antiviral activity against herpes simplex virus type 2 and *Enterococcus faecium* NCIMB 10415 antiviral effect against influenza virus (Jung et al. 2017). Likewise, the gut commensal *Bacteroides fragilis* NCTC9343 and its capsular polysaccharide A (PSA) has reported important results of antiviral activity against herpes virus simplex virus 1, strain 17+ syn causing encephalitis, with Vero cell culture in vitro and in vivo with 129 WT and Rag2−/− mice. They found that virus replication decreased and viability was not affected, in vivo they observed a decrease in the inflammatory response, even only mice not treated with PSA died, a decrease in brain stem inflammation was also observed, and EPS promoted an immune response with interleukins (IL-10) to control inflammation (Ramakrishna et al. 2019).

5 Anticancer activity of Bacterial Polysaccharides

Nowadays, cancer is a disease that affects the entire population and is considered one of the leading causes of death worldwide. Therefore, various therapies of natural origin have been sought to generate fewer side effects. There are several studies that indicate that the metabolites like bacterial polysaccharides (BPS) produced by lactic acid bacteria (LAB) have anticancer and antiproliferative activity by activating various cell signaling pathways, it modulates the host's immune defense mechanism, promotes apoptosis, inhibits the onset of carcinogenesis and blocks its progression, and can also interfere with the downregulation of genes involved in tumor cell proliferation (Deepak et al. 2016; Saber et al. 2017). Furthermore, the PS being obtained from natural sources, have been shown to have few side effects and low cytotoxicity compared to the anticancer effect it generates, so it could be considered a good alternative for an antitumor agent. Another mechanism of action of BPS is the stimulation of components of the immune system such as macrophages, lymphocytes, NK cells and the induction of interleukins and decreases angiogenesis because it inhibits the growth of the vascular endothelium (Ismail and Nampoothiri 2013). In colon cancer cells, the effect of various EPS obtained from *Lactobacillus spp*. isolated from various dairy products was evaluated, observing a downregulation of the ErbB-2 and ErbB-3 genes, indicating an anticancer effect caused by the treatment of EPS in vitro (Faghfoori et al. 2017).

Other authors isolated and purified three EPS fractions of *Lactobacillus helveticus* MB2–1 from traditional fermented milk (LHEPS-1, LHEPS-2, LHEPS-3), elucidated their chemical structure, and incubated them with BGC-823 cells from human gastric cancer. They found an antiproliferative effect with all EPS but with LHEPS-2 they observed that it had a better growth inhibitory effect of up to 31.8% at a dose of 600 μm/mL, while LHEPS-1 of 27% and LHEPS- 3 25%, reporting that this polysaccharide has a different structure from the others (Li et al. 2014b).

Moreover, a dose-dependent effect was observed in colon cancer Caco and HCT 15 cells with EPS obtained from *Lactobacillus acidophilus*. They determined the toxic dose of EPS that was 5 mg/mL and evaluated the effect of EPS on the messenger RNA (mRNA) of cells, where they found a downregulated expression of vascular endothelial growth factor and hypoxia-inducible factor (HIF-1α and HIF-2 α), also an increase in plasminogen activator inhibitor-1. This suggests that EPS induces an inhibitory effect on the expression of genes related to tumor angiogenesis (Deepak et al. 2016).

In addition, in another colon cancer line (HT-29) the effect of EPS on the apoptotic pathway was evaluated, also to the composition of various EPS obtained from *L. delbrueckii* ssp. *bulgaricus* B3 isolated yogurt and *L. plantarum* GD2*, L. rhamnosus* E9*, L. brevis* LB63 isolated from healthy infant feces. These authors report a significant effect dependent on the variation of the molecular composition of EPS and time dependent. EPS induced apoptosis at 24 and 48 h, decreasing Bcl-2 and increasing expression of BAX, caspase 3 and 9, *L. delbrueckii ssp. bulgaricus B3* being the best inducer of apoptosis of up to 42%. These analyses suggest that the high mannose and low glucose composition of EPS influences the apoptotic effect in

tumor cells, because it is a signaling pathway that is commonly altered in cancer (Tukenmez et al. 2019).

In general, the anticancer activity of BPS is reported to be related to its structure, chemical composition, such as molecular weight or sugar composition, as well as in a dose-dependent and time-dependent manner with BPS and the type of cancer. It also depends on the signaling or metabolic pathway that is intended to be modulated, such as apoptosis, angiogenesis, necrosis, immunotoxicity, or expression of oncogenes.

6 Other Bioactivities

Polysaccharides are widespread in plants, animals, and microorganisms, and display chemical diversity, physiological properties, and biological activities. As it was stated, they could be divided into capsule polysaccharides, lipopolysaccharide, and EPS according to their locations in microorganisms. EPS, a biopolymer synthesized extracellularly or secreted into the extracellular of the microorganism during its growth for their survival in the increased temperature and pH conditions. The bacterial polysaccharides are of great interest for their immunostimulatory, immunomodulatory, antitumor, antiviral, anti-inflammatory, and antioxidant properties. EPS contribute to their inherent physiochemical and biological properties and render a multitude of functional activities that can be exploited for additives in health product or therapeutic medicine and adjunctive therapy in curing inflammation. It also has immunostimulatory, immunomodulatory, antitumor, antiviral, anti-inflammatory, cholesterol-lowering and antioxidant properties (Kanmani et al. 2013; Abdhul et al. 2014; Chen and Huang 2018).

Bacterial polysaccharides are always anchored to the cell surface by lipids. They are nontoxic natural biopolymers, and are reported as a kind of effective free radical scavenger and antioxidants, playing a critical role in protecting against oxidation damage in living organisms. On the other hand, many diseases, such as asthma, chronic obstructive pulmonary disease, inflammation, diabetes, myocardial infarction, and cardiovascular diseases, are reported to associate with oxidative stress (Wang et al. 2016).

Numerous polysaccharides are able to activate cellular components involved in host defense mechanisms. Extracellular polysaccharides purified from *Aureobasidium pullulans* SM-2001 (Polycan) (EAP) have exhibited favorable anti-inflammatory activities, potent immunomodulatory activities in immunosuppressed mice, showing powerful immunomodulatory, antioxidant, and anti-inflammatory mechanisms (Lim et al. 2018).

6.1 Cholesterol-Lowering Effect

The bacterial EPS is either covalently associated with the cell surface or loosely attached, or secreted into the surrounding environment. From studies, it was found that certain EPS-producing probiotic strains could bind free bile acids, hence

increasing their discharge after assimilation. EPS has been reported to contribute toward autoaggregation and cell surface adhesion, thus enabling the colonization of probiotics in the host. Probiotic EPS has been reported to possess hypocholesterolemic bioactivities; this could bring about the synthesis of new bile acids from cholesterol by the liver and thus lower the level of circulating cholesterol (Zhang et al. 2016; Kanmani et al. 2018; Sasikumar et al. 2017).

Cholesterol-lowering effect, related to EPS produced by *Enterococcus faecium*, displayed the cholesterol-lowering property compared with a negative control group. Since cardiovascular diseases are one of the major causes of global mortality, a decrease in serum cholesterol may decline the incidences of heart diseases. Incorporation of *E. faecium* K1 EPS in functional foods may help lowering the raised cholesterol level. Similarly, in an in vitro assay, 45% cholesterol level could be reduced by an EPS from *Lactobacillus plantarum* BR2 (Bhat and Bajaj 2018; Sasikumar et al. 2017; Zhou et al. 2019).

6.2 Antioxidant Effect

Exogenous antioxidants are frequently added to food to aid preservation; however, many synthetic antioxidants, such as butylated hydroxyanisole and butylated hydroxytoluene, may exhibit cytotoxicity. For this reason, more attention has been paid to natural nontoxic antioxidants in an effort to protect the human body from free radicals and retard the progress of many chronic diseases (Pan and Mei 2010).

Antioxidant activity has different reaction mechanisms including free radical scavenging, reductive capacity, binding of transition metal ion catalysts, inhibition of lipid chains oxidation, etc., and it could be due to the presence of the hydroxyl group and other functional groups of EPS, which converts the free radical into a more stable form (Kanmani et al. 2018).

There is an increasing attention in exploiting the EPS-producing LAB, as the antioxidant activity (Abdhul et al. 2014) exhibited equal scavenging activity compared to the known standard, ascorbic acid. Kodali and Sen reported the EPS of probiotic strain *B. coagulans* Rk-02 to have strong super oxide and hydroxyl radical activity in vitro. One mechanism of action of EPS may be alleviation of cancers through the antioxidant (free radical scavenging) activity (Kodali and Sen 2008).

Wang et al. (2016) reported that the EPS of *Paenibacillus* sp. TKU023 showed the strongest antioxidant activity. The antioxidant activity of various natural polysaccharides was examined and suggested that the chelating ions, including Fe^{2+} and Cu^{2+}, play role in inhibition of hydroxyl radicals but not directly.

6.3 Immunomodulator Effect

One of the major pharmacological effect of EPSs lies in the modulation of immune system response, and its diverse composition dictates different immunomodulatory properties and their derivatives can enhance the body immunity. A large number of

experiments show that polysaccharides can play an important role in regulating the immune system as an immunomodulator and accelerator, activate T lymphocytes, B lymphocytes, macrophages, and other immune cells to exert their immune activity. The regulatory effects of polysaccharides on macrophages include the number, morphology, phagocytic capacity, and cell secretion capacity (Chen and Huang 2018).

Lactobacillus paraplantarum BGCG11, isolated from cheese, produces EPS composed of glucose, rhamnose, galactose, and mannose and exhibits an anti-inflammatory effect on peripheral blood mononuclear cells (PBMC), unlike the non-EPS derivative strains, which induce the higher proinflammatory response of PBMC. Polysaccharides have been recognized as molecules with anti-inflammatory activity (Caggianiello et al. 2016; Dinic et al. 2018).

Therefore, in the light of the rising application of bacterial EPSs, here we demonstrate the novel ability of EPS CG11 to suppress a pain sensation via regulation of inflammatory response. *L. paraplantarum* BGCG11 produces ropy, big-size polymer around 2×10^6 Da, mainly composed of glucose and rhamnose with the traces of galactose and mannose, but further information regarding stability of the polymer is missing (Dinic et al. 2018).

An indirect benefit is prebiotic action. A "prebiotic" is a "selectively fermented ingredient that results in specific changes in the composition and/or activity of the gastrointestinal microbiota, and thus, conferring health benefits on the host" (Caggianiello et al. 2016). A prebiotic effect has been found in EPS produced by LAB. For example, it was found that with α-D-glucan produced by *L. plantarum*, which had little digestibility in artificial gastric juice, it had a prebiotic effect in vitro, which was corroborated by the low growth of non-probiotic bacteria such as Enterobacteriaceae. Exopolysacharides of *Weissella cibaria*, *Weissella confusa*, *L. plantarum,* and *Pediococcus pentosaceus* had a high resistance to gastric and intestinal digestion as well as a selective increase in beneficial bacteria, in particular bifidobacteria (Caggianiello et al. 2016).

LAB's EPSs have an important role in biofilm formation and adhesion to surfaces, allowing colonization of different environments. Furthermore, EPS from probiotic bacteria adhere to the intestinal mucosa. These bacteria are associated with the digestion of nutrients, development and maintenance of the proper immune functions of the intestinal mucosa. Additionally, several strains provide essential vitamins such as folate, biotin, vitamin K, and produce short-chain fatty acids that are used as an energy source by cells in the colon (Caggianiello et al. 2016).

Intestinal epithelial cells (IECs) or dendritic cells (DCs) can communicate with the intestinal microbiota through their pattern recognition receptors (PRRs), which can detect microorganisms-associated molecular patterns (MAMPs). The interaction between MAMPs and PRRs results in the induction of signaling cascades, which will determine a molecular response against the detected microorganisms, for example: cytokines, chemokines, and the immunomodulation of antimicrobial agents. The cell wall of Gram-positive bacteria has several structures that are essential in the interaction between probiotics and host receptors (Caggianiello et al. 2016).

Some EPSs have immunomodulatory properties, with potential effect on human health. Some polysaccharides have a protective role for cells against the intestinal

environment. For example, an *L. lactis* EPS film has been reported to have a protective effect against host phagocytosis by murine macrophages (Caggianiello et al. 2016).

The ability of EPS to activate immune responses is different between LAB species and strains; these differences are related to the structure and size of the EPS produced. For example, acidic HePs are characterized by having phosphate in their composition and induce an immune response. Those with high molecular weight, on the other hand, appear to suppress the immune response. In *Lactobacillus casei* Shirota, high molecular weight EPS induced the production of various cytokines by macrophages (Caggianiello et al. 2016).

Biofilms are resistant to the intestine environment and produce extracellular factors that have both immunomodulatory properties and the ability to inhibit the growth of pathogens (Caggianiello et al. 2016).

All of these effects, that is, colonization of the gastrointestinal tract increases both nutrient absorption and immunomodulating activity. In experiments with bacteria-free mice, it has been found that the proportion of CD4 + T, CD8 + T and B cells is much lower than in mice colonized by bacteria. That is, the lack of colonization of the intestine by beneficial bacteria results in a defective development of the immune system (Mazmanian and Kasper 2006). Not only does the evidence show that certain bacteria are beneficial to humans, but it also appears that the host provides the right conditions for their growth.

Several epidemiological data show that there has been an increase in allergies in recent decades, especially in developed countries. This seems to be influenced by an "excess" of hygiene, that is, by reducing exposure to microorganisms, especially in early stages of life, so that later in life, diseases and exaggerated responses to relatively innocuous antigens develop (Strachan 1989; Willis-Karp et al. 2001). A "balanced" gut microbiota has been found to contribute to an adequate state of health, whereas disturbances in either the type and/or amounts of non-beneficial microorganisms result in allergies, dermatitis, asthma, inflammatory bowel disease, etc. These changes are related to diet and modern lifestyle. It was found in an experiment that children from a developed country compared to a developing country had less colonization of *Bacteroides* species, which had a higher incidence of allergies (Alm et al. 2002). Because many of the beneficial effects of bacterial EPS come from LAB, which are present in fermented foods, the consumption of these foods may be related to these same beneficial effects.

7 Production and Extraction of Polysaccharides of Microbial Origin

Bacteria produce exopolysaccharides for cell protection from phagocytosis by protozoans or white blood cells and to protect them from stressful environments. They are also produced for cell adherence and biofilm formation (Nwodo et al. 2012). Polysaccharide production is normally highest under aerobic conditions. Therefore, more polymer is usually excreted during growth on solid media than obtained from

comparable amounts of cells grown in liquid media. The composition of most EPS found in bacterial slime or capsule appears to be independent of the carbon and energy source provided for growth and polymer synthesis (Cerning 1990). Exopolysaccharides produced by microorganisms represents an industrially untapped market; some microorganisms can produce and excrete over 40 gL^{-1} of EPS in simple but costly production conditions (Donot et al. 2012).

Submerged fermentation (SmF) and Solid-State fermentation (SSF) are the methods used for the production of intracellular and extracellular polysaccharides. Both methods have been used to produce the maximum amount of microbial EPS and improve their bioactivities and other properties (Li et al. 2016). Fungi, bacteria, and archaea are the microorganisms responsible for synthesizing extracellular polysaccharides (Chaisuwan et al. 2020). The former is the cultivation of microorganisms in a liquid medium, while the latter involves the culture of microorganisms on solid supports in the absence of or with small amounts of free water. The selection of a method should consider the nature of microbes, the available equipment, and the production yield (Nwodo et al. 2012).

The factors affecting the yield of EPS are inoculum age, inoculum size, compositions of the medium, and the physical parameters of growth conditions (Chaisuwan et al. 2020; Jenny Angel et al. 2018). The medium's compositions, especially carbon and nitrogen sources, mainly involve microbial growth and production of EPS. Glucose is a common monosaccharide used as the sole carbon source for the production in many species, but some species use other types of sugar for fermentation that produce a higher yield of EPS (Chaisuwan et al. 2020). Some microorganisms prefer another carbon source as sucrose (glucose and fructose) that is suitable for *Agrobacterium radiobacter*, *Bacillus* spp., *Leuconostoc mesenteroides*, and *Weissella confuse* (Chaisuwan et al. 2020; Jenny Angel et al. 2018; Seesuriyachan et al. 2014). In case of xylose, maltose, sorbitol, lactose, and galactose are preferred by *Streptomyces nasri*, *Brevibacillus thermoruber, Candida famata, Mycoleptodonoides aitchisonii,* and *Paenibacillus polymyxa,* respectively (Chaisuwan et al. 2020; Choi et al. 2011; Gientka et al. 2016; Radchenkova et al. 2011; Raza et al. 2011).

During fermentation is important to have an organic or inorganic such as yeast extract and/or $(NH_4)_2HPO_4$. This is the case for development of *Bacillus velezensis* and *Brevibacillus thermoruber* (Radchenkova et al. 2011; Moghannem et al. 2018).

Solid-state fermentation is a useful method for EPS production of microorganisms, especially lactic acid bacteria. The cultivation of some microbes on a solid medium increases EPS production. Seesuriyachan et al. (2014) reported that dextran from *Weissella confusa* cultivated by SSF was 86.4 g/l in 40.8 h, while by SmF it was 63.8 g/l in 24 h. Although SSF gave a higher amount of dextran, it took a long time more than SmF. *Weissella confusa* produced a higher amount of dextran on a modified MRS agar more than in the liquid medium at optimized conditions (Donot et al. 2012; Chaisuwan et al. 2020).

Once the process of obtaining EPS has concluded, it is important to remove the biomass that has been generated. Usually, biomass is the easiest to remove impurities and it can be removed by decantation, filtration, centrifugation, etc. In the case of

centrifugation, it is required to separate microbial cells, and ethanol precipitation is a widely used technique for precipitation of the EPS (Chaisuwan et al. 2020). These stages are where the product is concentrated and further purified. Here, the product is concentrated and renatured to move onto the final purification stages. These depend on the required final product purity. Pharmaceutical products require high purity while industrial products require lower purity. Chromatography and aqueous two-phase extraction are popular techniques (Aguilar et al. 2019).

Isolation of EPS generally presents a few problems when it is secreted as an exocellular slime. The lack of physical attachment between polysaccharide and cell permits the use of differential centrifugation to be employed. The main problem in such preparations is the high viscosity of the slime solutions, which hinders the deposition of the cells. Different slime preparations vary in their intrinsic viscosity and there are no general rules for centrifugal speeds necessary to obtain an adequate separation. The addition of electrolytes (salts) may be useful in the precipitation by neutralizing charges on the polysaccharide. If the polysaccharide is in capsule form it must be detached from the cells. Again, it is difficult to generalize, as capsules are much more readily removed from some strains than from others. Gentle stirring or mixing in a homogenizer may suffice, but sometimes more drastic procedures may have to be employed. Depending on the culture medium used or methods employed, the isolation of a polymer containing various contaminations and some degradation may also occur (Cerning 1990).

The importance of the quest for new chemical compounds from natural resources and bacteria both for food additives and for use in medicine is an active field (Mason et al. 2010). The application of green technologies as Ultrasound-assisted extraction, microwave-assisted extraction, supercritical fluid extraction, and hot-water extraction are an excellent alternative for obtaining and recovery chemical compounds as the polysaccharides (intracellular polysaccharides and exocellular polysaccharides) that could be used in food industry, pharmaceutical industry, and medicine. These methods have gained increasing attention due their environmentally friendly process, higher extraction efficiency, cost effectiveness, and structure preservation ability (Liu et al. 2015).

Ultrasound-assisted extraction is a process that uses acoustic energy and solvents to extract target compounds from various plant matrices and biofilms. The generally accepted explanation for solvent extraction enhancement by using ultrasound is the propagation of ultrasound pressure waves and resulting cavitation phenomena, whereby the collapse of cavitation bubbles and highly localized temperature breaks cell walls and releases the contents of the cell into the extraction medium (Ebringerová and Hromádková 2010). Alboofetileh et al. 2019 studied the extraction of fucoidan from *Nizamuddinia zanardini*. They applied enzyme, ultrasound, enzyme-ultrasound methods, and evaluated their implications on yield, chemical and molecular structure, anticancer, and immunostimulatory properties. The results showed that the enzyme-ultrasonic isolated fucoidan showed the maximum extraction yield (7.87%) while that obtained by ultrasonic had the minimum value (3.6%). Fucoidans were composed of different levels of carbohydrates (52.78–58.65%), proteins (6.98–8.91%), sulfates (21.78–29.6%), and uronic acids (0.42–1.08%).

On the other hand, microwave heating (2.45 GHz) occurs as the result of the dissipation of electromagnetic waves in the irradiated medium. The dissipated power in a medium depends on the dielectric properties and the local time average electric field strength. So, there is a fundamental difference between microwave and conventional extraction (Mason et al. 2010). De Cássia Da Fonseca et al. (2009) used a microwave oven to accelerate the levan acid hydrolysis from *Zymomonas mobilis* envisaging the production of oligofructans for antitumor uses. They concluded that the use of microwave oven in the levan acid hydrolysis is viable and leads to a considerable reduction in the hydrolysis time (5 min), and obtained oligofructans or fructooligosaccharides with a degree of polymerization varying from 4 to 14.

But the traditional production of EPS always been an excellent alternative. An example of classic EPS production is hyaluronic acid. *Streptococci* strains for hyaluronan production generally use glucose as a carbon source, which is significantly higher than typical yields for complex polysaccharides in lactic acid bacteria (Duan et al. 2009). Extracting hyaluronan from microbial fermentation broth is a relatively simple process with high yields. An additional and important advantage of microbial hyaluronan production is that microbial cells can be physiologically and/or metabolically adapted to produce more hyaluronan of high molecular weight. Therefore, microbial hyaluronan production using either pathogenic streptococci or safe recombinant hosts, containing the necessary hyaluronan synthase, is nowadays more and more preferred (Boeriu et al. 2013). Due to the characteristics of hyaluronic acid (units containing N-acetyl-D-glucosamine and glucuronic acid), high molecular weight and unique viscoelastic and rheological properties, the principal applications are in biomedicine and cosmetic industry (Kogan et al. 2007).

The EPS of LAB, as already mentioned, have the capacity of modifying the rheological properties, texture, and "mouthfeel" of food products, so with that they can find applications in the food industry as viscosifiers, stabilizers, emulsifiers, or gelling agents. Furthermore, LABs are "generally recognized as safe" (GRAS), due to their history in food production. One of the most used polysaccharides is xanthan gum, produced by *Xanthomonas campestris*, of which up to 50 g / L has been produced, which modifies the rheology of food. These substances, called "gums," can be added to yogurth or bread, and they can be cheaper than the use of vegetable hydrocolloids (Caggianiello et al. 2016).

8 Conclusion

In conclusion, bacterial polysaccharides, specifically EPS, are of great interest not only for their biological actions but also for their possible industrial applications. EPS, due to its biological actions, can prevent the prevention of diseases that are detected by oxidative stress or by the effect of high levels of cholesterol in the blood. Likewise, since EPS is the means by which the microorganism interacts with the environment, this molecule is capable of promoting, directing the development, or modulating the action of the host's immune system. Obviously, there are EPS that are not beneficial, but there are many more that can be used to improve the quality of life

of humans. This is closely related to the increase in recent decades of diseases such as allergies to foods or substances that are normally innocuous and this may be related to "exaggerated" hygiene, as well as the use of antimicrobial agents in every day products such as soap and antibiotics, so exposure to microorganisms and their respective EPS have been diminished resulting in these health conditions. As it was seen throughout the chapter, many of these microorganisms have coevolved with humans and one of its most important effects is the stimulation of the immune system, mainly through the intestinal mucosa; therefore contact with microorganisms through of their EPS is very important to achieve a good development of the immune system. Likewise, many of these polysaccharides not only serve for this stimulation, to prevent diseases such as cancer, or to take advantage of their antioxidant, antiviral, and even antibacterial properties, but they may also be important in various industries such as food industry to achieve the development of pleasant textures and flavors in food or the pharmaceutical industry as an agent for drug delivery. All in all, microbial polysaccharides are a group of very interesting molecules that have a lot of different applications.

References

Abdhul K, Ganesh M, Shanmughapriya S, et al. Antioxidant activity of exopolysaccharide from probiotic strain *Enterococcus faecium* (BDU7) from Ngari. Int J Biol Macromol. 2014. https://doi.org/10.1016/j.ijbiomac.2014.07.026.

Abid Y, Casillo A, Gharsallah, et al. Production and structural characterization of exopolysaccharides from newly isolated probiotic lactic acid bacteria. Int J Biol Macromol. 2018;108:719–28. https://doi.org/10.1016/j.ijbiomac.2017.10.155.

Aguilar CN, Ruiz HA, Rubio Rios A, et al. Emerging strategies for the development of food industries. Bioengineered. 2019;10(1):522–37.

Alayande BA, Aung MM, Kim IS. Correlation Between Quorum Sensing Signal Molecules and Pseudomonas aeruginosa's Biofilm Development and Virulency. Curr Microbiol. 2018;75:787–93. https://doi.org/10.1007/s00284-018-1449-5.

Alboofetileh M, Rezaei M, Tabarsa M, et al. Bioactivities of Nizamuddinia zanardinii sulfated polysaccharides extracted by enzyme, ultrasound and enzyme-ultrasound methods. J Food Sci Technol. 2019;56(3):1212–20. https://doi.org/10.1007/s13197-019-03584-1.

Alm JS, Swartz J, Björkstén B, et al. An anthroposophic lifestyle and intestinal microflora in infancy. Pediatr Allergy Immunol. 2002;13:402–11.

Aullybux AA, Puchooa D, Bahorun T. Phylogenetics and antibacterial properties of exopolysaccharides from marine bacteria isolated from Mauritius seawater. Ann Microbiol. 2019;69:957–72. https://doi.org/10.1007/s13213-019-01487-2.

Bhat B, Bajaj BK. Hypocholesterolemic and bioactive potential of exopolysaccharide from a probiotic *Enterococcus faecium* K1 isolated from kalarei. Bioresour Technol. 2018:264–7. https://doi.org/10.1016/j.biortech.2018.01.078.

Biliavska L, Pankivska Y, Povnitsa O, et al. Antiviral Activity of Exopolysaccharides Produced by Lactic Acid Bacteria of the Genera Pediococcus, Leuconostoc and Lactobacillus against Human Adenovirus Type 5. Medicina. 2019;55:519. https://doi.org/10.3390/medicina55090519.

Boeriu CG, Springer J, Kooy FK, et al. Production methods for hyaluronan. Int J Carbohydr Chem. 2013;2013:1–14.

Brandenburg K, Andrä J, Müller M, et al. Physicochemical properties of bacterial glycopolymers in relation to bioactivity. Carbohydr Res. 2003;338(23):2477–89.

Caggianiello G, Kleerebezem M, Spano G. Exopolysaccharides produced by lactic acid bacteria: from health-promoting benefits to stress tolerance mechanisms. Appl Microbiol Biotechnol. 2016;100(9):3877–86.
Cerning J. Exocellular polysaccharides produced by lactic acid bacteria. FEMS Microbiol Lett. 1990;87(1–2):113–30.
Chaisuwan W, Jantanasakulwong K, Wangtueai S, et al. Microbial exopolysaccharides for immune enhancement: Fermentation, modifications and bioactivities. Food Biosci. 2020;35:100564. https://doi.org/10.1016/j.fbio.2020.100564.
Chen F, Huang G. Preparation and immunological activity of polysaccharides and their derivatives. Int J Biol Macromol. 2018. https://doi.org/10.1016/j.ijbiomac.2018.01.169.
Chen Y, Liu T, Wang K, et al. Baicalein inhibits staphylococcus aureus biofilm formation and the quorum sensing system in vitro. PLoS One. 2016;11:1–18. https://doi.org/10.1371/journal.pone.0153468.
Chen L, Ge MD, Zhu Y, et al. Structure, bioactivity and applications of natural hyperbranched polysaccharides. Carbohydr Polym. 2019. https://doi.org/10.1016/j.carbpol.2019.115076.
Choi DB, Lee JH, Kim YS, et al. A study of mycelial growth and exopolysaccharide production from a submerged culture of mycoleptodonoides aitchisonii in an air-lift bioreactor. Korean J Chem Eng. 2011;28(6):1427–32.
De Cássia Da Fonseca V, Haminiuk C, Izydoro DRW, et al. Stability and rheological behaviour of salad dressing obtained with whey and different combinations of stabilizers. Int J Food Sci Technol. 2009;44:777–83.
Deepak V, Ramachandran S, Balahmar RM, et al. In vitro evaluation of anticancer properties of exopolysaccharides from Lactobacillus acidophilus in colon cancer cell lines. In Vitro Cell Dev Biol Anim. 2016;52(2):163–73. https://doi.org/10.1007/s11626-015-9970-3.
Dinic M, Pecikoza U, Djokic J, et al. Exopolysaccharide produced by probiotic strain lactobacillus paraplantarum BGCG11 reduces inflammatory hyperalgesia in rats. Front Pharmacol. 2018;9:1–12.
Donot F, Fontana A, Baccou JC, et al. Microbial exopolysaccharides: main examples of synthesis, excretion, genetics and extraction. Carbohydr Polym. 2012;87(2):951–62. https://doi.org/10.1016/j.carbpol.2011.08.083.
Duan XJ, Niu HX, Tan WS, et al. Mechanism analysis of effect of oxygen on molecular weight of hyaluronic acid produced by Streptococcus zooepidemicus. J Microbiol Biotechnol. 2009;19(3):299–306.
Ebringerová A, Hromádková Z. An overview on the application of ultrasound in extraction, separation and purification of plant polysaccharides. Cent Eur J Chem. 2010;8(2):243–57.
Faghfoori Z, Gargari BP, Saber A, et al. Prophylactic effects of secretion metabolites of dairy lactobacilli through downregulation of ErbB-2 and ErbB-3 genes on colon cancer cells. Eur J Cancer Prev. 2017:1–9. https://doi.org/10.1097/CEJ.0000000000000393.
Gientka I, Bzducha-Wróbel A, Stasiak-Różańska, et al. The exopolysaccharides biosynthesis by Candida yeast depends on carbon sources. Electron J Biotechnol. 2016;22:31–7.
Hooper LV, Stappenbeck TS, Hong CV, et al. Angiogenins: a new class of microbicidal proteinas involved in innate immunity. Nat Immunol. 2003;4:269–73.
Ismail B, Nampoothiri KM. Exposition of antitumor activity of a chemically characterized exopolysaccharide from a probiotic Lactobacillus plantarum MTCC 9510. Biologia. 2013;68(6):1041–7. https://doi.org/10.2478/s11756-013-0275-2.
Jenny Angel S, Vidyadharani G, Santhosh S, et al. Optimization and characterisation of thermo stable exopolysaccharide produced from bacillus licheniformis WSF-1 strain. J Polym Environ. 2018;26(9):3824–33. https://doi.org/10.1007/s10924-018-1261-0.
Jeong D, Kim D, Kang I, et al. Characterization and antibacterial activity of a novel exopolysaccharide produced by Lactobacillus kefiranofaciens DN1 isolated from kefir. Food Control. 2017;78:436–42. https://doi.org/10.1016/j.foodcont.2017.02.033.
Jung YJ, Lee YT, Ngo VL, et al. Heat-killed Lactobacillus casei confers broad protection against influenza A virus primary infection and develops heterosubtypic immunity against future secondary infection. Sci Rep. 2017;7:17360. https://doi.org/10.1038/s41598-017-17487-8.

Kanmani P, Suganya K, Satish Kumar R, et al. Synthesis and functional characterization of antibiofilm exopolysaccharide produced by enterococcus faecium mc13 isolated from the gut of fish. Appl Biochem Biotechnol. 2013;169(3):1001–15.
Kanmani P, Albarracin L, Kobayashi H et al. Exopolysaccharides from Lactobacillus delbrueckii OLL1073R-1 modulate innate antiviral immune response in porcine intestinal epithelial cells. Mol Immunol. 2018;93:253–65. https://doi.org/10.1016/j.molimm.2017.07.009.
Kim K, Lee G, Thanh HD, et al. Exopolysaccharide from Lactobacillus plantarum LRCC5310 offers protection against rotavirus-induced diarrhea and regulates inflammatory response. J Dairy Sci. 2018;101:5702–12. https://doi.org/10.3168/jds.2017-14151.
Kodali VP, Sen R. Antioxidant and free radical scavenging activities of an exopolysaccharide from a probiotic bacterium. Biotechnol J. 2008;3(2):245–51.
Kogan G, Šoltés L, Stern R, et al. Hyaluronic acid: A natural biopolymer with a broad range of biomedical and industrial applications. Biotechnol Lett. 2007;29(1):17–25.
Li S, Huang R, Shah NP, et al. Antioxidant and antibacterial activities of exopolysaccharides from Bifidobacterium bifidum WBIN03 and Lactobacillus plantarum R315. J Dairy Sci. 2014a;97:7334–43. https://doi.org/10.3168/jds.2014-7912.
Li W, Ji J, Tang W, et al. Characterization of an antiproliferative exopolysaccharide (LHEPS-2) from Lactobacillus helveticus MB2-1. Carbohydrate Polymers. 2014b;105(2014):334–40. https://doi.org/10.1016/j.carbpol.2014.01.093.
Li S, Xiong Q, Lai X, et al. Molecular modification of polysaccharides and resulting bioactivities. Compr Rev Food Sci Food Saf. 2016;15(2):237–50.
Lim JM, Lee YJ, Cho HR, et al. Extracellular polysaccharides purified from Aureobasidium pullulans SM-2001 (Polycan) inhibit dexamethasone-induced muscle atrophy in mice. Int J Mol Med. 2018;41(3):1245–64.
Liu J, Willför S, Xu C. A review of bioactive plant polysaccharides: biological activities, functionalization, and biomedical applications. Bioact Carbohydr Diet Fibre. 2015;5(1):31–61.
Lynch KM, Zannini E, Coffey A, et al. Lactic acid bacteria exopolysaccharides in foods and beverages: isolation, properties, characterization, and health benefits. Annu Rev Food Sci Technol Lact. 2018;9:155–76. https://doi.org/10.1146/annurev-food-030117-012537.
Mason TJ, Chemat F, Vinatoru M. The extraction of natural products using ultrasound or microwaves. Curr Org Chem. 2010;15(2):237–47.
Mazmanian SK, Kasper DL. The love-hate relationship between bacterial polysaccharides and the host immune system. Nat Rev Immunol. 2006;6(11):849–58.
Mcpherson AJ, Harris NL. Interactions between commensal intestinal bacteria and the immune system. Nat Rev Immunol. 2004;4:478–85.
Moghannem SAM, Farag MMS, Shehab AM, et al. Exopolysaccharide production from Bacillus velezensis KY471306 using statistical experimental design. Braz J Microbiol. 2018;49(3):452–62. https://doi.org/10.1016/j.bjm.2017.05.012.
Nehal F, Sahnoun M, Smaoui S, et al. Characterization, high production and antimicrobial activity of exopolysaccharides from Lactococcus lactis F-mou. Microb Pthogenes. 2019;132:10–9. https://doi.org/10.1016/j.micpath.2019.04.018.
Nikaido H. Structure and functions of the cell envelope of Gram-negative bacteria. Rev Infect Dis. 1988;10:S279–81.
Nwodo UU, Green E, Okoh AI. Bacterial exopolysaccharides: functionality and prospects. Int J Mol Sci. 2012;13(11):14002–15.
Pan D, Mei X. Antioxidant activity of an exopolysaccharide purified from Lactococcus lactis subsp. lactis 12. Carbohydr Polym. 2010;80(3):908–14. https://doi.org/10.1016/j.carbpol.2010.01.005.
Radchenkova N, Tomova A, Kambourova M. Biosynthesis of an exopolysaccharide produced by Brevibacillus thermoruber 438. Biotechnol Biotechnol Equip. 2011;25:77–9.
Ramakrishna C, Kujawski M, Chu H, et al. Bacteroides fragilis polysaccharide A induces IL-10 secreting B and T cells that prevent viral encephalitis. Nat Commun. 2019;10:2153. https://doi.org/10.1038/s41467-019-09884-6.

Raza W, Makeen K, Wang Y, et al. Optimization, purification, characterization and antioxidant activity of an extracellular polysaccharide produced by Paenibacillus polymyxa SQR-21. Bioresour Technol. 2011;102(10):6095–103. https://doi.org/10.1016/j.biortech.2011.02.033.

Saber A, Alipour B, Faghfoori Z, et al. Cellular and molecular effects of yeast probiotics on cancer. Crit Rev Microbiol. 2017;43(1):96–115. https://doi.org/10.1080/1040841X.2016.1179622.

Sasikumar K, Kozhummal Vaikkath D, Devendra L, et al. An exopolysaccharide (EPS) from a Lactobacillus plantarum BR2 with potential benefits for making functional foods. Bioresour Technol. 2017. https://doi.org/10.1016/j.biortech.2017.05.075.

Seesuriyachan P, Kuntiya A, Chaiyaso T, et al. Enhancement and optimization of exo-polysaccharide production by Weissella confusa TISTR 1498 in pH controlled submerged fermentation under high salinity stress. Chiang Mai J Sci. 2014;41(3):503–12.

Shahid M, Rajoka R, Mehwish HM, et al. Characterization, the antioxidant and antimicrobial activity of exopolysaccharide isolated from poultry origin lactobacilli. Probiotics Antimicrob Proteins. 2018;11:1132–42.

Spano A, Lagana P, Visalli G, et al. In vitro antibiofilm activity of an exopolysaccharide from the marine thermophilic bacillus licheniformis T14. Curr Microbiol. 2016;75:518–28. https://doi.org/10.1007/s00284-015-0981-9.

Strachan DP. Hay fever, hygiene, and household size. BMJ. 1989;299:1259–60.

Trabelsi I, Ktari N, Ben SS, et al. Evaluation of dermal wound healing activity and in vitro antibacterial and antioxidant activities of a new exopolysaccharide produced by Lactobacillus sp.Ca6. Int J Biol Macromol. 2017;103:194–201. https://doi.org/10.1016/j.ijbiomac.2017.05.017.

Tukenmez U, Aktas B, Aslim B, et al. The relationship between the structural characteristics of lactobacilli-EPS and its ability to induce apoptosis in colon cancer cells in vitro. Nat Sci Rep. 2019;9:8268. https://doi.org/10.1038/s41598-019-44753-8.

Upreti RK, Kumar M, Shankar V. Bacterial glycoproteins: functions, biosynthesis and applications. PRO. 2003;3:363–79.

Wang J, Hu S, Nie S, et al. Reviews on mechanisms of in vitro antioxidant activity of polysaccharides. Oxid Med Cell Longev. 2016;2016:5692852.

Willis-Karp M, Santeliz J, Karp CL. The germless theory of allergic disease: revisiting the hygiene hypothesis. Nat Rev Immunol. 2001;1:69–75.

Yang Y, Song H, Wang L, et al. Antiviral effects of a probiotic metabolic products against transmissible gastroenteritis coronavirus. J Prob Health. 2017a;5:184. https://doi.org/10.4172/2329-8901.1000184.

Yang W-T, Yang G-L, Shi S-H, et al. Protection of chickens against H9N2 avian influenza virus challenge with recombinant Lactobacillus plantarum expressing conserved antigens. Appl Microbiol Biotechnol. 2017b;101:4593–603. https://doi.org/10.1007/s00253-017-8230-8.

Zeidan AA, Poulsen VK, Janzen T, et al. Polysaccharide production by lactic acid bacteria: from genes to industrial applications. FFEMS Microbiol Rev. 2017;41:168–200. https://doi.org/10.1093/femsre/fux017.

Zhang Z, Liu Z, Tao X, et al. Characterization and sulfated modification of an exopolysaccharide from Lactobacillus plantarum ZDY2013 and its biological activities. Carbohydr Polym. 2016;153:25–33. https://doi.org/10.1016/j.carbpol.2016.07.084.

Zhou Y, Cui Y, Qu X. Exopolysaccharides of lactic acid bacteria: Structure, bioactivity and associations: A review. Carbohydr Polym J. 2019;207:317–32. https://doi.org/10.1016/j.carbpol.2018.11.093.

Surface Properties of Polysaccharides

24

Evrim Umut

Contents

Abstract

Polysaccharides are sugar derivative polymers with high natural abundance existing in microorganisms, algae, plants, insects, and animals, where they play vital role in realizing cellular functions. On the contrary to synthetic polymers,

E. Umut (✉)
Department of Medical Imaging Techniques, Dokuz Eylul University, School of Healthcare, Izmir, Turkey
e-mail: evrim.umut@deu.edu.tr

J. M. Oliveira et al. (eds.), *Polysaccharides of Microbial Origin*,
https://doi.org/10.1007/978-3-030-42215-8_31

polysaccharides stem as a renewable source of value-added chemicals that can be synthesized from microorganisms (bacteria and fungi) by fermentation without harmful side products, although with relatively higher costs. Most of the microbial polysaccharides are hydrophilic, biocompatible, and biodegradable, which make them used in food, cosmetics, pharmaceutical, and biomedical applications. Moreover, their physicochemical properties can be improved either by in situ changing the bacterial culture conditions or post synthesis surface modification, that is, cross linking to fulfill the requirement of more applications. In this review, the structure-function relationship of the most commercially used polysaccharides is summarized and different surface modification strategies are discussed with the focus on microbial polysaccharides. Recent advances in the applications of microbial polysaccharides are given with examples from literature.

Keywords

Microbial polysaccharides · Surface modification · Food applications · Pharmaceutical applications · Biomedical applications

1 Introduction

Polysaccharides are long chained biomacromolecules constituted of repeating monosaccharide (sugar) units either in neutral, that is, glucose, galactose, fructose, rhamnose, or acidic form such as glucuronic- and galacturonic acid. Their molecular weight may change in a broad range starting from a few kDa up to tens of thousands of kDa, in which the same (homopolysaccharides) or different types (heteropolysaccharides) of monosaccharides are linked to each other in varying sequences mostly forming linear shaped polymers for low molecular weights (cellulose, chitin) and branched or helical shaped ones (κ-carrageenan, xanthan gum) for high molecular weights. Polysaccharides are found in natural sources like plants (starch, cellulose, pectin), seaweeds (alginate, agar, carrageenan), animals (chitin, chitosan, gelatin), and microorganisms (dextran, xanthan gum, gellan gum, curdlan, pullulan) (Shanmugam and Abirami 2019). In the living organisms they can be located either inside (intracellular polysaccharides) or outside of the cells (extracellular polysaccharides) that are attached on the cell membrane in the form of capsules, granules, or slimes (Ahmad et al. 2015). Intracellular polysaccharides, that is, starch, glycogen store energy for the cell, tend to be branched or folded upon themselves and are generally insoluble in water, whereas extracellular polysaccharides (EPS) are responsible for maintaining the structural integrity of the cells (like cellulose in plants and chitin in insects) and have linear, rigid structures. Another important biological function of the EPS is that they act as targeting mediators for transferring the communication signals between different cells by conjugating to lipids and proteins hence forming glycolipids and glycoproteins. These EPS are generally in the form of water-soluble slimes that are released (secreted) from cells naturally or under stress into the external cell culture medium, hence it is easier to isolate such

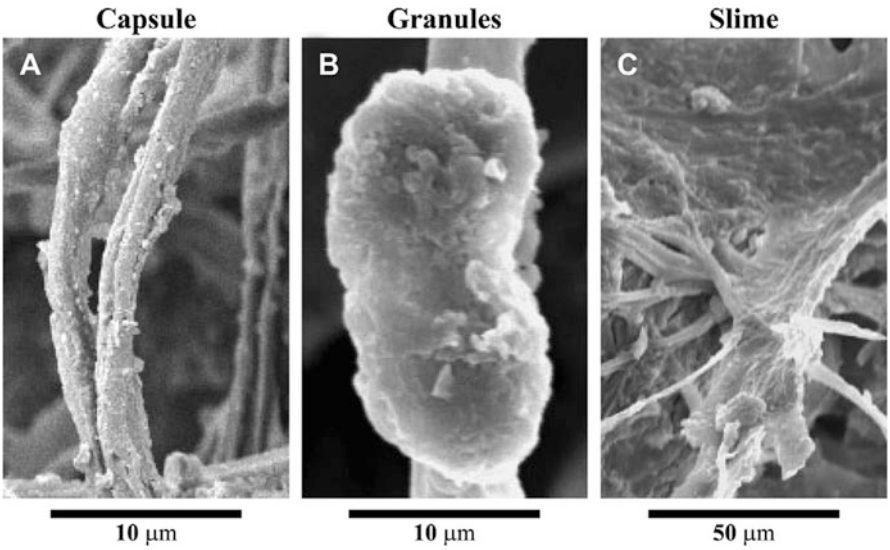


Fig. 1 Scanning electron microscopy (SEM) images EPS found on cyanobacterial soil crusts. (Reprinted from Mager and Thomas 2011 with permission of Elsevier Inc.)

EPS as compared to the ones attached on cell walls (Jindal and Khattar 2018). Figure 1 shows electron microscopy images of different forms of EPS found on cyanobacterial soil crusts (Mager and Thomas 2011).

2 Production, Extraction, and Purification/Isolation of Exopolysaccharides

The microbial polysaccharides exhibit unique physicochemical properties which make them commonly used in food industry, pharmaceutics, regenerative medicine, and cosmetics (Jindal and Khattar 2018; Smelcerovic et al. 2008; Ng et al. 2020). For this reason the production of pure and high quality EPS from microbial sources has crucial importance. EPS are synthesized via fermentation, that is, bacterial growth though enzymatic activity in the presence of nutrients, where fermentation parameters like pH, temperature, oxygen concentration as well as the strain (type) of the microorganisms determine the production amount and quality of the EPS. For the synthesis of each type of polysaccharide there is one or few specific type of bacteria (see Table 1) and regardless of the type of EPS that will be produced, in terms of the nitrogen-rich nutrient (biomass) availability as a general rule the carbon/nitrogen ratio should be kept maximum in batch culture medium (Oner 2013; Zhang et al. 2015; Jiang 2013; Sarilmiser 2015).

The production of EPS is followed by the extraction, purification, and isolation steps that can include different processes depending on the type of produced EPS. The extraction of EPS in slime form or as capsules that are weakly associated to the

Table 1 Different types of microbial polysaccharides and corresponding bacterial sources

Polysaccharide	Microbial source	Reference
Dextran	*Leuconostoc mesenteroides*	Siddiqui et al. (2014)
Xanthan gum	*Xanthomonas campestris*, and *Xanthomonas pelargonii*	Niknezhad et al. (2016)
Cellulose	*Gluconacetobacter xylinum*	Ruka et al. (2012)
Glucan	*Saccharomyces cerevisiae*	Hunter et al. (2002)
Gellan gum	*Sphingomonas elodea, Sphingomonas paucimobilis*	Zhang et al. (2015)
Alginate	*Pseudomonas aeruginosa, Pseudomonas putida*	Chang et al. (2007)
Curdlan	*A. faecalis*	Jiang (2013)
Levan	*Halomonas eurihalina, Zymomonas mobilis*	Sarilmiser (2015)

cell membrane is done by centrifugation or preferably by ultracentrifugation also in order to filter the residuals and biomass remained in the culture. The centrifugation speed and time may change depending on the viscosity and molecular weight of EPS and sometimes performed with the aid of thermal treatment. On the other hand, for the case of capsular EPS that is tightly associated to microorganisms some chemical pretreatments such as alkaline, sodium chloride, EDTA, or alcohol precipitation or additional thermal steps, that is, heat bath in saline, phenol-water mixtures, are required before centrifugation. After the extraction, the EPS should be still purified and isolated from the crude extract including proteins and the secondary metabolites, which is achieved by using columns, membranes, or chromatography techniques, that is, size-exclusion chromatography, liquid chromatography, and anion-exchange chromatography (Harding 2005).

3 Physicochemical Properties of Microbial Polysaccharides

The microbial EPS are rendered with a large variety of physicochemical properties in terms of charge neutrality (neutral, anionic, or cationic), hydrophilicity, water solubility, and viscosity. Since the structural units of an EPS are the monosaccharides, at first stage these properties naturally depend on the types of monomers, their absolute configuration (D or L), and anomeric configuration (α or β). The monosaccharides that most commonly involved in microbial EPS are D-glucose, D-galactose, D-mannose; L-rhamnose, L-fructose; and N-acetylhexosamines-N-acetyl-D-glucosamine and N-acetyl-D-galactosamine. Equally important factor is the glycosidic linkages (1 → 2), (1 → 3), (1 → 4) and the sequence of monomers. For example, helix structured EPS including β(1 → 4) linkages between monosaccharides are mostly water insoluble due to the strong interchain hydrogen bonding and exclusion of water, while EPS with β(1 → 2) linkages have flexible coil structure and show substantial steric hindrance (Venugopal 2011). Another aspect affecting the physicochemical properties is the substitution groups, that is, sulfates and phosphates. Microbial EPS commonly include acyl substituents like ester-linked acetate or ketal-linked pyruvate, where in terms of the charge neutrality of the polysaccharide

molecule the first does not contribute to the overall charge on the contrary to the second. Other ester-linked substituents have been also identified in microbial EPS such as hydroxybutanyl, propionyl, and glyceryl residues (Ahmad et al. 2015). The ring type (size) constituting the EPS chain, that is, pyranose (six carbon – one oxygen) or furanose (five carbon – one oxygen) also was shown to be effective determining the rheological and mechanical properties. Marszalek et al. have shown that the elasticity of some microbial EPS (dextran, pullulan, amylose) results from the force-induced deformation of the pyranose rings eventually transforming from chair-like structures to boat-like structures (Marszalek et al. 1998).

3.1 Dextran

Dextan consists of linear α(1 → 6) and branched α(1 → 3) glycosidic linkages between D-glucose units, where the latter distinguishes it from dextrin which have branched α(1 → 4) linkages (see Fig. 2) (Ng et al. 2020). It is the first commercially available microbial polysaccharide and produced by the fermentation of sucrose via the so-called dextransucrase enzyme secreted from *Leuconostoc*, *Streptococcus*, and *Lactobacillus* bacteria. Dextran synthesized by *Leuconostoc mesenteroides* mostly include α(1 → 6) glycosidic linkages with rare α(1 → 3) bonded side chains and are readily soluble in water, stable under mild acidic and basic conditions (Smelcerovic et al. 2008). On the contrary, dextrans including 50% branching through α(1 → 3) linkages are water insoluble. It is a neutral biopolymer and available in a large range of molecular weights between 10 and 50,000 kDa (Kothari et al. 2014). Dextran forms highly viscous liquids at the same time easily degraded in human digestive tract, which makes it a promising material as viscosifier, emulsifier, gelling, and texturing agent for food industry. On the other hand, it is not degraded in blood and filtered by kidney allowing it to be used as blood plasma expander. Moreover, it has

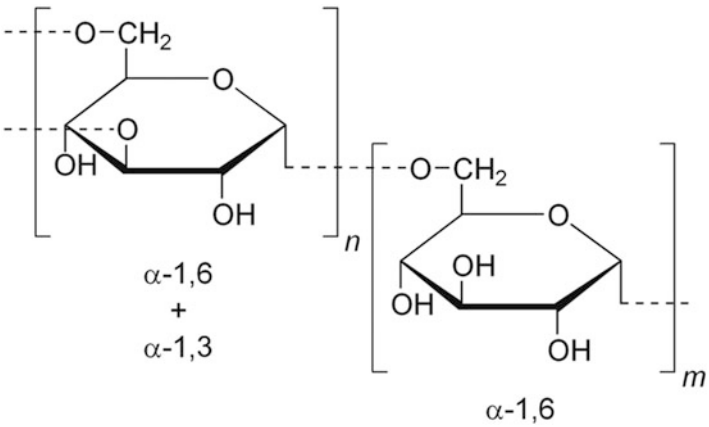


Fig. 2 Chemical structure of dextran. (Reused from wikimedia.org under common license)

been reported that water-soluble dextran derivatives including some extent of sulfonate groups, carboxymethyl, and benzylamide show antiviral properties (Neyts et al. 1995).

3.2 Xanthan

Xanthan gum is one of the first FDA (US Food and Drug Administration) approved microbial polysaccharide. It has a molecular weight around 2000 kDa and produced by the enzymatic activity of *Xanthomonas campestris* bacteria either from sucrose or glucose. Xanthan consists of D-glucose, D-mannose, and D-glucuronic acid in the molar ratio of 2:2:1, respectively, with lots of trisaccharide side chains (Ng et al. 2020). In particular, repeating β(1 → 4) linked D-glucose builds the backbone and α(1 → 3) linked side chains include D-glucuronic acid placed between two D-mannose units with β(1 → 2) and β(1 → 4) linkages. The presence of D-glucuronic acid attributes anionic character to branched xanthan polymer, which eventually causes the charged side chains to wrap around the backbone leading to helix structures. Depending on this ionic strength xanthan polymer can also get easily hydrated and dissolved in water. The solutions of xanthan gum show relatively high viscosity at low concentrations and for this reason they are used as thickeners and emulsion stabilizers in food products.

3.3 Gellan

Gellan is a linear, anionic, hetero-EPS produced from the bacteria *Sphingomonas paucimobilis*, also called as *Pseudomonas elodea*. It is built by β(1 → 4) linked D-glucose, D-glucuronic acid, and α(1 → 3) linked L-rhamnose sugar units and natively contains two acyl groups linked as side chains. It exists in two- or three-folded helix structure and can be soluble in water forming elastic, thermally reversible gels depending on the concentration. These gelation properties can be altered by deacylation of gellan, particularly O-acetylation on one of the two glucose residues, leading to a harder, brittle, and thermally irreversible form. Gellan gum is first approved in Japan and used in Japanese foods as gelation agent instead of agar (Jindal and Khattar 2018). Then, it became widespread and drawn attention as a modifiable biopolymer, whose gelation properties can be controlled by changing pH and temperature. In this respect in pharmaceutics, gellan was also shown to be a promising drug carrier for oral, nasal, and ophthalmic administration due to its in situ gelling capabilities (Coviello et al. 2007; Rajinikanth and Mishra 2008).

3.4 Cellulose

Cellulose is the most abundant biopolymer in nature as it exists in microorganisms, plants, and animals. It can be synthesized in large amounts from *Acetobacter xylinum* in

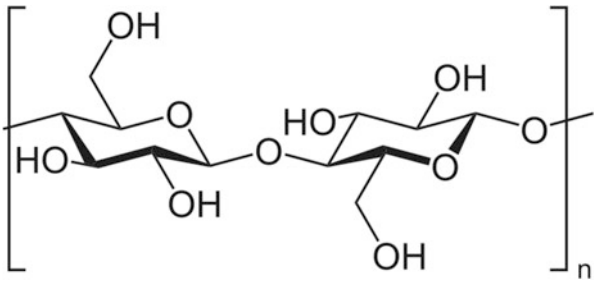


Fig. 3 Chemical structure of cellulose. (Reused from wikimedia.org under common license)

biofilm form under static culture conditions. Independent of its source, molecular formulation of cellulose is same as it consists of β(1 → 4) linked glucan chains (Fig. 3). However, only bacterial cellulose organizes as three-dimensional network of entangled nanofibers, and unlike the mostly crystalline form plant cellulose which shows very high mechanical strength, it exhibits great elasticity and hydrophilicity (Trovatti 2012). Thanks to its nanoporous surface, microbial cellulose can absorb water as hundreds times greater of its dry mass. This high amount of water content when combined with its flexibility makes cellulose very similar to extracellular matrix in human skin. Therefore, cellulose carries the unique properties to be used in regenerative medicine as it can both accommodate the drug molecules and serves as a physical barrier against any external infection (Czaja et al. 2006).

3.5 Chitin and Chitosan

Chitin is the second most abundant polysaccharide in nature after cellulose and its chemical structure can be described similar to cellulose with the difference that instead of a hydroxyl group on each D-glucose unit there exists an acetyl amine group, that is, the so-called N-acetyl-D-glucosamine. Chitin can be obtained from different microorganisms such as *Allomyces*, *Penicillium*, *Aspergillus*, and *Fusarium*. As in the case of most microbial EPS, chitin has been reported to show antiviral, antimicrobial, and antitumor activity as well as wound dressing property due to its nanofibrous structure similar to cellulose (Trovatti 2012). Chitosan, a much more popular biopolymer employed in many applications (cosmetics, pharmaceutics, food, biomedical, energy storage, etc.), is obtained by the deacetylation of chitin with NaOH. It is composed of randomly distributed anhydro-N-acetyl-D-glucosamine and anhydro-D-glucosamine residues with different degrees of deacetylation. This deacetylation pattern together with the molecular weight spanning from 10 kDA to more than 300 kDa determines the different physicochemical characteristics of chitosan. On the contrary to chitin, the native chitosan indeed has some disadvantageous such as poor water solubility, low surface area, and low porosity (Rajoka et al. 2020). However, it includes both hydroxyl and amino groups hence can be easily N-, O-, or N,O-modified to many derivatives each of them possessing a

forthcoming property, that is, enhanced water solubility and pH or temperature resistance depending on the requirements of diverse applications. Chitosan derivatives like carboxymethyl chitosan (Shariatinia 2018), diethylaminoethyl chitosan (Viegas de Souza et al. 2018), trimethyl chitosan (Mourya and Inamdar 2009), and N-dodecylated chitosan (Liu et al. 2001) have been used in tissue engineering, gene delivery, and targeted and controlled drug delivery. Functionalized chitosan also was shown to exhibit enhanced antibacterial, antiviral, and antitumor properties.

3.6 Curdlan

Curdlan is a microbial EPS secreted by the bacteria *Agrobacterium biobar* and *A. faecalis*. Its building blocks are β(1 → 3) bonded D-glucans, which are again polysaccharides constituted of D-glucose with α- or β-condensation linkages. In the linear chain these monosaccharides are partially esterified with succinic acid forming succinoglucan (Wustenberg 2014). Some intermolecular (1 → 2) or (1 → 6) linkages were also reported, which leads to branched and cyclic form of curdlan, respectively (McIntosh et al. 2005). Curdlan is a neutral EPS in triple helix structure with molecular weights changing between 50 kDa and 2000 kDa. At room temperature curdlan is insoluble in water, alcohols, and most organic solvents; whereas it can be dissolved in water at elevated temperatures. When heated up to 55 °C aqueous solutions of curdlan form thermo-reversible gels dissociating to singe chain helix and further heating above 80 °C and cooling results in thermo-irreversible gel form as it is used in most of the food applications. Due to its water and organic binding capabilities curdlan is proposed to be used in drug delivery applications as well. Carboxymethylether and sulfate modified curdlan reported to show enhanced water solubility and biological activity, that is, curdlan sulfates exhibit anticoagulant and anti-HIV properties (McIntosh et al. 2005).

3.7 Alginate

Alginate, also known as alginic acid, is a linear anionic copolymer constituting of β(1 → 4) linked D-mannuronic acid (M) and α(1 → 4) linked L-guluronic acid (G) residues both in homopolymeric (M or G) and heteropolymeric (MG) sequences. Alginate can be extracted from marine algae (seaweed) or produced by microorganisms *Azotobacter vinelandii* and *Pseudomonas aeruginosa* as biofilms. In terms of the homopolymeric chain lengths, M/G ratio and molecular weight of alginate synthesized from bacterial sources and algal sources generally differ from each other (Ahmad et al. 2015). Another aspect, which determines the viscosity and elasticity, is the acetylation degree i.e. alginates from algal origin do not include acetyl groups unlike the microbial alginates. Alginates from two different bacteria also show differences among each other. For example, the one produced from *A. vinelandii* contains G homopolymer component and interacts with (generally used) Ca^{+2} metal ions producing stronger gels, whereas alginate synthesized from *P. aeruginosa* produce flexible gels in the presence

of Ca^{+2} (Urtuvia et al. 2017). Owing to their high water binding capacity and gelation properties alginates are good candidates as blood expanders, drug delivery vehicles, and scaffolds for tissues. Moreover, they are used as stabilizer, emulsifier, and thickeners in food products.

3.8 Other Examples

Some other microbial polysaccharides that are commercially produced and employed in biomedical, food, and pharmaceutical applications are Pullulan, Levan, and Scleroglucan. Pullulan composed of α(1 → 6) bonded maltotriose units, which are three D-glucose linked with α(1 → 4) condensation linkages and produced from fungi *Aureobasidium pullulans* and *Cryphonectria parasitica*. It dissolves in water forming slightly viscous solutions that is stable in wide range of pH, but does not form a gel even in the presence of ions. In food industry pullulan is used as a low-calorie food additive, binder, thickener, and coating agent. Scleroglucan is another fungal polysaccharide as it name comes from the fungi *Sclerothinia* and composed of β(1 → 3) linked glucan backbone with β(1 → 6) linked glucose as side chain for every three glucan residues (Jindal and Khattar 2018). It is neutral and soluble in water, and aqueous solutions of Scleroglucan are stable in a wide range of pH, temperature, and presence of ions. Scleroglucan is very commonly used in cosmetics and food products. Levan is produced by *Bacillus subtilis*, *Zymomonas mobilis*, *Halomonas* sp. and consists of many β(2 → 6) linked fructan, a polymer of fructose. It can be both in linear and branched structure with relatively low molecular weight, where the latter is more stable and forms spherical shape. Levan shows cholesterol-lowering and prebiotic activity in food industry besides its use in some pharmaceutical and cosmetic applications (Oner et al. 2016).

Although they cannot be exactly classified as microbial polysaccharides, due to their importance in industrial use two polysaccharides are worth to mention here: carrageenan and hyaluronan. Carrageenan cannot be produced by bacteria; however, it is extracted by processing of marine algae *Kappaphycus alvarezii* and *Eucheuma denticulatum*. It has a sulfated D-galactose based linear chain alternatively linked by β(1 → 4) and α(1 → 3) linkages (Ficko-Blean et al. 2017). Depending on the number and the location of the sulfate groups it takes different names such as (kappa) κ-, (iota) I-, and (lambda) λ-carrageenan including one, two, and three sulfate groups per disaccharide, respectively. Only λ-carrageenan is soluble in water at room temperature forming viscous solution and does not form a gel, whereas other two forms of carrageennan can be dissolved in water at higher temperatures and form thermo-reversible gels (see Fig. 4) (Blakemore 2016). Although there are continuing discussions about its safety for human health (David et al. 2018), due to its gelling and protein-binding capabilities carrageenan is commonly utilized in food industry and in cosmetic products.

Another carbohydrate polymer, which is most commonly used in cosmetic and biomedical applications, is hyaluronan, which is also known as hyaluronic acid (HA). HA consists of repeating disaccharide units of D-glucuronic acid and N-acetyl-D-glucosamine linked with alternating β(1 → 4) and α(1 → 3) bonds among each

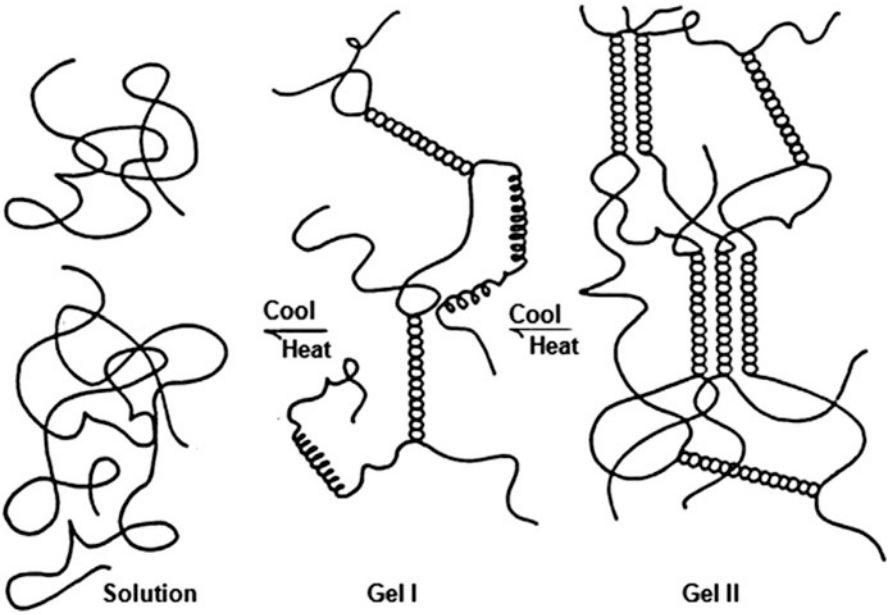


Fig. 4 Thermo-reversible gelation mechanism of carrageenan. (Reprinted from Blakemore 2016 with permission of Elsevier Inc.)

other. Its molecular weight varies between 5 and 2000 kDa. HA is found in all living organisms as a main component of extracellular matrix and is particularly concentrated in synovial fluid, the vitreous fluid of the eye, and umbilical cords (Necas et al. 2008). HA is highly hydrophilic and besides its main role as hydrating the tissues and lubricating the joints and muscles, it realizes many other functions like cell proliferation and migration, tumor development, metastasis, and inflammation (Seton-Rogers 2012). HA is present in the skin and soft connective tissues in high concentrations, which together with abovementioned functions makes it a perfect material to be used as skin moisturizer, dermal fillers, and surgical wound repairers in cosmetics. Due to its viscoelasticity maintaining function HA is used in the local treatment for osteoarthritis via intra-articular injection. HA solutions also serve as a viscosity-enhancing component of eye drops and facilitated in dry eye care in ophtalmotology (Necas et al. 2008). Cross-linked HA hydrogels are utilized in sustained and controlled drug delivery as drug carrier agents.

4 Surface Modification and Functionalization of Microbial Polysaccharides

Although most of the microbial EPS natively carry unique physicochemical characteristics, which make them promising materials for many abovementioned applications, their properties can be further improved or extended in order to suit the

requirements of more specific applications. The modification of microbial polysaccharides can be performed yet in the synthesis step by changing the fermentation conditions, that is, pH, temperature, oxygen, and nitrogen concentration in bacterial culture. The strain (family) of the source bacteria shown to be also effective on the structure and physicochemical characteristics of secreted EPS, hence this opens the way of synthesizing high quality EPS with desired properties by manipulating the bacterial genes encoding the enzymatic pathways, with a process so-called metabolic engineering (Schilling et al. 2020). This strategy successfully applied to produce functionalized xanthan, cellulose, and alginates so far and the biggest challenge seems to be to clarify the individual roles of genes in biosynthesis of EPS. All microbial polysaccharides include many hydroxyl groups, while some of them include carboxyl and amine groups, where the presence of such reactive groups allows their chemical modification and/or attachment of different moieties, that is, proteins, enzymes, drug molecules, etc. Esterification of EPS can be performed with compounds including carboxylic acid or in the presence of coupling agent N,N-′-dicyclohexylcarbodiimide at room temperature (Smelcerovic et al. 2008). Periodate oxidation, that is, another way of modification of polysaccharides with compounds lacking carboxylic acid function, is shown to be a proper way of conjugating proteins without any loss in their pharmacologic activity.

4.1 Copolymerization

The forthcoming properties of synthetic polymers and EPS can be combined either with the graft copolymerization of the synthetic polymers onto the backbone of EPS or synthesis of new amphiphilic block copolymers (Volokhova et al. 2020). Some examples are combinations of HEMA, PEG, PMAA, PLLA with alginate, dextran, chitosan, and pullulan. Synthesis of polysaccharide-based block copolymers can be done via direct coupling (linking a preformed polymer) or by chain extension approach including insertion of a polymer into the polysaccharide chain. The latter can be achieved by the polymerization of monomers with radical polymerization, ring opening polymerization, enzymatic polymerization, and atom transfer radical polymerization. On the other side, the graft copolymerization can be via microwave, UV, and gamma-ray irradiation, radical and atom transfer radical polymerization. Since polysaccharides are highly hydrophilic, both polysaccharide-based graft and block copolymers tend to form self-assembled structures in water as to maximize the water-polysaccharide interface area (see Fig. 5).

4.2 Cross-Linking

Polysaccharides easily undergo enzymatic degradation in physiological media and for this reason they are natively not suitable for some biomedical applications, that is, drug delivery. Besides the abovementioned preparation of graft and block copolymers, another way to protect the carbohydrate backbone from rapid degradation is

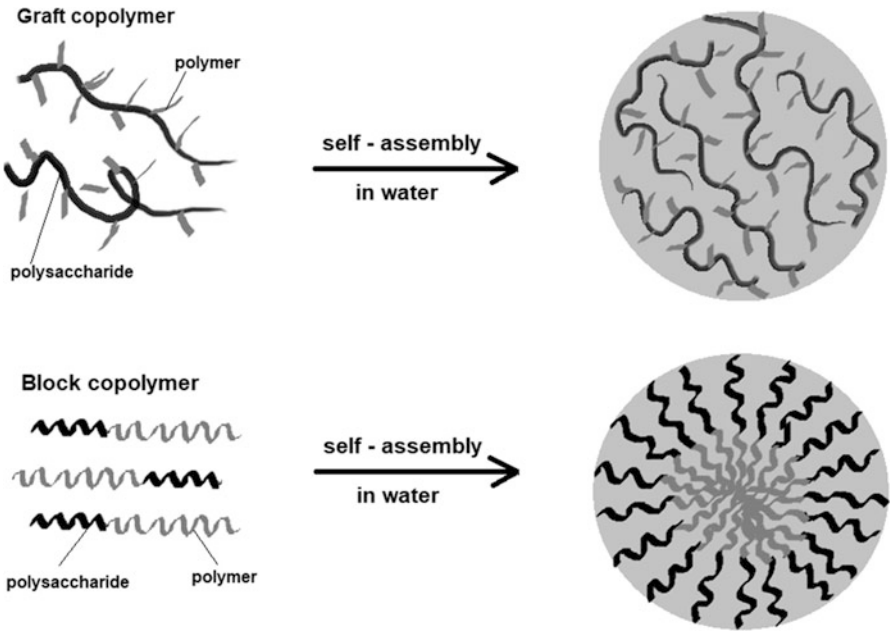


Fig. 5 In water self-assembly of graft and block copolymer based on polysaccharides

cross-linking. The cross-linked polysaccharides both show mechanical stiffness at the same time preserve their hydrophilic and gelation properties leading to the formation of hydrogels, which are three-dimensional polymeric networks that can imbibe water much larger than their dry mass. Cross-linking of polysaccharides can be done following chemical or physical routes. Chemical cross-linking includes covalent bonding of polymers using of a cross-linking agent (cross-linker), hence forms hydrogels with higher mechanical stiffness compared to the ones prepared by physical cross-linking. However, these cross-linkers are generally toxic and should be avoided before using in vivo (Ng et al. 2020).

4.2.1 Physical Cross-Linking

Physical interactions used in cross-linking are relatively weak and reversible, which is favorable for some applications where polysaccharide hydrogels should temporarily preserve its structural integrity but easily decompose under the influence of stimuli (pH, temperature change, etc.) as in the case of stimuli responsive controlled drug delivery. First approach to physically cross-link the polysaccharide chains is to facilitate –OH, COOH, $–NH_2$ groups on their backbones that can form hydrogen bonds. Since protonation/deprotonation strongly depends on the pH, temperature, and the used solvent, such parameters should be selected as to promote the weak hydrogen bonding during cross-linking. For the case of anionic and cationic polysaccharides however, polyelectrolyte complexation between two oppositely charged polysaccharides or cross-linking of anionic polysaccharides in the presence of

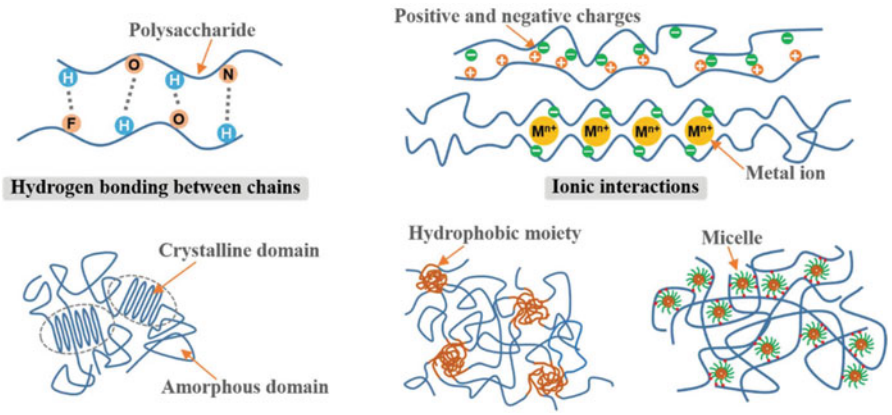


Fig. 6 Different physical approaches used for the cross-linking of polysaccharides. (Reproduced from Hu and Xu 2020 with permission of Royal Society of Chemistry)

counter metal ions, so-called as ionotropic gelation, is a better strategy to choose. This approach is particularly useful for sodium alginate, where the alpha-L-guluronate (G) blocks can electrostatically bind to divalent ions like Ca^{+2} and Cu^{+2} (Hu et al. 2018). Both divalent Ba^{+2} and trivalent Al^{+3} were chelated with gellan and due to its additional positive charge as compared to divalent Ba^{+2}, trivalent Al^{+3} can bind one more carboxylate group on gellan hence forming stronger Al^{+3}/gellan network (Patil et al. 2006; Reddy and Tammishetti 2002). Another physical and well-known method to form hydrogels is crystallization by repetitive freezing and thawing of aqueous polysaccharide solutions. Almost all polysaccharides can be gelated with this approach, where the microcrystals formed by subsequent freeze-thaw cycles function as joints for entangled polysaccharides. Crystallization can be performed with one type of polysaccharide or in the coexistence of another polymer such as polyvinyl alcohol (PVA) (Xiao and Gao 2008). An alternative way to form entangled hydrogels is introducing hydrophobic chemical moieties or micelles as joints onto the hydrophilic polysaccharide. In this approach the hydrophobic interactions lead to the organization of resulting amphiphilic polymers in a cross-linked structure. Figure 6 depicts the physical cross-linking methods applied to polysaccharides (Hu and Xu 2020).

4.2.2 Chemical Cross-Linking

On the contrary to physical interactions chemical cross-linking includes strong covalent bonding of hydroxyl, carboxyl, amide, and amine groups on the backbone of one polysaccharide with a complementary group on another polysaccharide or on a molecular cross-linking agent. Depending on which of these reactive groups are present on the polysaccharide to be studied, different cross-linkers are used such as glutaraldehyde (GA), glycerin, epichlorohydrin, ethylene glycol, triethylene glycol, polyethylene glycol diglycidylether, diviniyl sulfone (DVS), and N,N′-methylene-bisacrylamide (MBA) (Sahiner 2013). Most of these cross-linkers are hazardous and

toxic hence their residuals should be totally removed after the synthesis in order to be used in biomedical applications. On this respect, a safer additive-free method is polymerization triggered by high energy radiation. In this technique, aqueous solution of polysaccharides are irradiated either by microwaves, ultraviolet (UV), or gamma rays, which both decomposes the water molecules and breaks the C-H bonds on polysaccharide chain creating radicals, which then initiate cross-linkages with another polysaccharide (Dave and Gor 2018). By this method simultaneous sterilization of the resulting hydrogels is also achieved; however, the irradiation conditions, that is, irradiation time and radioactivity level of the gamma source should be considered in order to prohibit any degradation (scission) of the polysaccharide (see Fig. 7). Finally, an efficient though rarely employed alternative method is enzyme mediated cross-linking. As in the case of radiation initiated polymerization, no additional toxic cross-linkers are needed in this relatively novel method. However, for certain type of polysaccharides there are specific enzymes that catalyze the cross-linking, where this substrate specificity and high costs limit the application of the method. Recently, preparation of hydrogels based on ferrulic acid (FA) grafted neutral pullulan has been reported by the cross-linking using laccase enzyme obtained from *Trametes versicolor*, a common polypore mushroom (Hadrich et al. 2020).

Cross-linking plays a crucial role in the mechanical and swelling properties of hydrogels, where the latter is closely related with the amount of water (or solvent) that the polysaccharide gel can absorb. Hydrogels are porous polymeric networks and when immersed in water, swelling (volume expansion) of a dry gel starts by the penetration of water into these interconnected pores. First, all hydrophilic groups of polysaccharide matrix on pore walls are bonded with water (first hydration shell). Subsequently more water molecules get partially bonded on the first layer building a second hydration layer, which is in continuous chemical exchange with bulk-like water that fills the rest of the pore volume. Finally, an equilibrium state is reached when all the pores are saturated by water and the osmotic pressure is balanced with elastic retraction. Such swelling dynamics are strongly dependent on the pore size distribution, which in turn is related with cross-linking density and the type of cross-linker. By increasing the concentration of the cross-linking agent during gel formation, more points on different polysaccharide backbones are connected to each other, building smaller meshes – the repeating units of gel network.

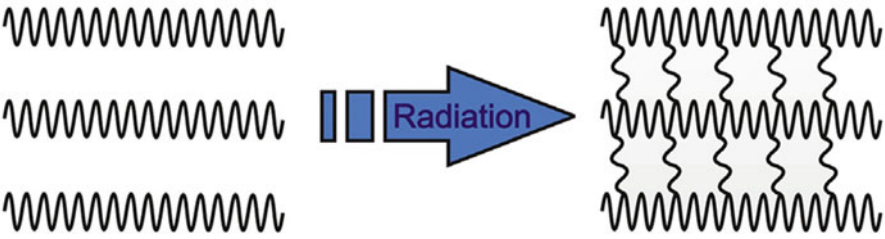


Fig. 7 Radiation-induced cross-linking among polysaccharides. (Reproduced from Gull et al. 2018 with permission of Elsevier Inc.)

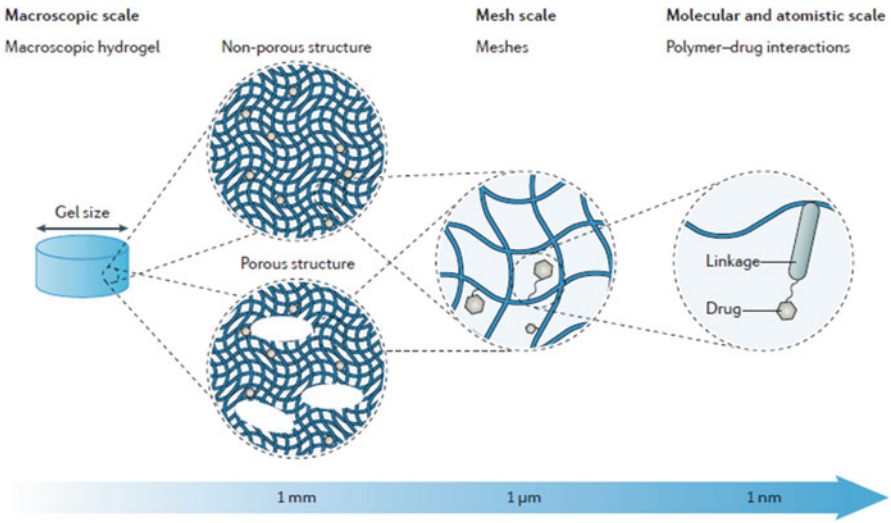


Fig. 8 Representative sketch showing the multiscale structure of a hydrogel for drug delivery application. (Reprinted from Li and Mooney 2016 with permission of Springer Nature Ltd)

Mesh size, which should not be confused with pore size, is another parameter determining the permeability against water or any other solute that is trying to diffuse through the gel matrix. This is especially important in drug release kinetics in sustained drug delivery application with hydrogels such that well-balanced drug encapsulation capacity and drug diffusivity depends on the mesh size (see Fig. 8) (Li and Mooney 2016).

4.3 Functionalization

Polysaccharide-based hydrogels often should be further modified and functionalized according to the special requirements of each different application. For example for wound dressing application in regenerative medicine, the hydrophilic nature of the polysaccharides is not favorable since it is repelled by the hydrophobic cell surface. Incorporation of many cell adhesion sites through the hydrogel network and further modifications to promote the cell proliferation is needed, besides a good mechanical strength and stretchability. On the contrary, for stimuli responsive drug delivery application hydrogel matrix should be degradable as to release its drug content under the influence of external stimuli (Gholamali 2019). This could be achieved by introducing cleavable bonds and groups on to the polysaccharide backbone, such that these bonds and groups break through hydrolysis by physiological enzymes or by the change of pH and temperature. For both procedures if transdermal application is planned, then the hydrogels should be injectable as well, that is, in the form of viscous liquid in vitro and gelation under the skin again by the effect of environmental conditions like pH and temperature (Liu et al. 2018).

There are also numerous publications reporting the functionalization of polysaccharide-based hydrogels with a large variety of inorganic materials like metals, metal-oxides, magnetic nanoparticles, carbon nanofillers (graphene, graphene-oxide, carbon nanotube), and clays (Mergen et al. 2020). These materials are added into the gel matrix either by direct material blending during gelation or enzymatic incorporation, electrospinning, and electropolymerization.

The main purpose of such studies is both to improve the intrinsic properties of hydrogels, that is, mechanical strength, elasticity, swelling capacity, and to design multifunctional composites, in which these improved properties are combined with individual forthcoming functions of the inorganic materials hosted in biocompatible gel matrix (Zheng et al. 2015).

5 Applications of Microbial Polysaccharides

5.1 Environmental Applications

One of the biggest sources of environmental pollution is the heavy metal ions like Cobalt (II), Nickel (II), Zinc (II), Aluminum (III), Chromium (VI), Mercury (II), and Lead (II), which are revealed during many industrial applications as side products and contaminates into the waste water, ground water, and soil. These heavy metal ions are threatening the living bodies' health even in trace amounts, such that clinical reports showed development retardation, kidney damage, various cancers, and death cases for human patients who are continuously exposed to these ions. Among the recent technologies used for the clearance of heavy metal ions, ion-exchange, cation precipitation into inert form, and using of nanomaterials have certain limitations and include contaminants, which themselves indirectly cause pollution. On this respect, removal of heavy metal ions by microbial polysaccharide biosorbents stems as much cheaper and green chemistry approach. Many microorganisms (algae, fungi, bacteria) as part of their defense system are already able to remove the toxic metal ions by biosorption thanks to the EPS on their cell walls. These EPS have hydroxyl, carboxyl, amine, amide, sulfonate, and phosphonate groups that can bind the metal ions through different mechanisms like physical adsorption, ion exchange, complexation, and precipitation (Muthu et al. 2017). As mentioned above, the number of metal-binding sites and hence the affinity to metal ion uptake of EPS systems can be further improved by preparing nanocomposites based on polysaccharide hydrogels functionalized with magnetic nanoparticles, carbon nanotubes, titanium dioxide, zeolites, and clays benefiting the enhanced surface-to-volume ratio and porous structure.

Among microbial polysaccharides chitin, chitosan, cellulose, and alginate are the most commonly studied ones for heavy metal ion removal due to the presence of multiple functional groups on their backbone (Na et al. 2020). For example Gokila et al. synthesized chitosan and alginate nanoparticles for Cr(IV) removal from wastewater and by investigating the adsorption kinetics they showed sorption capacity reaches maximum at pH = 5 (Gokila et al. 2017). In another study, preparation of carboxymethyl chitosan (CMC)/sodium alginate (SA) hydrogel

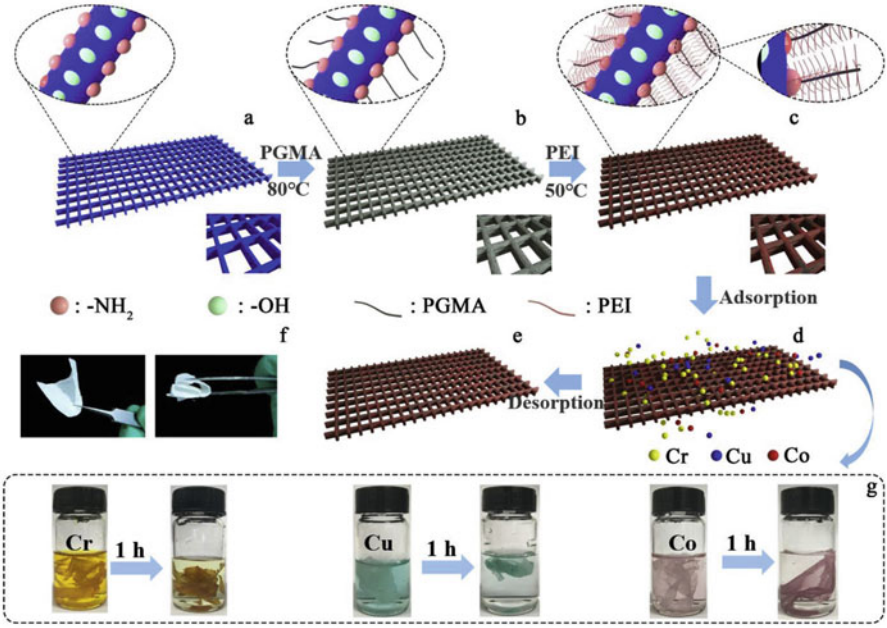


Fig. 9 CS-PGMA-PEI nanofiber membranes for Cr(VI), Cu(II), and Co(II) removal. (Reprinted from Yang et al. 2019 with permission from Elsevier Inc.)

nanocomposites that are functionalized by graphene oxide modified magnetite (Fe_3O_4) nanoparticles was described. It was shown that (CMC/SA/graphene oxide@Fe_3O_4) magnetic beads can successfully remove Cu(II), Cd(II), and Pd (II) ions and have 90% of the adsorption rates even after five cycles (Wu et al. 2019). A kind of different approach for the heavy metal removal was reported by Yang et al., where they prepared poly(glycidyl methacrylate) (PGMA) grafted chitosan electrospun nanofiber membranes, whose surface is further functionalized with polyethyleneimine (PEI) (Yang et al. 2019). They reported high adsorption rates and reusability of these CS-PGMA-PEI membranes for Co(II) and Cr (VI) removal (Fig. 9).

5.2 Biological and Pharmaceutical Applications

Native polysaccharides or its derivatives are known to exhibit many biological functions like antioxidant, antibacterial, antiviral, anticoagulant, antidiabetes, and antitumor activity. Unnecessarily high amount of reactive oxygen species, that is, superoxide anion, hydrogen peroxide, nitric oxide, and hydroxyl radicals, which are generated by cells during reduction of the molecular oxygen to water for energy production, cause oxidative stress, aging, and decrease in immune functions. Microbial EPS have the ability to remove these excess reactive oxygen species and in this

manner said to carry antioxidant and antiaging activities (Yu et al. 2018). EPS were also shown to have hypoglycemic activity by increasing plasma insulin levels and decreasing plasma sugar levels, hence are used as a main component of several antidiabetic herbal medicines. Sulfated EPS, due to their anticoagulant activities, are good alternatives to heparin, the commonly used drug in medicine for preventing the blood coagulation, avoiding its severe side effects like thrombocytopenia and thrombosis syndrome. Regarding the antiviral activity, again mostly the sulfated EPS reported to inhibit the rapid replication of a wide spectrum of enveloped viruses including herpes simplex virus (HSV), human immunodeficiency virus (HIV) and influenza virus (Boission-Vidal et al. 1995). Nevertheless, among the different bioactivities of polysaccharides, maybe the most promising and investigated one is their antitumor/anticancer activity. It was demonstrated that EPS opposes to cancer in more than one way: i) inhibiting the growth of tumor cells and metastasis, ii) triggering the tumor cell apoptosis through different signaling pathways, iii) enhancing the immune functions by activating macrophages, T cells, and natural killer cells, and iv) potentiating the effect of chemotherapy drugs, where these functions were shown to be dependent on many factors like the molecular size, branching degree, type, and sequencing of monosaccharide units and number of sulfate groups. The long list of biological functions of EPS can be further extended, that is, involving hypocholesterolemic-, hypoglycemic-, hypolipidemic-effect, prebiotic activity (Liu et al. 2015).

One of the most promising pharmaceutical application, in which the microbial polysaccharides are effectively used, is drug delivery. Drug delivery concerns with concentrating only the required quantity (dose) of drugs at target-specific locations with minimized side effects on healthy tissues both in spatial and temporal manner. The mechanical and compositional similarity of polysaccharide hydrogels to native extracellular matrix, when combined with advantages of nanoscale, that is, possibility of targeting to the close proximity of diseased tissues and enhanced surface-to-volume ratio promoting much higher amount of drug upload/release, make hydrogel micro/nanoparticulate systems excellent candidates for drug delivery. Such polysaccharide-based micro/nanogels are prepared via emulsion cross-linking, reverse micelle, spray-drying, coacervation/precipitation, and emulsion droplet coalescence methods (Smelcerovic et al. 2008). The drug molecules that are incorporated into the gel matrix either by encapsulation or chemical binding can be released instantly by the influence of external (magnetic field, electric field, irradiation) or internal (pH, temperature, osmolality) stimuli via so-called stimuli responsive controlled drug delivery. As an example, pH-responsive xanthan gum and sodium alginate including hydrogels loaded with hydrophilic drug hydrocortisone sodium succinate (HSS) were site-specifically targeted to colon and used for ulcerative colitis therapy (You et al. 2015). However, in most cases, as similar in cancer therapy with chemotherapy agents, drug release should be realized in a time-controlled sustained process that the body can tolerate the released amount of drug in a certain time interval (sustained drug delivery). In contrast to other class of drug delivery vehicles, which mostly release its total drug content at an instant generally by the decomposition of their drug carrier matrix, polysaccharide-based hydrogels can do this job with a release rate (starting from a few days up to a few weeks) dependent on the diffusivity of active molecules or water through the gel

network. In drug delivery, EPS-based hydrogels were applied in different particulate formulations such as micro/nanoparticles, liposomes, films, and membranes, and applied in almost all types of drug administration ways like oral, nasal, ocular, and transdermal routes (Li and Mooney 2016).

Chitosan, alginate, and dextran are the most frequently studied EPS, where a wide spectrum of drugs including insulin, calcitonin, melatonin, paracetamol, ibuprofen, ampicilin, 5-Fluorouracil as well as proteins and genes were loaded on to the different forms of the hydrogels composed of these EPS (see Fig. 10) (Suner et al. 2020). Gellan due to its gelation tendency in tear fluid is utilized as drug carrier in ocular drug delivery. Despite their non-gelating characteristics in native form, derivatized and functionalized pullulan and hyaluronic acid (HA) are also used as drug delivery systems. Recently, Sahiner et al. synthesized HA-based hydrogel microparticles, which are loaded with several cancer drugs, corticosteroid, and antibiotics by encapsulation and conjugation methods. After performing blood compatibility tests and drug release studies in physiological conditions, they showed that prepared HA-microgels show sustainable and up to 300 hours long-term release for 5-fluorouracil, mitomycin C, doxorubicin, dexamethasone, and ciprofloxacin (Sahiner et al. 2020).

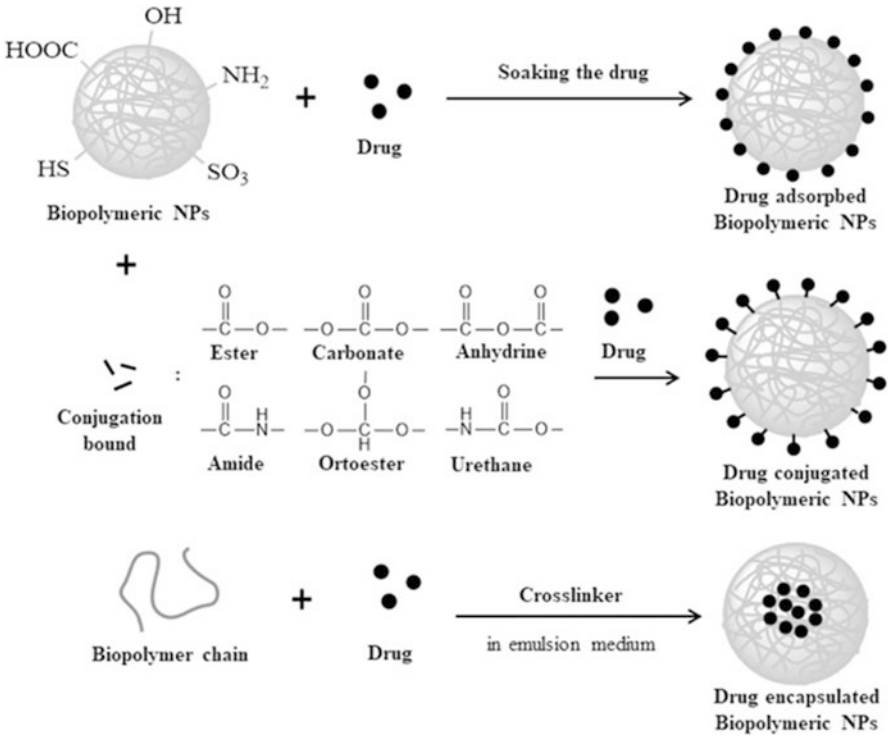


Fig. 10 Synthesis of drug-loaded biopolymer microparticles. (Reprinted from Suner et al. 2020 with permission from Springer Nature Ltd.)

5.3 Tissue Engineering Applications

Actually in some extent being closely related with the field of abovementioned control drug delivery, tissue engineering and regenerative medicine define the replacement or repairing of damaged soft tissues often due to the injuries, burns, or infections. Traditional approaches for the treatment include surgical transplantation of healthy tissues obtained again from patient's own body or from a donor, which is subject to considerable level of risks like tissue rejection and infections (Ng et al. 2020). For this reason, soft biopolymers that will inhibit the infection, trigger the proliferation of a small number of residual healthy cells, and eventually promote the formation of a new tissue on the wound are highly appealing. Microbial EPS have unique advantages due to their biocompatibility, biodegradability, anti-inflammation, and antibacterial activities, and hence can be utilized in tissue engineering applications. There are already some commercial products like chitin-based Beschitin® and oxidized cellulose-based Surgicel®, which are proved to accelerate postoperative wound healing and promote dermal regeneration in addition to anticoagulant activity (Smelcerovic et al. 2008). Owing to their additional porosity and mechanical stability, hydrogels prepared from EPS can be promising materials as tissue mimicking scaffolds. However, scaffolds should adhere on the hydrophobic surface of extracellular matrix containing integrin – a transmembrane protein responsible for cell-cell or cell-extracellular matrix adhesion; therefore the entirely hydrophilic EPS should be further derivatized and functionalized with integrin binding ligands, that is, collagen, laminin, fibronectin, and vitronectin. Such connections in microcellular environment are crucial for the signaling pathways can take place, which are necessary for cell viability, cell differentiation hence a good integration with surrounding tissues. Chitosan-collagen scaffolds were widely employed in cartilage, skin, and stem cell tissue engineering. Recently, injectable stem cell loaded alginate-laminin hydrogel microparticles showing in-situ gelation property were introduced for potential use as scaffolds in breast regeneration after lumpectomy (Yang et al. 2021).

In the last decade parallel to the developments in biomedical technology, namely in three-dimensional (3D) printing of biopolymers, tissue engineering was pushed one step forward such that the first applications of skin replacements in this field are now extended to the fabrication of hydrogel-based scaffolds for bones, cartilage, blood vessels, and even organs. At his point, medical imaging techniques such as computed tomography (CT) and magnetic resonance imaging (MRI) provide high-resolution detailed structures (3D maps) of the bones and tissues that will be printed. Most microbial EPS-based hydrogels suffer from the lack of mechanical strength required for 3D printing. Therefore particle-, fiber-, anisotropic filler-based reinforcements or another type of polymer/hydrogel (interpenetrating networks – IPN) are introduced in the host EPS-based hydrogels in order to tune the mechanical and rheological properties as to suit for printing purposes (Jang et al. 2018). Among different EPS chitosan, alginate, gelatin, hyaluronic acid based hydrogels, which are reinforced by the addition of nanocellulose, gellan gum, PEG, PLA, collagen, fibrinogen, hydroxyapatite, and laponite were frequently used as scaffold materials.

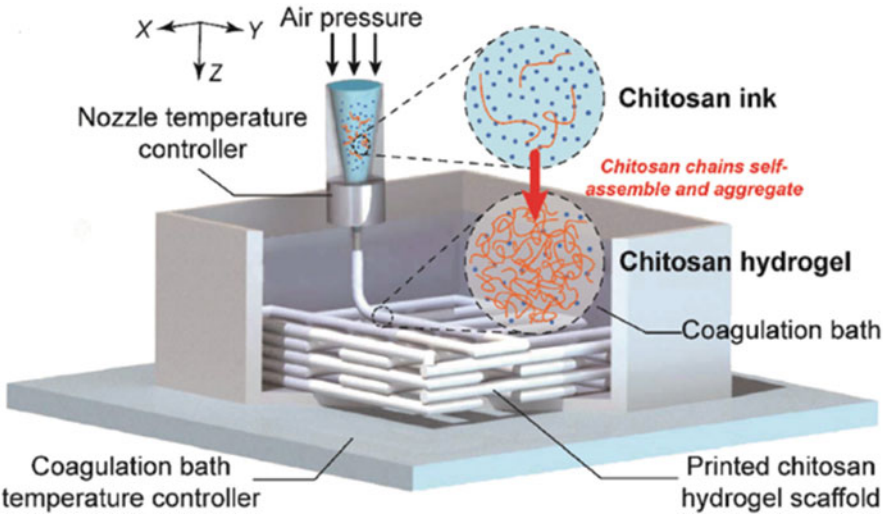


Fig. 11 Three-dimensional printing of chitosan hydrogel-based scaffolds. (Reproduced from Zhou et al. 2020 with the permission of Royal Society of Chemistry)

These composite polymers, mostly in viscous solution form, are layer-by-layer deposited according to the 3D model using laser-based, nozzle-based, or inkjet printer-based 3D printing methods (see Fig. 11) (Zhou et al. 2020).

5.4 Food Applications

Food quality and safety is one of the most important aspects regarding the public health. Chemical ingredients used in the food products should meet the allowed limitations put by health authorities such as the FDA (US Food and Drug Administration). On this respect, naturally occurring polysaccharides that are inherently biocompatible and biodegradable have crucial importance in healthy and additive-free food production. Lots of attention are also focused on the taste, flavor, and mouthfeel of the food products, which are closely related with the texture and viscosity of these highly complex mixtures including proteins, lipids, fat acids, vitamins, minerals, sugar, and of course water. Another concern of the food industry is also to design food with unique textures to fulfill the requirements of some special groups of consumers, who have difficulty consuming normal food, that is, gluten-free, porcine-free, bovine-free, dietary products. Microbial polysaccharides are tasteless, odorless, and water soluble, hence can change the texture of the products by manipulating its rheological properties without interfering the original taste. Most of the foods exist in emulsion form, that is, composed of two or more nonmiscible components like oil-water, hence depending on the type and fraction of these components they can have very different viscosities from fluids (milk, fruit juice) to creamy (sauce, salad dressing, mayonnaise) and elastic materials (butter, cheese) (Yang et al. 2020). Here

the low viscosity products suffer from time-dependent precipitation or separation of different phases and microbial EPS are used as emulsion stabilizers (emulsifiers), which ensure the preservation of the emulsion during shelf life. Dextran, alginate, and emulsan are the most commonly used emulsifiers that can significant increase the viscosity of added product even at very low concentrations (0.1%–1%). Gelation is another forthcoming characteristic of EPS in terms of applicability to food products. Thermo-reversible gelation is often required in the preparation of confectionary products, jellies, and jams. As gelling agent gelatin, which can be obtained from bovine and pork, is the most commonly used polysaccharide in the food market, but it is not preferred by vegetarian or Islamic consumers. For this reason gellan, xanthan, carrageenan, and agar are used as alternative gelling agents in food industry (Jindal and Khattar 2018).

Recently, frozen foods are even more popular among consumers, so that it is practical and almost all types of products (meat, fish, vegetables, bakery, etc.) can be stored as frozen food. However, melting of the ice crystals present in frozen food matrix results in the loss of texture and softening. One way to overcome this quality retrogression is to reduce the amount of freezable water, namely the free water fraction in the food matrix. Due to their highly hydrophilic nature and water binding capacity, microbial EPS can do this job serving as water-binding agents and preserve the moisture in the food matrix during many freeze-thaw cycles. Dextran and curdlan were often employed as ice inhibitors in different kinds of frozen food products. The last but not least application of microbial EPS in food industry is the edible coatings on foods. This application is mostly based on the biofilm formation capability of some derivatized EPS and oriented to increase the shelf life of products. One typical example is the sausage casings made of cellulose or collagen, which are used in other protective coatings as well. Edible coatings are also used on some confectionary products both for protecting and decoration purposes.

Finally, as a recent trend in food industry one should also mention the utilization of EPS-based hydrogel microparticles in fat replacement in dietary products, regulation of gastrointestinal functions, and satiety control. In this relatively new field, as similar to the case in drug delivery application different nutrients, reduced fat or prebiotics are encapsulated on microgels, which then release its content on target sites in digestive tract through swelling-deswelling (shrinking) mechanisms. The use of such colloidal microgel suspensions in food products was shown to increase the mouth-feel and satiety, while preserving the necessary sensitive and volatile ingredients in the product (Shewan and Stokes 2013).

6 Concluding Remarks and Future Perspectives

As discussed throughout the chapter, owing to their biocompatible, hydrophilic, and gellation properties together with suitability for further functionalization, microbial polysaccharides always have been popular in soft matter science. Especially in last two decades, developments in cutting-edge-technology characterization methods, such as small angle x-ray scattering (SAXS), x-ray crystallography, cryogenic

electron microscopy (cryo-EM), and magic-angle spinning (MAS) NMR spectroscopy, allowed scientists to reveal the structure-function relationship in polysaccharides. Meanwhile, by the introduction of novel chemical approaches polysaccharide-based nanocomposites utilizing the size advantageous and enhanced surface-to-volume ratio in nanoscale was synthesized and replaced their bulk counterparts in biomedical, pharmaceutical, cosmetic, and food applications. Nevertheless, there are still remaining challenges in the commercialization of polysaccharides mostly related with the production costs. Commercial microbial polysaccharides are produced through fermentation by heterotrophic bacteria, which unfortunately require expensive organic substrates. Limited geographic availability of natural sources, nutrients, difficulties in cultivation, and downstream processing are other drawbacks in front of high purity large amount production of polysaccharides. At this point, cyanobacteria, which obtain energy via photosynthesis and able to grow also on inorganic media, are cheaper alternative sources of polysaccharides. Cyanobacterial polysaccharides have no noteworthy differences in monosaccharide composition found in EPS extracted from fungi, plants, and animals. Although their production amount is lesser than other bacteria and fungi, they show diverse functionalities which are similar to other microbial polysaccharides, and hence have the potential to substitute them in industrial applications (Bhatnagar and Bhatnagar 2019).

References

Ahmad NH, Mustafa S, Man YBC. Microbial polysaccharides and their modification approaches: a review. Int J Food Prop. 2015;18(2):332–47.

Bhatnagar M, Bhatnagar A. Diversity of polysaccharides in Cyanobacteria. In: Satyanarayana T, et al., editors. Microbial diversity in ecosystem sustainability and biotechnological applications. Singapore: Springer Nature; 2019. p. 447–96.

Blakemore WR. Polysaccharide ingredients: carrageenan. In: Reference module in food sciences. Amsterdam: Elsevier; 2016.

Boission-Vidal C, et al. Biological activities of polysaccharides from marine algae. Drugs Future. 1995;20(12):1237–49.

Chang WS, et al. Alginate production by Pseudomonas putida creates a hydrated microenvironment and contributes to biofilm architecture and stress tolerance under water-limiting conditions. J Bacteriol. 2007;189:8290–9.

Coviello T, Matricardi P, Marianecci C, Alhaique F. Polysaccharide hydrogels for modified release formulations. J Control Release. 2007;119:5–24.

Czaja W, Krystynowicz A, Bielecki S, Brown RM. Microbial cellulose—the natural power to heal wounds. Biomaterials. 2006;27:145–51.

Dave PN, Gor A. Natural polysaccharide-based hydrogels and nanomaterials: recent trends and their applications. In: Hussain CM, editor. Handbook of nanomaterials for industry applications. Amsterdam: Elsevier; 2018. p. 36–66.

David S, et al. Revisiting the carrageenan controversy: do we really understand the digestive fate and safety of carrageenan in our foods? Food Funct. 2018;9:1344–52.

Ficko-Blean E, et al. Carrageenan catabolism is encoded by a complex regulon in marine heterotrophic bacteria. Nat Commun. 2017;8:1685.

Gholamali I. Stimuli-responsive polysaccharide hydrogels for biomedical applications: a review. Regen Eng Transl Med. 2019;7:91–114.

Gokila S, et al. Removal of the heavy metal ion chromiuim(VI) using chitosan and alginate nanocomposites. Int J Biol Macromol. 2017;104:1459–68.
Gull N, et al. Modification of polyaniline. In: Visakh PM, Pina CD, Falletta E, editors. Polyaniline blends, composites, and nanocomposites. Amsterdam: Elsevier; 2018. p. 61–93.
Hadrich A, et al. Biomimetic hydrogel by enzymatic crosslinking of pullulan grafted with ferulic acid. Carbohydr Polym. 2020;250:116967.
Harding SE. Analysis of polysaccharides by ultracentrifugation, size, conformation and interactions in solutions. Adv Polym Sci. 2005;186:211–54.
Hu H, Xu F-J. Rational design and latest advances of polysaccharide-based hydrogels for wound healing. Biometar Sci. 2020;8:2084.
Hu Y, et al. Dual-crosslinked amorphous polysaccharide hydrogels based on chitosan/alginate for wound healing applications. Macromol Rapid Commun. 2018;39(20):1800069.
Hunter KW, Gault RA, Berner MD. Preparation of microparticulate β-glucan from *Saccharomyces cerevisiae* for use in immune potentiation. Lett Appl Microbiol. 2002;35:267–71.
Jang T-S, Jung HD, Pan HW, et al. 3D printing of hydrogel composite systems: recent advances in technology for tissue engineering. Int J Bioprinting. 2018;4(1):126.
Jiang L. Effect of nitrogen source on curdlan production by Alcaligenes faecalis ATCC 31749. Int J Biol Macromol. 2013;52:218–20.
Jindal N, Khattar JS. Microbial polysaccharides in food industry. In: Grumezescu AM, Holban AM, editors. Biopolymers for food design. Amsterdam: Elsevier; 2018. p. 95–123.
Kothari D, Das D, Patel S, Goyal A. Dextran and food application. In: Ramawat KG, Merillon J-M, editors. Polysaccharides, vol. 2014. Cham: Springer; 2014. p. 1–16.
Li J, Mooney DJ. Designing hydrogels for controlled drug delivery. Nat Rev Mater. 2016;1:16071.
Liu WG, Yao KG, Liu QG. Formation of a DNA/*N*-dodecylated chitosan complex and salt-induced gene delivery. J Appl Polym Sci. 2001;82:3391–5.
Liu J, Willför S, Xu C. A review of bioactive plant polysaccharides: biological activities, functionalization, and biomedical applications. Bioact Carbohydr Diet Fibre. 2015;5:31–61.
Liu H, et al. Self-healing and injectable polysaccharide hydrogels with tunable mechanical properties. Cellulose. 2018;25:559–71.
Mager DM, Thomas AD. Extracellular polysaccharides from cyanobacterial soil crusts: a review of their role in dryland soil processes. J Arid Environ. 2011;75:91–7.
Marszalek PE, Oberhauser AF, Pang Y-P, Fernandez JM. Polysaccharide elasticity governed by chair–boat transitions of the glucopyranose ring. Nature. 1998;396:661–4.
McIntosh M, Stone BA, Stanisich VA. Curdlan and other bacterial (1→3)-β-D-glucans. Appl Microbiol Biotechnol. 2005;68:163–73.
Mergen ÖB, Umut E, Arda E, Kara S. A comparative study on the AC/DC conductivity, dielectric and optical properties of polystyrene/graphene nanoplatelets (PS/GNP) and multi-walled carbon nanotube (PS/MWCNT) nanocomposites. Polym Test. 2020;90:106682.
Mourya VK, Inamdar NN. Trimethyl chitosan and its applications in drug delivery. J Mater Sci Mater Med. 2009;20:1057–79.
Muthu M, et al. Exploiting microbial polysaccharides for biosorption of trace elements in aqueous environments—scope for expansion via nanomaterial intervention. Polymers. 2017;9:721.
Na Y, et al. Removal of heavy metals by polysaccharide: a review. Polym-Plast Technol Mat. 2020;59(16):1770–90.
Necas J, Bartosikova L, Brauner P, Kolar J. Hyaluronic acid (hyaluronan): a review. Veterinari Medicina. 2008;53(8):397–411.
Neyts J, et al. Differential antiviral activity of derivatized dextrans. Biochem Pharmacol. 1995;50: 743–51.
Ng JY, et al. Biomimicry of microbial polysaccharide hydrogels for tissue engineering and regenerative medicine – a review. Carbohydr Polym. 2020;241:116345.
Niknezhad NV, et al. Production of xanthan gum by free and immobilized cells of Xanthomonas campestris and Xanthomonas pelargonii. Int J Biol Macromol. 2016;82:751–6.

Oner ET. Microbial production of extracellular polysaccharides from biomass. In: Fang Z, editor. Pretreatment techniques for biofuels and biorefineries. Berlin: Springer; 2013. p. 35–56.
Oner ET, Hernandez L, Combie J. Review of Levan polysaccharide: from a century of past experiences to future prospects. Biotechnol Adv. 2016;34(5):827–44.
Patil S, Sharma S, Nimbalkar A, Pawar A. Study of formulation variables on properties of drug-gellan beads by factorial design. Drug Dev Ind Pharm. 2006;32:315–26.
Rajinikanth PS, Mishra B. Floating in situ gelling system for stomach site-specific delivery of clarithromycin to eradicate *H. pylori*. J Control Release. 2008;125:33–41.
Rajoka MSR, et al. Chitin/chitosan derivatives and their interactions with microorganisms: a comprehensive review and future perspectives. Crit Rev Biotechnol. 2020;40(3):365–79.
Reddy T, Tammishetti S. Gastric resistant microbeads of metal ion cross-linked carboxymethyl guar gum for oral drug delivery. J Microencapsul. 2002;19(3):311–8.
Ruka DR, Simon GP, Dean KM. Altering the growth conditions of Gluconacetobacter xylinus to maximize the yield of bacterial cellulose. Carbohydr Polym. 2012;89:613–22.
Sahiner N. Soft and flexible hydrogel templates of different sizes and various functionalities for metal nanoparticle preparation and their use in catalysis. Prog Polym Sci. 2013;38(9):1329–56.
Sahiner N, et al. HA particles as resourceful cancer, steroidal and antibiotic drug delivery device with sustainable and multiple drug release capability. J Macromol Sci Part A. 2020; https://doi.org/10.1080/10601325.2020.1832518.
Sarilmiser HK. Effective stimulating factors for microbial Levan production by Halomonas smyrnensis AAD6T. J Biosci Bioeng. 2015;119(4):455–63.
Schilling C, Badri A, Sieber V, Koffas M, Schmid J. Metabolic engineering for production of functional polysaccharides. Curr Opin Biotechnol. 2020;66:44–51.
Seton-Rogers S. Multitasking hyaluronic acid. Nat Rev Cancer. 2012;12:228.
Shanmugam M, Abirami RG. Microbial polysaccharides -chemistry and applications. J Biol Act Prod Nat. 2019;9(1):73–8.
Shariatinia Z. Carboxymethyl chitosan: properties and biomedical applications. Int J Biol Macromol. 2018;120:1406–19.
Shewan HM, Stokes JR. Review of techniques to manufacture micro-hydrogel particles for the food industry and their applications. J Food Eng. 2013;119:781–92.
Siddiqui NN, et al. Structural analysis and characterization of dextran produced by wild and mutant strains of Leuconostoc mesenteroides. Carbohydr Polym. 2014;99:331–8.
Smelcerovic A, Knezevic-Jugovic Z, Petronijevic Z. Microbial polysaccharides and their derivatives as current and prospective pharmaceuticals. Curr Pharm Des. 2008;14:3168–95.
Suner SS, et al. Tunable biopolymeric drug carrier Nanovehicles and their safety. In: Das MK, Pathak YV, editors. Nano medicine and Nano safety. Singapore: Springer; 2020. p. 405–32.
Trovatti E. The future of bacterial cellulose and other microbial polysaccharides. J Renew Mater. 2012;1:28–41.
Urtuvia V, et al. Bacterial alginate production: an overview of its biosynthesis and potential industrial production. World J Microbiol Biotechnol. 2017;33:198.
Venugopal V. Polysaccharides: their characteristics and marine sources. Marine polysaccharides food applications. Boca Raton: CRC Press; 2011. p. 3–27.
Viegas de Souza RHF, et al. Diethylaminoethyl- chitosan as an efficient carrier for siRNA delivery: improving the condensation process and the nanoparticles properties. Int J Biol Macromol. 2018;119:186–97.
Volokhova AS, Edgar KJ, Matson JB. Polysaccharide-containing block copolymers: synthesis and applications. Mater Chem Front. 2020;4:99–112.
Wu Z, et al. Novel magnetic polysaccharide/graphene oxide@Fe_3O_4 gel beads for adsorbing heavy metal ions. Carbohydr Polym. 2019;216:119–28.
Wustenberg T. General overview of food hydrocolloids. Cellulose and cellulose derivatives in the food industry: fundamentals and applications. Weinheim: Wiley-VCH; 2014. p. 1–68.

Xiao C, Gao Y. Preparation and properties of physically crosslinked sodium carboxymethylcellulose/poly(vinyl alcohol) complex hydrogels. J Appl Polym Sci. 2008;107:1568–72.
Yang D, et al. Functionalized chitosan electrospun nanofiber membranes for heavy-metal removal. Polymer. 2019;163:74–85.
Yang X, et al. An overview of classifications, properties of food polysaccharides and their links to applications in improving food textures. Trends Food Sci Technol. 2020;102:1–15.
Yang I-H, et al. The development of laminin-alginate microspheres encapsulated with Ginsenoside Rg1 and ADSCs for breast reconstruction after lumpectomy. Bioact Mater. 2021;6(6): 1699–710.
You, YC et al. In vitro and in vivo application of pH-sensitive colon-targeting polysaccharide hydrogel used for ulcerative colitis therapy. Carbohydr Polym. 2015;130:243–253.
Yu Y, et al. Biological activities and pharmaceutical applications of polysaccharide from natural resources: a review. Carbohydr Polym. 2018;183:91–101.
Zhang J, et al. Optimization of culture medium compositions for gellan gum production by a halobacterium Sphingomonas paucimobilis. Carbohydr Polym. 2015;115:694–700.
Zheng Y, Monty J, Linhardt RJ. Polysaccharide-based nanocomposites and their applications. Carbohydr Polym. 2015;405:23–32.
Zhou L, et al. 3D printing of high-strength chitosan hydrogel scaffolds without any organic solvents. Biomater Sci. 2020;8:5020–8.

Antioxidant and Antibacterial Activities of Polysaccharides

25

S. Chandra Mohan and Anand Thirupathi

Contents

Abstract

Polysaccharide is a high molecular weight polymer, consisting of at least ten monosaccharides mutually joined by glycosidic linkages. Polysaccharides, present in almost all organisms, are important functional biological macromolecules because of their significant benefit to human health such as antioxidant, antidiabetic, immune potentiation, antitumor, anti-inflammatory, and hypoglycemic activities. More and more studies have shown evidence that polysaccharides have the capacity of scavenging free radicals and may be potential natural antioxidants. The antioxidant activity was found to be determined by multiple factors, including molecular weight, monosaccharide composition, sulfate position and its degree. To address this issue, this chapter summarizes the latest discoveries and advancements in the study of sources, chemical composition, structural characteristics, antimicrobial and antioxidant capacity of polysaccharides, and gives a detailed description of the possible mechanisms.

S. C. Mohan (✉)
Division of Phytochemistry, Shanmuga Centre for Medicinal Plants Research, Thanjavur, Tamil Nadu, India

A. Thirupathi
Faculty of Sports Science, Ningbo University, Ningbo, China

J. M. Oliveira et al. (eds.), *Polysaccharides of Microbial Origin*,
https://doi.org/10.1007/978-3-030-42215-8_32

Keywords

Antioxidant activity · Polysaccharides · Chemical composition · Structural characteristics · Antibacterial activity · Mechanism · Factors

1 Introduction

Polysaccharides are polymeric carbohydrates, formed by repeating units joined together by glycosidic bonds. They have widely been investigated due to their chemical and biological activities (Rubavathi and Ramya 2016). Polysaccharides are also widely used as emulsifiers, gelling agents, thickeners, and fat replacers in functional food, cosmetics industries, and biological medicine, including drug delivery and tissue engineering (Bais et al. 2005). Polysaccharides present a large diversity of structures attributable to their variety in composition, substitutions, and glycosidic bonds. Polysaccharides widely distributed in plants, animals, fungi, yeasts, and algae have attracted considerable attention for their biological activities. Marine algae macromolecules include sulfated polysaccharides such as carrageenan and agar from red marine algae; alginate, fucan, and laminarin from brown marine algae; and cellulose and ulvan from green marine algae (Shannon and Ghannam 2016). Polysaccharides have proved to be potential sources of natural antibacterial, antioxidants, immunomodulatory, antitumor, hepato-cardioprotective, and neuroprotective compounds (Xie et al. 2016; Wei et al. 2015; Rjeibi et al. 2019). They have been increasingly applied because they are sourced naturally, and they impart less toxicity, biodegradability, and fewer side effects than synthetic ones. Antioxidant activity, a common bioactivity of natural-derived polysaccharides, was widely investigated for the prevention and treatment of diseases associated with oxidation damage (Wang et al. 2016). It is widely believed that the antioxidant activity of natural polysaccharides was influenced by raw materials, extraction procedures, and drying methods (Zhu et al. 2012; Zhao et al. 2015). The chemical mechanisms behind polysaccharide antioxidant activity have not systematically been elucidated to date. However, as literature suggest, the higher degree of sulfation in polysaccharides might be attributed to the observed antioxidant activity (Ananthi et al. 2010). Polyphenols bound to polysaccharides might also contribute to the antioxidant activity. Further investigation is needed to separate and further purify these polysaccharides and to identify their sequence, monosaccharide composition, and other structural properties. This chapter aims to review the antioxidative polysaccharides and summarize the possible mechanisms.

2 Types of In Vitro Antioxidant Activity

Many different in vitro models have been introduced to evaluate the antioxidant activities. The hydrogen atom transfer (HAT)-based methods usually measure the ability of quench free radical by hydrogen donation, that is, oxygen radical

absorbance capacity (ORAC), total radical-trapping antioxidant parameter (TRAP), inhibition of induced low-density lipoprotein (LDL) oxidation, total oxyradical scavenging capacity assay, and so forth. On the other hand, single electron transfer (SET)-based methods detect the ability of transferring one electron to reduce any compound, including metals, carbonyls, and radicals, and result in a change in color when this compound is reduced, such as ferric ion reducing antioxidant power (FRAP) assay, and 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) scavenging. Other assays, for example, superoxide radical scavenging, hydrogen peroxide scavenging, and singlet oxygen quenching, evaluate the scavenging ability for oxidants (Wang et al. 2016).

3 Antioxidant Activity of Polysaccharides

According to the extensive in vitro antioxidant studies, the polysaccharide is indeed an effective antioxidant. The underlying mechanism is, however, uncertain as the relationships between antioxidant activity and physicochemical properties or structural features are not comprehensively elucidated and confirmed. Besides, it is worth noting that conflicting results are observed in comparison with a number of literatures, since different sources, extraction methods, and even drying procedures can influence the evaluated polysaccharides. On the other hand, limited information is available on antioxidant activity of high-purity polysaccharides, and therefore other antioxidant substances, for instance, protein, peptide, and polyphenol, that may always be retained in polysaccharides in a form of either conjugation or mixture should be taken into account. Overall, the antioxidant potentials of polysaccharides are not determined by a single factor but a combination of several related factors. Numerous antioxidative polysaccharides have been discovered from plants, fungi, bacteria, and algae which are shown in Table 1.

4 Mechanism and Factors Affecting the Antioxidant Activity of Polysaccharide

Polysaccharides and polysaccharide-complex extracted from many natural sources. However, despite the great antioxidant potentials of polysaccharides exerted, their underlying mechanism is not systematically elucidated. Limited information is available on antioxidant activity of high-purity polysaccharides, and therefore other antioxidant substances, for instance, protein, peptide, and polyphenol, that may always be retained in polysaccharides in a form of either conjugation or mixture should be taken into account. As a result, the following sections summarize the current understanding of possible antioxidant mechanisms of polysaccharides.

Structural Characteristics of Polysaccharide: It is widely believed that the bioactivity of polysaccharides is affected by their structure characteristics, such as chemical composition, molecular mass, types of glycosidic linkage, and conformation. Differences in origin materials, extraction procedures, and even drying

Table 1 Polysaccharides from various sources and their antioxidant activity

Source	Name	Polysaccharides	Antioxidant activity	Ref.
Brown Algae	*Chnoospora minima*	Crude polysaccharides	DPPH (IC_{50} = 3.22 g/mL); hydroxyl (IC_{50} = 48.35 g/mL)	Fernando et al. 2017
	Costaria costata	Fucoidan	Hydroxyl (63.3% at 10.0 mg/mL)	Wang et al. 2014b
	Dictyota ciliolata	Fucoidan fraction	DPPH (27% at 2.0 mg/mL)	Chale-Dzul et al. 2017
	Ecklonia cava	Fucoidan	DPPH (IC_{50} = 0.73 mg/mL); peroxyl (IC50 = 0.48 mg/mL)	Kim et al. 2014
	Laminaria japonica	Pigments free fucoidans	DPPH (IC_{50} = 4.64 mg/mL)	Zhao et al. 2018
	Lobophora variegate	Fucoidan	DPPH (36.3% at 10 mg/mL)	Castro et al. 2015; Castro et al. 2016
	Nemacystus decipients	Fucoidan	DPPH (IC_{50} = 3.96 mg/mL); hydroxyl (IC_{50} = 4.12 mg/mL)	Li et al. 2017
	Padinasanctae cruces	Fucoidan	DPPH (22% at 2.0 mg/mL)	Chale-Dzul et al. 2017
	Sargassum cinereum	Fucoidan	DPPH (51.99% at 80 μg/mL)	Somasundaram et al. 2016
	Sargassum fluitans	Fucoidan	DPPH (14% at 2.0 mg/mL)	Chale-Dzul et al. 2017
	Sargassum polycystum	Fucoidan contains the monosaccharide composition such as fucose, galactose, mannose, and xylose	DPPH (61.22% at 1.0 mg/mL); reducing (67.56% at 1.0 mg/mL); TAC (65.30% at 1.0 mg/mL)	Palanisamy et al. 2018
	Turbinaria conoides	Fucoidan	DPPH (IC_{50} = 534.45 μg/mL); ABTS (IC50 = 323.8 μg/mL)	Delma et al. 2015
	Turbinaria ornata	Crude sulfated polysaccharides (CSP)	ABTS (IC_{50} = 88.71 ± 1.01 μg/mL); DPPH (IC_{50} = 440.07 ± 4.43 μg/mL); superoxide (IC_{50} = 352 ± 4.58 μg/mL)	Guru et al. 2015

Red algae	*Gracilaria corticata* var. *ramalinoides*	Crude polysaccharides	DPPH (>2,000 μg/mL); alkyl (367.43 ± 1.74 μg/mL); hydroxyl (654.13 ± 9.14 μg/mL)	Fernando et al. 2017
	Gracilaria foliifera	Crude polysaccharides	DPPH (1,654 ± 37.46 μg/mL); alkyl (382.55 ± 1.23 μg/mL); hydroxyl (582.47 ± 9.29 μg/mL)	Fernando et al. 2017
	Ahnfeltiopsis pygmaea	Crude polysaccharides	DPPH (>2,000 μg/mL); alkyl (377.24 ± 6.10 μg/mL); hydroxyl (768.92 ± 8.10 μg/mL)	Fernando et al. 2017
	Gracilaria corticata	Crude polysaccharides	DPPH (603.38 ± 40.3 μg/mL); alkyl (332.33 ± 15.29 μg/mL); hydroxyl (287.63 ± 13.68 μg/mL)	Fernando et al. 2017
	Jania adhaerens	Crude polysaccharides	DPPH (>2,000 μg/mL); alkyl (114.59 ± 5.01 μg/mL); hydroxyl (281.70 ± 4.96 μg/mL)	Fernando et al. 2017
	Gracilaria edulis	Crude polysaccharides	DPPH (>2,000 μg/mL); alkyl (113.09 ± 7.13 μg/mL); hydroxyl (602.95 ± 12.26 μg/mL)	Fernando et al. 2017
	Gloiopeltis furcata	Sulfated polysaccharide	Superoxide (64.37% at 90 μg/mL); DPPH (23.49% at 0.1 mg/mL)	Shao et al. 2013
	Sarcodia ceylonensis	The four seaweed polysaccharides belong to β-type polysaccharides with pyranose groups, and have uronic acids	Hydroxyl (83.33% at 4 mg/mL); ABTS (IC50 = 3.99 mg/mL)	He et al. 2016
	Solieria filiformis	Sulfated polysaccharides, main constituent of the SFP is *iota-carrageenan*	DPPH (88.93% at 4.0 mg/mL); ABTS (IC50 = 2.01 mg/mL)	Sousa et al. 2016
Green algae	*Chaetomorpha antennina*	Crude polysaccharides	DPPH (>2,000 μg/mL); alkyl (278.18 ± 0.75 μg/mL); hydroxyl (102.68 ± 16.00 μg/mL)	Fernando et al. 2017
	Halimeda discoidea	Crude polysaccharides	DPPH (>2,000 μg/mL); alkyl (110.06 ± 2.98 μg/mL); hydroxyl (1008.65 ± 8.19 μg/mL)	Fernando et al. 2017

(continued)

Table 1 (continued)

Source	Name	Polysaccharides	Antioxidant activity	Ref.
	Halimeda gracilis	Crude polysaccharides	DPPH (>2,000 μg/mL); alkyl (116.60 ± 2.59 μg/mL); hydroxyl (1,006.90 ± 6.40 μg/mL)	Fernando et al. 2017
	Caulerpa racemosa var. *racemosa f. Remota*	Crude polysaccharides	DPPH (>2,000 μg/mL); alkyl (359.48 ± 20.54 μg/mL); hydroxyl (200.08 ± 8.17 μg/mL)	Fernando et al. 2017
	Ulva fasciata	Sulfated polysaccharide	Superoxide (81.45% at 90 μg/mL); DPPH (37.63% at 0.1 mg/mL)	Shao et al., 2013
	Ulva intestinalis	Sulfated polysaccharide	DPPH (the highest 82.23%)	Peasura et al. 2016
Micro algae	*Odontellaaurita* K-1251	Chrysolaminarin, a glucan	DPPH (42.45% at 0.1 mg/mL); hydroxyl (83.54% at 10 mg/mL)	Xia et al. 2014
	Graesiella sp.	Hetero-sulfated-anionic polysaccharides	Hydroxyl (IC_{50} = 0.87 mg/mL); ferrous ion-chelating (IC_{50} = 0.33 mg/mL)	Trabelsi et al. 2016
	Isochrysis galbana	β-type heteropolysaccharide with a pyran group	Superoxide (53.5% at 3.2 mg/mL)	Sun et al., 2014a
	Pavlova viridis	Water-soluble polysaccharides which had β-pyranose and α-pyranose configurations	DPPH (IC_{50} = 0.77 mg/mL); hydroxyl (IC50 = 0.70 mg/mL)	Sun et al. 2014b
	Sarcinochrysis marina Geitler	Hetero-sulfated-anionic polysaccharides	DPPH (IC_{50} = 0.91 mg/mL); hydroxyl (IC_{50} = 0.91 mg/mL)	Trabelsi et al. 2016
Fungi	*Alternaria* sp. SP-32	A novel homogeneous extracellular polysaccharide	DPPH (EC_{50} = 3.4 mg/mL); hydroxyl (EC_{50} = 4.2 mg/mL)	Chen et al. 2016
	Aspergillus terreus	An extracellular polysaccharide with a novel branched galactomannan	Hydroxyl (EC_{50} = 2.8 mg/mL)	Wang et al. 2013a
	Aspergillus versicolor N(2) bC	Exopolysaccharide, the main chain consists of glucopyranose and mannopyranose units	Superoxide (EC_{50} = 2.20 mg/mL); DPPH (EC_{50} = 0.97 mg/mL)	Yan et al. 2016

	Fusarium oxysporum	Novel extracellular polysaccharide, the structure contains a backbone of (1 → 6)-linked β-D-galactofuranose residues with multiple side chains	Hydroxyl (EC_{50} = 1.1 mg/mL); superoxide (EC_{50} = 2.0 mg/mL); DPPH (EC_{50} = 2.1 mg/mL)	Chen et al. 2015
	Streptomyces violaceus MM72	An extracellular polysaccharide	DPPH (IC_{50} = 76.38 mg/mL); superoxide (IC_{50} = 67.85 mg/mL)	Manivasagan et al. 2013
Bacteria	*Aerococcus uriaeequi*	Extracellular polysaccharide, mainly composed of glucose and mannose with β-configurations	Hydroxyl (45.65% at 100 μg/mL); superoxide (67.31% at 250 μg/mL)	Wang et al. 2018
	Alteromonas sp. PRIM-21	Sulfated polysaccharides	DPPH (IC_{50} = 0.61 mg/mL); superoxide (IC_{50} = 0.65 mg/mL)	Priyanka et al. 2015
	Bacillus amyloliquefaciens 3MS 2017	Acidic exopolysaccharide contained uronic acid (12.3%) and sulfate (22.8%) with constitution of glucose, galactose, and glucuronic acid	DPPH (IC_{50} = 0.21 μg/mL); H_2O_2 (IC_{50} = 30.04 μg/mL); superoxide (IC_{50} = 35.28 μg/mL)	El-Newary et al. 2017
	Bacillus thuringiensis	Extracellular polysaccharide. Fructose and galactose were found as the major monosaccharides in the EPS	DPPH (79% at 1.0 mg/mL); superoxide (75.12% at 1.0 mg/mL)	Sathishkumar et al. 2018
	Enterobacter sp. PRIM-26	Exopolysaccharide; galactose and glucose were found as main monosaccharides	DPPH (IC_{50} = 0.44 mg/mL); superoxide (IC_{50} = 0.33 mg/mL)	Priyanka et al. 2015
	Halolactibacillus miurensis	Exopolysaccharide; galactose and glucose were found as main monosaccharides	DPPH (84% at 10 mg/mL); superoxide (89.15% at 0.5 mg/mL); hydroxyl (61% at 3.2 mg/mL)	Arun et al. 2017
	Haloterrigena turkmenica	Sulfated heteropolysaccharide containing glucose, galactose, glucosamine, galactosamine, and glucuronic acid	DPPH (IC_{50} = 6.03 mg/mL)	Squillaci et al. 2015
	Labrenzia sp. PRIM-30	Sulfated polysaccharide	DPPH (IC_{50} = 0.64 mg/mL); superoxide (IC_{50} = 0.19 mg/mL)	Priyanka et al. 2015
	Nitratireductor sp. PRIM-24	Sulfated polysaccharide	DPPH (IC_{50} = 0.49 mg/mL); superoxide (not scavenging activity)	Priyanka et al. 2015

(continued)

Table 1 (continued)

Source	Name	Polysaccharides	Antioxidant activity	Ref.
	Polaribacter sp. SM1127	Exopolysaccharides and it mainly comprises N-acetyl glucosamine, mannose, and glucuronic acid residues bound by heterogeneous linkages	DPPH (55.40% at 10 mg/mL); hydroxyl (52.1% at 10 mg/mL)	Sun et al. 2015
Plants	Leaves of *Arctium lappa* var. *herkules*,*Aloe barbadensis*, *Althaea officinalis* var. *robusta*, *Plantago lanceolata* var. *libor*, aerial parts and roots of *Rudbeckia fulgida* var. *sullivantii*, stems of *Mahonia aquifolium*, and peach-tree (*Prunus persica*) gum exudates	Eleven polysaccharides have been isolated	The polysaccharides were investigated for their ability to inhibit peroxidation of soyabean lecithin liposomes by OH radicals. The highest inhibition was found with glucuronoxylans of *A. officinalis* var. *robusta* and *P. lanceolata* var. *libor*, aerial parts. Their antioxidant activity accounted for ~ 69% of the activity of the reference compound α-tocopherol.	Kardošová and Machová (2006)
	Amaranthus hybridus L.	Crude polysaccharides AHP-M-1 and AHP-M-2	The IC_{50} values of AHP-M-1 and AHP-M-2 were 1.194 mg/mL and 0.67 mg/mL, respectively	Tang et al. 2020
	Chrysanthemum morifolium cv. Hangju	Crude polysaccharides extracted by ultrasonic assisted extraction (CMP-U) and with heat reflex extraction (CMP-H)	DPPH (IC_{50} for CMP-U and CMP-H were 1.850 mg/mL and 3.587 mg/mL), BHA (0.047 mg/mL). Hydroxyl radical (IC_{50} for CMP-U and CMP-H were 2.612 mg/mL and 4.236 mg/mL, respectively). Ferrous chelating activity (IC_{50} for CMP-U and CMP-H were 2.523 mg/mL and 3.982 mg/mL)	Hou et al. 2020

technologies that influence the physiochemical properties, structure, or conformation of polysaccharides will lead to differences in antioxidant activity, speculating their possible relationships (Ma et al. 2013a; Chen et al. 2009; Shen et al. 2014; Gou et al. 2014). Specifically, a correlation between molecular weight and radical scavenging activity was well documented (Cheung et al. 2012). Additionally, it is suggested that the overall radical scavenging ability was associated with the number of hydroxyl or amino groups in polysaccharide molecules such as chitosan (Guo et al. 2005).

Molecular weight of polysaccharides: The molecular weight affects the antioxidant ability of the polysaccharide. In addition, optimal antioxidant ability will vary depending on the type of polysaccharide. Liu et al. (2010a) reported that *Ganoderma lucidum* polysaccharide (GLP_L1) with low molecular weight (5.2 kDa) has a higher ability to scavenge free radicals, superoxide radicals, and hydrogen peroxide than the component (GLP_L2) with high molecular weight (15.4 kDa). At a concentration of 10 mg/mL, the scavenging rates of GLP_L1 to hydroxyl radical and superoxide anion are close to 75% and 90%, respectively, whereas the scavenging ability of GLP_L2 is only 50% and 60%, respectively. Ma et al. (2013) isolated four kinds of polysaccharides, namely, GLP1 (>10 kDa), GLP2 (8–10 kDa), GLP3 (2.5–8 kDa), and GLP4 (<2.5 kDa), from *G. lucidum* by ultrasonic method. At the concentration of 0.5 mg/mL, the reducing ability was in the following order: GLP2 > GLP1 > GLP3 > GLP4, showing that the GLP2 polysaccharide with moderate molecular weight had the best reducing ability. Zha et al. (2009) obtained three polysaccharides from rice bran with a molecular weight ranging from 1.2×10^5 to 6.3×10^6 Da (PW1), 3.5×10^4 to 7.4×10^4 Da (PW2), and 5.3×10^3 to 2.3×10^4 Da (PW3), respectively. Results showed that PW3 exhibited the best potentials of reducing power, chelating metal ion, and scavenging abilities against DPPH and ABTS radical among three fractions, revealing that a relative low molecular weight fraction had high antioxidant abilities.

Crude polysaccharides: In many reports, crude polysaccharide extracts exhibited notable antioxidant activity, but after further fractionation, the final purified polysaccharide showed moderate or low activity. It seemed that other antioxidant substances contained in the crude polysaccharide extract, such as pigments, flavones, peptide, protein, and polyphenol, might contribute to the antioxidant activity (Fan et al. 2014). Wang et al. (2013b) investigated the role of tea polyphenol (EGCG) in crude polysaccharide extracts from tea leaves (TPS) in the view of antioxidant ability. Results showed that the crude TPS exhibited strong antioxidant functions, whereas the further purified TPS fractions were hardly effective. But in the presence of EGCG, the reducing power and DPPH radical scavenging ability of TPS fractions were obviously enhanced. Meanwhile, the same results were also observed in dextran-EGCG system, indicating EGCG caused a synergistic increase in the antioxidant activity and tea polyphenol was the major antioxidant in the crude TPS. Mu et al. (2012) illustrated the existence of protein and pigment would influence the scavenging effect of both water-soluble and alkali-soluble crude polysaccharides from *Inonotus obliquus*. Wei et al. (2010) purified an acidic polysaccharide from *Prunella vulgaris* Linn., of which scavenging abilities against DPPH and hydroxyl radical were significantly lower than crude polysaccharide, possibly ascribed to

other antioxidants, such as flavones and pigments contained in the crude polysaccharide extracts. Lin et al. (2009) compared the antioxidant properties of different polysaccharide fractions isolated from *Lycium barbarum* Linnaeus, including crude polysaccharide (CP), crude extract of polysaccharide (CE), deproteinated polysaccharide (DP), and deproteinated and dialyzed polysaccharide (DDP), as well as four purified fractions (one neutral and three acidic polysaccharides, named as LBPN, LBPa1, LBPa2, and LBPa3, respectively). In their study, it was suggested that the inhibition effect of superoxide and hydroxyl radical by hydroxyl groups in polysaccharides was minor due to lacking phenolic-type structure which was essential for scavenging free radicals. Many other factors, such as molecular weight, galacturonic acid, and other chemical components in polysaccharide fractions, were also supposed to play a role in their antioxidant activities. Crude and purified polysaccharide, obtained from *G. atrum*, was compared in terms of DPPH scavenging ability and self-oxidation of 1,2,3-phentriol. Although the high concentration of purified polysaccharide, PSG-1, showed noticeable antioxidant ability, it was much lower than the crude polysaccharide, probably attributing to other constituents contained in crude polysaccharides extracts, such as proteins, amino acids, peptides, cellulose, phytosterol, ascorbic acid, thiamine, nucleotide, nicotinic acid, organic acids, and microelements (Chen et al. 2008).

Polysaccharide Chelating Metal: It is worth noting that one mechanism of antioxidant activity is to inhibit the generation of free radicals by chelating ions such as ferrous and copper instead of directly scavenging them. Transition metal ions could catalyze the generation of extremely reactive hydroxyl radicals from superoxide and hydrogen peroxide, known as Fenton reaction ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^{\cdot}$), especially ferrous ion, which is the most effective prooxidant in the food system (Yamauchi et al. 1998). Two polysaccharide fractions (GAPS-1 and SAPS-1) from *A. barbadensis* Miller were isolated and purified. The hydroxyl radical scavenging activity of GAPS-1 was significantly higher than SAPS-1. Meanwhile, GAPS-1 had a higher chelating ability against ferrous ion, indicating that the chelating effect might impart polysaccharides capable of antioxidant potentials (Chun-hui et al. 2007). A similar correlation was also revealed by Li et al. (2011) investigating an extracellular polysaccharide from *N. commune*.

Generally, the structure of compounds containing more than one of the following functional groups, that is, -OH, -SH, -COOH, $-PO_3H_2$, -C=O, $-NR_2$, -S-, and -O-, is in favor of chelating ability (Gulcin 2006). Therefore, presence of uronic acid and sulfate groups appeared to be essential in demonstrating the chelating ability of polysaccharides. Chang et al. (2010) illustrated that the larger the content of galacturonic acid in polysaccharide, the higher the ability of chelating ferrous ion. Fan et al. (2014) fractionated four polysaccharides from the leaves of *Ilex latifolia* Thunb. by DEAE cellulose-52 chromatography (ILPS1, ILPS2, ILPS3, and ILPS4) with ILPS4 having the highest contents of sulfuric radical (3.7%) and uronic acid (23.2%). Results showed that IC_{50} of ferrous chelating activity for ILPS4 was 1965 $\pm$ 8.1 μg/mL, while for other fractions the abilities were 4.7%, 11.3%, and 46.7%, respectively. This observation confirmed that the chelating effect might partly be due to the presence of functional groups such as carboxyl group and sulfuric radical in

the polysaccharide structure. However, the ferrous ion chelating effect of a carboxymethylated polysaccharide (C-GLP) from *G. lucidum* was weak as compared to EDTA (Xu et al. 2009). The reason was probably attributed to its structural features unsuitability for chelating metal ion, as the chelating ability of ferrous was dependent upon hydroxyl numbers and the hydroxyl substitution in the ortho position (Wang et al. 2008).

Types of substitution groups and degrees of substitution: Types of substitution groups and degrees of substitution (DS) appeared to have an effect on the physicochemical properties and conformation of native polysaccharides, such as molecular weight, polarity, solubility, and charge density. DS may also affect the activity through interruption of inter- and intramolecular hydrogen bonds. Chen et al. (2014) found that not only did the total sugar content of acetylated and carboxymethylated derivatives decrease significantly, but also its molecular weight was reduced in contrast to native *G. atrum* polysaccharide. Liu et al. (2010b) proved that sulfation effectively improved the water solubility and bile acid-binding capacities of a water-insoluble polysaccharide from *G. lucidum* (GLP). Furthermore, ^{13}C NMR results showed that C-2, C-4, and C-6 position might be partially substituted, and C-4 was the most reactive. It was probably due to its special structure features and the influence of steric hindrance.

A linear relationship between the degree of substitution and antioxidant potentials was not always observed, suggesting high DS was not necessary for antioxidant behavior. Xie et al. (2015) revealed that antioxidant activity of sulfated CP with a highest DS of 0.55 was not as effective as derivatives with middle DS (0.42 and 0.06). However, the influence of DS was still disputable, as a high DS could enhance the antioxidant activity evidenced in many reports. Yan et al. (2012) pointed out that the sulfation of exopolysaccharide, produced by *Cordyceps sinensis* fungus (Cs-HK1), occurred most frequently at hydroxyl groups of C-6 and caused a conformation change from random coils or aggregates to single helices in aqueous solution. The antioxidant activity of the sulfated derivatives for hydroxyl radical and ABTS radical scavenging effect was significantly enhanced with increasing DS and reducing molecular weight. Wang et al. (2014a) showed that C-6 substitution was predominately in phosphorylated derivatives of galactomannan (PGG) from guar gum according to ^{13}C NMR analysis and PGG with high DS achieved a higher radical scavenging effect and stronger chelating ability than PGG with lower DS. Jung et al. found that DPPH radical scavenging ability of polysaccharide from *Pleurotus eryngii* was improved with increasing degree of sulfation (Jung et al. 2011). This finding was also consistent with the report that high degree of sulfated substitution (0.90) was more effective than that of low DS (0.43) in scavenging DPPH (Wang et al. 2013c). Another study also revealed a positive relationship between the degrees of acetylated substitution and scavenging effects against DPPH and superoxide radical, as well as reducing power (Song et al. 2013).

One mechanism is that the introduction of these substitution groups into polysaccharide molecules leads to weaker dissociation energy of hydrogen bond. Therefore, the hydrogen donating ability of polysaccharide derivatives was increased. Another mechanism is speculated to activate the abstraction of the anomeric carbon.

On the other hand, the chemical modification is sometimes accompanied with a decrease of molecular weight, hence improving the antioxidant potentials of polysaccharides. Among the derivatives, the sulfate polysaccharide is commonly reported as a stronger antioxidant, which is partly due to its ordered, extended structure. The sulfated polysaccharide usually traps free radicals in an electrostatic manner since the sulfate groups usually generate a highly acidic environment and the sulfur substitution may also weaken hydrogen bond interactions between polysaccharides (Wang et al. 2016).

Sulfated polysaccharides: Sulfated polysaccharides demonstrated stronger antioxidant capacities than de-sulfated polysaccharides (Hu et al. 2010; Yang et al. 2011). Thus, the high degree of sulfation and low molecular weight showed the best antioxidant capacities (Wang et al. 2010). Yang et al. (2011) compared the antioxidant activity of sulfated polysaccharide from *Corallina officinalis* and its desulfated derivatives; the results showed that the native sulfated polysaccharides possessed more excellent radical scavenging activity and reducing power than the desulfated fractions. Generally, the biological activity of sulfated polysaccharides from marine algae is related to the molecular size, type of sugar, sulfate content, sulfate position, and type of linkage; also, molecular geometry is known to play a role in activity (Shanmugan and Mody 2000).

Selenium enriched polysaccharides: Selenium (Se) is an essential trace element for nutrition of a capital importance in the human biology. The Se does not directly act as a ROS/RNS scavenger but is a cofactor of selenoprotein, for example, glutathione peroxidase, which exerts various antioxidant activities in vivo. Confirmed by FT-IR and NMR spectra, the selenylation modification by H_2SeO_3/HNO_3 method predominantly happened at the C-6 position of polysaccharides and a distinct decrease of molecular weight was also induced due to the acid environment of selenized reaction. Additionally, it was proposed that the combination of Se in polysaccharides was possibly in the form of selenyl group (-SeH) or selenoacid ester (Xu and Huang 1994). Wei et al. (2015) synthesized a series of selenylated polysaccharide from *Radix hedysari* (Se-RHP), the content of Se ranging from 1.04 to 3.29 mg/g and the molecular weight decreasing from 62.7 kDa to 27.7 kDa, which showed better scavenging activity and reducing power in contrast to the native RHP.

Se-containing derivatives from *Artemisia sphaerocephala* (Wang et al. 2012c) and *Potentilla anserina* L. (Zhao et al. 2013) have been acknowledged to improve the antioxidant activity compared to the native polysaccharides. The proposed mechanism might be involved in changes of conformation structure of polysaccharides and emerged the increasing amount of hydroxyl group, resulting in an influence on the antioxidant activity.

Further analysis on polysaccharide obtained from Se-enriched materials confirmed the important role of Se in enhancing the antioxidant potentials of polysaccharides. As evidenced by the results of Yu et al. (2007), polysaccharide from Se-enriched green tea presented significant higher antioxidant capacity than that from regular green tea. In addition, all polysaccharides isolated from Se-enriched *G. lucidum* were more effective on attenuating the production of superoxide radicals (Zhao et al. 2008). Mao et al. (2014) revealed that although there was no significant

difference of polysaccharide content and molecular weight of each Se-enriched *G. frondosa* polysaccharide (Se-GP) fraction and the corresponding GP, except for the Se content, Se-GPs were a more effective scavenger (against DPPH, ABTS, and hydroxyl radicals), especially for hydroxyl radical, reaching 71.32% at a concentration of 2 mg/mL. On the other hand, selenium-polysaccharide synthesized by adding selenium chloride oxide ($SeCl_2O$) also exhibited a higher total antioxidant capacity, superoxide radical, and hydroxyl radical scavenging effect as reported by Guo et al. (2013).

Phenolic compounds: Phenolic compounds, especially phenolic acids, play an important role in the overall radical scavenging ability of xylans and xylo-oligosaccharides from the wheat bran (Hromádková et al. 2008; Veenashri and Muralikrishna 2011). Hromádková et al. (2013) pointed out that both protein and phenolic compounds contributed to the radical scavenging effects of xylans, and the protein-free fraction displayed the highest hydroxyl radical scavenging ability indicating the distinct role of phenolic acids. Siu et al. (2014) study revealed that the antioxidant activities of all polysaccharide fractions from three mushrooms (*L. edodes, G. frondosa*, and *T. versicolor*) were significantly correlated with the total phenolic and protein content according to three in vitro assessments, including TEAC, FRAP, and ferrous ion chelating activity assay. However, no significant correlation was observed between the total sugar content and any of tested antioxidant assays. The results were similar to a study carried out by Wang et al. (2012a) that the neutral content was not apparently correlated with DPPH and FRAP antioxidant actions of polysaccharides from oolong tea. Furthermore, purified polysaccharide fractions, free of phenolics and proteins, hardly showed significant antioxidant activities. Indeed, polysaccharide-polyphenol residues have been demonstrated to have noticeable antioxidant functions in many reports. Li et al. (2007) found no statistical difference in scavenging linoleic acid radicals between the polysaccharides from *Lycium barbarum* fruits and the positive control (BHT). The coupled oxidation of β-carotene and linoleic acid developed free radicals, which oxidize unsaturated β-carotene molecules, leading to the discoloration of the system. In this model, the proposed mechanism in hindering β-carotene oxidation could be attributed to the polyphenolic-associated polysaccharide neutralizing the free radicals. In DPPH radical scavenging assay, the polysaccharide showed pronounced antioxidant ability as well, possibly attributing to polyphenolic-associated polysaccharide fraction formed between high molecular weight phenolics and polysaccharides.

However, not all the conjugated moiety of polysaccharides was responsible for antioxidant power. After removing polyphenols, the tea polysaccharide conjugate from low grade green tea was found to possess strong antioxidant properties based on the results of free radical scavenging and lipid peroxidation inhibitory effect (Chen et al. 2005). Likewise, Wang et al. (2012b) evidenced that the DPPH radical scavenging effect of another tea polysaccharide fraction (TPS1) was beyond 90%, close to that of ascorbic acid, although both of the protein and polyphenol content were relatively low in TPS1, suggesting other factors such as carboxyl group other than polyphenol compounds that are of concern. In order to determine the molecular

interactions between tea polyphenols and oat β-glucan, Wu et al. (2011) prepared complex and physical mixture of oat β-glucan and tea polyphenols, further using four in vitro antioxidant evaluations (DPPH radical, hydroxyl radical, superoxide radical, and reducing power) to compare the activity among tea polyphenols, β-glucan, their complex, and physical mixture. Results showed that the complex had the strongest effect against superoxide radical, whereas the mixture had the strongest hydroxyl radical scavenging effect in the concentration of 0.5–2.5 mg/mL. With regard to reducing power assay, no synergistic effect was found between tea polyphenols and β-glucan, but it was observed in DPPH scavenging assay when β-glucan was combined with tea polyphenols at low concentration (<0.05 mg/mL). However, when tea polyphenol was used at a high concentration (0.09 mg/mL), it was changed to antagonistic effect in scavenging DPPH radical. The inconsistent antioxidant outcomes of tea polyphenols and oat β-glucan complex might be dependent on its structure and provided dose, as well as the strong hydrogen bonds between them.

Ferulic acid, a kind of phenolic acid and a strong antioxidant, was shuttled to wall matrices via attachment to structural polysaccharides. Feruloylation, in certain cases, occurs on the arabinose or galactose side chains of pectin polysaccharides and influences their chemical properties. The attachment of ferulic acid is covalently via an ester linkage formed between carboxylic acid group and the primary hydroxyl at carbon-5 position of α-L-arabinofuranosyl residues (Hatfield et al. 1997; Ishii 1997). Therefore, the content of total phenolic compounds conjugated in the polysaccharide extracts might explain their high antioxidant potentials.

On the other hand, the definite role of monosaccharide or glycosidic linkages in antioxidant activity of polysaccharide remained confused. Lo et al. (2011) investigated the relationship between antioxidant properties of polysaccharides and monosaccharide or glycosyl linkages using four conventional antioxidant models (conjugated diene, reducing power, DPPH scavenging, and ferrous ions chelating) on multiple linear regression analysis (MLRA). Results revealed that compositions and ratios of monosaccharide as well as types of glycosyl linkages would be of concern in modulating the antioxidant properties. Specifically, rhamnose and mannose showed positive coefficients in all the four MLRA models. Meanwhile, glycosyl linkages, specifically arabinose $1 \rightarrow 4$ and mannose $1 \rightarrow 2$ of the side chain, were significantly related to the reducing power, whereas glucose $1 \rightarrow 6$ and arabinose $1 \rightarrow 4$ were closely in relation to DPPH radical scavenging effect. Tsiapali et al. (2001) pointed out that a portion of antioxidant ability of the carbohydrate appeared to correlate with the monosaccharide composition rather than types of intrachain linkages, molecular weight, or degree of branching, since either dextrose or mannose showed weaker free radical scavenging ability than the polymer. It was interesting to find that the polymer had better radical scavenging effect than either of the monosaccharides, suggesting that the polymeric structure conferred additional activity of the carbohydrates. Meng et al. (2015) adopted Pearson correlation analysis test and linear regression analysis to explore the relationship between the monosaccharide composition of polysaccharides and the antioxidant activity. Results showed that the antioxidant activity was significantly correlated with the content of mannose

($P < 0.01$) and glucose ($P < 0.05$), whereas galactose was not correlated ($P < 0.05$). Furthermore, both the contents of monosaccharide were observed to have high correlation coefficients concerning radical scavenging activity with mannose the positive ($r = 0.942$) and glucose the negative ($r = -0.905$).

Antioxidant enzymes: It is found that polysaccharides in many plants and microorganisms have significant antioxidant effects, mainly through the endogenous antioxidant stress Nrf2/ARE pathway to regulate the expression of downstream antioxidant enzymes. These antioxidant enzymes can further block the free radical chain reaction, thus reducing the generation of free radicals. Secondly, by inhibiting the expression of iNOS mRNA and reducing NO production, it can significantly increase the antioxidant capacity and reduce oxidative stress injury. Polysaccharides are rich in natural resources, their multi-channel, multi-target, multi-effect, and other characteristics have made great progress in antioxidants (Mu et al. 2020).

Chitosan and chitosan derivatives: Chitosan is a biopolysaccharide, which is obtained from alkaline deacetylation of chitin, and acetamide groups are transformed into primary amino groups during the deacetylation. The diverse biological activities of chitosan and its derivatives are extensively studied that allows to widening the application fields in various sectors especially in biomedical science. The biological properties of chitosan are strongly depending on the solubility in water and other solvents. Deacetylation degree (DDA) and molecular weight (MW) are the most decisive parameters on the bioactivities since the primary amino groups are the key functional groups of chitosan where permits to interact with other molecules. Higher DDA and lower MW of chitosan and chitosan derivatives demonstrated higher antimicrobial, antioxidant, and anticancer capacities (Kim 2018).

The antioxidant activity of chitosan has been getting high attention from many scientists. Chitosan has shown a notable scavenging activity against different radical species presenting a great potential for an extensive applications. The scavenging activity of chitosan derivatives against free radicals comes through donating hydrogen atom, and several theories were proposed by Xie et al. (2001):

(i) The hydroxyl groups in the polysaccharide unit can react with hydroxyl radicals by the typical H-abstraction reaction.
(ii) OH can react with the residual-free amino groups NH_2 to form stable macromolecules radicals.
(iii) The NH_2 groups can form ammonium groups NH_3^+ by absorbing H^+ from the solution, and then they react with OH through addition reactions.

The DDA and MW of chitosan are also the major factors deciding the scavenging capacity of chitosan (Aranaz et al. 2009). Different with chitosan, chitin is an insoluble polymer in water and thus the major limitation exists for being a useful antioxidant agent. The NH_2 groups in chitosan are responsible for free radical scavenging, and they can be protonated in acidic solution.

The antioxidant potentials of polysaccharides are determined by combination of several related factors.

5 Antibacterial Activity of Polysaccharides

Marine algae or macroalgae provide a variety of natural metabolites and bioactive compounds with antimicrobial activity, such as polysaccharides, polyunsaturated fatty acids, phlorotannins, and other phenolic compounds, and carotenoids. (Jun et al. 2018). The marine algae cell wall consists of various polysaccharides including alginic acid and alginates, carrageenans and agar, laminarans, fucoidans, ulvans, and their derivatives (Shannon and Ghannam 2016), which have been employed in dietary fibers as functional ingredients for human gut health or industrially utilized as colloidal materials to improve the rheological characteristics of food matrices (O'Sullivan et al. 2014). Besides these utilizations, several studies demonstrated the potential of sulfated polysaccharides, such as fucoidan, ulvan, and carrageenan, as antimicrobial agents against a wide range of human bacterial pathogens (Yamashita et al. 2001; Li et al. 2010; Pierre et al. 2011; Marudhupandi and Kumar 2013; Lee et al. 2013). The antibacterial activities of some selected polysaccharides are shown in Table 2.

6 Mechanism and Factors Affecting the Antibacterial Activity of Polysaccharides

Most antimicrobial agents from marine algae have been identified as primary phenolic compounds and sulfated polysaccharides. The antimicrobial action of sulfated polysaccharides has not been clearly revealed (Jun et al. 2018). There are two theories explaining the antimicrobial action of sulfated polysaccharides proposed by Yamashita et al. (2001): one suggests the binding of the sulfated polysaccharides on the bacterial surface; the other suggests the trapping of nutrients or cationic minerals induced by the sulfated polysaccharides, which can reduce the bioavailability of nutrients (Yamashita et al. 2001). The main components of green, brown, and red marine algae are usually polysaccharides that have a variety of functions and structural (Shannon and Ghannam 2016). The activities of their antimicrobial depend on several factors, such as distribution, molecule weight, loads density, sulfate content (in sulfated polysaccharides), and structure and confirmation aspects. In addition, oligosaccharides obtained by depolymerization of marine algae polysaccharides also induce protection against viral, fungal, and bacterial infections in plants (Achmad and Huldani 2020).

G. lucidum as a medicinal mushroom has been widely used in China (named Ling Zhi) and Japan (named Reishi, Mannentake) for hundreds of years. The highest attention is paid to polysaccharides from *G. lucidum*, which activate macrophages, lymphocytes, NK cells, proinflammatory cytokines such as TNF or interleukins, essential for host survival from infection, and are also required for the repair of tissue injury (Gao et al. 2005; Ahmadi and Riazipour 2007). Polysaccharides extracted from the fruit bodies, mycelium, and spores of *G. lucidum* can promote the function of macrophages as well as B cells (Shao et al. 2004). Additionally, it has been shown that the antioxidant property of *Ganoderma* polysaccharide peptide decreased the oxidation of low density lipoprotein and exhibited antioxidant effect by scavenging

Table 2 Antibacterial activity of polysaccharides from selected sources

Source		Polysaccharides	Antibacterial activity	Ref.
Plant	*Crataegus azarolus* L. var. *aronia*	Polysaccharides from the pulps (CAP) and seeds (CAS)	CAP revealed the best antimicrobial effect against *L. monocytogenes* and *B. cereus*. However, CAS displayed the highest inhibition activity toward *E. faecalis*.	Rjeibi et al. 2020
Marine algae	*Fucus vesiculosus*	Fucoidan F85- (the structure of the fucoidan is composed of α-(1,3)-linked fucose with sulfate groups substituted at the C-4 position on some of the fucose residues); Fucoidan F95	Fucoidan F85, sulfated polysaccharides, which shows growth inhibitory effect on all dental plaque bacteria with *S. mutans*. In addition, it inhibits the growth of *Listeria monocytogenes, Staphylococcus aureus*, and two lactic acid bacteria. In contrast, Fucoidan F95 does not inhibit the growth of any bacterial strains, although it also comes from *F. vesiculosus*.	Jun et al. 2018
	Undaria pinnatifida and *Kjellmaniella crassifolia*	Sulfated polysaccharides	Two sulfated polysaccharides have growth inhibitory effects on *Salmonella typhimurium* at concentrations of 1000 μg mL^{-1} (both). Ampicillin MIC (as a standard antimicrobial agent) is in the range of 0.8–12.5 μg mL^{-1} for dental plaque bacteria	Jun et al. 2018
			Marine algae *Chlorophyta* and *Rhodophyta*; *C. lentifera*; and *K. alvarezii* each have antibacterial activity against the causative agent for oral infection; *S. aureus* and *S. mutans* using the disk diffusion method. Antibacterial activity indicates the possible value of marine algae therapy for oral infections.	Sabirin et al. 2015

(continued)

Table 2 (continued)

Source		Polysaccharides	Antibacterial activity	Ref.
Green algae	*Chlamydomonas reinhardtii*	Fucoidan F85	Fucoidan F85 at concentrations above 250 μg. mL^{-1} completely suppresses both the formation of biofilms and the growth of planktonic cells of *S. mutans* and *S. sobrinus*.	Vishwakarma and Vavilala 2019
Fungi	*Ganoderma lucidum*	Four strains of *Ganoderma lucidum* (GL01, GL02, GL03, and GL04) were cultivated. Even though the richest in polysaccharides was GL01 strain	The antibacterial activity of polysaccharides was determined in vitro using micro-dilution broth method. The panel of eight reference bacterial strains was used. All the polysaccharide samples tested showed the broad spectrum and the moderate antibacterial activity. *Micrococcus luteus* ATCC 10240 strain was the most sensitive with MIC (minimal inhibitory concentration) = 0.63 − 1.25 mg/mL.	Skalicka-Woźniak et al. 2012
	Pleurotus Florida, *Agrocybe cylindracea*	*Pleurotus Florida* polysaccharides (P1), *Agrocybe cylindracea* polysaccharides (P2)	The polysaccharide extracts had greatest activity against gram-negative bacterial strains *Klebsiella pneumonia* and ***Escherichia coli***. Gram-positive bacterial strain ***Staphylococcus aureus*** exhibited weak growth-inhibiting effect on both polysaccharides extract. ***Staphylococcus aureus*** is a gram-positive cocci, and catalase and coagulase positive bacterium.	Kumar et al. 2017

reactive oxygen species in mice (Paterson 2006). Other studies demonstrated that *G. lucidum* contained antibacterial constituents (Kim et al. 1996; Suay et al. 2000; Gao et al. 2003). The aqueous extract from the carpophores of *G. lucidum* inhibited 15 types of bacteria (Yoon et al. 1994).

Chitosan, a versatile hydrophilic polysaccharide derived from chitin, has a broad antimicrobial spectrum to which gram-negative and gram-positive bacteria and fungi are highly susceptible. Chitosan is with proved antimicrobial activity. The following antibacterial mechanisms have been proposed:

(i) The ionic surface interaction resulting in wall cell leakage.
(ii) The antimicrobial effect of chitosan is much higher comparing to chitin due to the numbers of the amine groups that is responsible for cationic property of chitosan. Positively charged chitosan at acidic condition might interact with negatively charged residues of carbohydrates, lipids, and proteins located on the cell surface of bacteria, which subsequently inhibit the growth of bacteria (Aranaz et al. 2009; Younes and Rinaudo 2015). Thus, the electronic property of chitosan plays a very important role in the inhibition mechanism of microorganisms. The high density of positive charge on the structure of chitosan or its derivatives generates strong electrostatic interaction that is affiliated with DDA. With this theory, chitosan is more promising for the inhibition of Gram-negative than Gram-positive bacterium since the negatively charged cell surfaces interact more with positively charged chitosan (Younes and Rinaudo 2015).
(iii) The inhibition of the mRNA and protein synthesis via the penetration of chitosan into the nuclei of the microorganisms.
(iv) The formation of an external barrier, chelating metals and provoking the suppression of essential nutrients to microbial growth. It is likely that all events occur simultaneously but at different intensities. The molecular weight (MW) and the degree of acetylation (DA) are also important factors in determining such activity. In general the lower the MW and the DA, the higher will be the effectiveness on reducing microorganism growth and multiplication. A study of previous work from the literature has not lead to any conclusive data as to whether the chitosan has higher activity on gram-positive or on gram-negative bacteria. On both species chitosan seems to act differently, though in both cases satisfactorily. Water-soluble derivatives, which can be attained by chemical introduction of CH_3 in the main chain, enhance the chitosan applicability in a large pH range and also improve the antimicrobial activity, opening up a broad range of possibilities (Goy et al. 2009).

Kumar et al. (2017) proposed the mechanism of antibacterial action is due to glycoprotein receptors present on the cell surface of polysaccharides which bind with compounds in the bacterial cell wall, cytoplasmic membrane, and DNA. This results in increased permeability of the cytoplasmic membrane, protein leakage, and binding of bacterial DNA.

Overall, the antioxidant and antibacterial activities of polysaccharides are not determined by a single factor but a combination of several related factors.

7 Conclusion

The total chemical evaluation of polysaccharides is particularly significant for development of an appreciative knowledge of the main features responsible for their antioxidant and antimicrobial activity. In the future, more studies should be concentrated on the exact mechanism of in vitro antioxidant activities of polysaccharides. Based on the various antioxidant mechanisms, different antioxidative results, such as DPPH radical scavenging, hydroxyl radical scavenging, ABTS radical scavenging, reducing power, and chelating ability, should be adopted to assess the respective influences of polysaccharides, especially their structural features. With regard to the intensive researches on natural antioxidants, the properties of polysaccharides from natural products useful for the antioxidant activity and healthy benefits should be clearly elucidated, and more specific research, therefore, on this topic is imperative. Further investigation of polysaccharides could facilitate the development of new pharmacological formulations.

References

Achmad H, Huldani RYF. Antimicrobial activity and sulfated polysaccharides antibiofilms in marine algae against dental plaque bacteria: a literature review. Sys Rev Pharm. 2020;11:459–65.

Ahmadi K, Riazipour M. Effect of *Ganoderma lucidum* on cytokine release by peritoneal macrophages. Iran J Immunol. 2007;4:220–6.

Ananthi S, Raghavendran HRB, Sunil AG, Gayathri V, Ramakrishnan G, Vasanthi HR. *In vitro* antioxidant and *in vivo* anti-inflammatory potential of crude polysaccharide from *Turbinaria ornata* (Marine Brown Alga). Food Chem Toxicol. 2010;48:187–92.

Aranaz I, Mengíbar M, Harris R, et al. Functional characterization of chitin and chitosan. Curr Chem Biol. 2009;3:203–30.

Arun J, Selvakumar S, Sathishkumar R, et al. In vitro antioxidant activities of an exopolysaccharide from a salt pan bacterium *Halolactibacillus miurensis*. Carbohydr Polym. 2017;155:400–6.

Bais D, Trevisan A, Lapasin R, et al. Rheological characterization of polysaccharide–surfactant matrices for cosmetic O/W emulsions. J Colloid Interface Sci. 2005;290:546–56.

Castro LSEPW, Pinheiro TD, Castro AJG, et al. Potential anti-angiogenic, antiproliferative, antioxidant, and anticoagulant activity of anionic polysaccharides, fucans, extracted from brown algae Lobophora variegata. J Appl Phycol. 2015;27:1315–25.

Castro LSEPW, Castro AJG, Santos MDN, et al. Effect of galactofucan sulfate of a brown seaweed on induced hepatotoxicity in rats, sodium pentobarbital-induced sleep, and anti-inflammatory activity. J Appl Phycol. 2016;28:2005–17.

Chale-Dzul J, Freile-Pelegrin Y, Robledo D, et al. Protective effect of fucoidans from tropical seaweeds against oxidative stress in HepG2 cells. J Appl Phycol. 2017;29:2229–38.

Chang SC, Hsu BY, Chen BH, et al. Structural characterization of polysaccharides from *Zizyphus jujuba* and evaluation of antioxidant activity. Int J Biol Macromol. 2010;47:445–53.

Chen H, Zhang M, Xie B, et al. Components and antioxidant activity of polysaccharide conjugate from green tea. Food Chem. 2005;90:17–21.

Chen Y, Xie MY, Nie SP, et al. Purification, composition analysis and antioxidant activity of a polysaccharide from the fruiting bodies of *Ganoderma atrum*. Food Chem. 2008;107:231–41.

Chen H, Qu Z, Fu L, et al. Physicochemical properties and antioxidant capacity of 3 polysaccharides from green tea, oolong tea, and black tea. J Food Sci. 2009;74:C469–74.

Chen Y, Zhang H, Wang Y, et al. Acetylation and carboxymethylation of the polysaccharide from *Ganoderma atrum* and their antioxidant and immunomodulating activities. Food Chem. 2014;156:279–88.
Chen YL, Mao WJ, Tao HW, et al. Preparation and characterization of a novel extracellular polysaccharide with antioxidant activity, from the mangrove-associated fungus *fusarium oxysporum*. Mar Biotechnol. 2015;17:219–28.
Chen Y, Yan WJ, Liu MX, et al. Purification, chemical characterization, and bioactivity of an extracellular polysaccharide produced by the marine sponge endogenous fungus *Alternaria sp* SP-32. Mar Biotechnol. 2016;18:301–13.
Cheung YC, Siu KC, Liu YS, et al. Molecular properties and antioxidant activities of polysaccharide-protein complexes from selected mushrooms by ultrasound-assisted extraction. Process Biochem. 2012;47:892–5.
Chun-hui L, Chang-hai W, Zhi-liang X, et al. Isolation, chemical characterization and antioxidant activities of two polysaccharides from the gel and the skin of *Aloe barbadensis* Miller irrigated with sea water. Process Biochem. 2007;42:961–70.
Delma CR, Somasundaram ST, Srinivasan GP, et al. Fucoidan from Turbinaria conoides: a multifaceted 'deliverable' to combat pancreatic cancer progression. Int J Biol Macromol. 2015;76:330.
El-Newary SA, Ibrahim AY, Asker MS, et al. Production, characterization and biological activities of acidic exopolysaccharide from marine *bacillus amyloliquefaciens* 3MS 2017. Asian Pac J Trop Biomed. 2017;10:715–25.
Fan J, Wu Z, Zhao T, et al. Characterization, antioxidant and hepatoprotective activities of polysaccharides from *Ilex latifolia* Thunb. Carbohydr Polym. 2014;101:990–7.
Fernando IPS, Sanjeewa KKA, Samarakoon KW, et al. FTIR characterization and antioxidant activity of water-soluble crude polysaccharides of Sri Lankan marine algae. Algae. 2017;32:75–86.
Gao Y, Zhou S, Huang M, Xu A. Antibacterial and antiviral value of the genus *Ganoderma P0. Karst. species (Aphyllophoromycetideae)*: a review. Int J Med Mushrooms. 2003;5:235–46.
Gao Y, Gao H, Chan E, et al. Antitumor activity and underlying mechanisms of ganopoly, the refined polysaccharides extracted from *Ganoderma lucidum*, in mice. Immunol Investig. 2005;34:171–98.
Gou X, Wang Q, Gao G, et al. Effects of extraction methods on antioxidant activities of polysaccharides from the *Curcuma phaeocaulis* rhizomes. Asian J Agric Food Sci. 2014;2(4). Available from: https://ajouronline.com/index.php/AJAFS/article/view/1481
Goy RC, de Britto D, Assis OBG. A review of the antimicrobial activity of chitosan. Polímeros. 2009;19:241–7. https://doi.org/10.1590/S0104-14282009000300013.
Gülçin I. Antioxidant and antiradical activities of L-carnitine. Life Sci. 2006;78:803–11.
Guo Z, Xing R, Liu S, et al. The synthesis and antioxidant activity of the Schiff bases of chitosan and carboxymethyl chitosan. Bioorg Med Chem Lett. 2005;15:4600–3.
Guo Y, Pan D, Li H, et al. Antioxidant and immunomodulatory activity of selenium exopolysaccharide produced by *Lactococcus lactis* subsp. *Lactis*. Food Chem. 2013;138:84–9.
Guru MMS, Vasanthi M, Achary A. Antioxidant and free radical scavenging potential of crude sulphated polysaccharides from *Turbinaria ornata*. Biologia. 2015;70:27–33.
Hatfield RD, Ralph J, Grabber JH, et al. Cell wall cross-linking by ferulates and diferulates in grasses. J Sci Food Agric. 1997;79:403–7.
He JZ, Xu YY, Chen HB, et al. Extraction, structural characterization, and potential antioxidant activity of the polysaccharides from four seaweeds. Int J Mol Sci. 2016;17:1988. https://doi.org/10.3390/ijms17121988.
Hou X, Huang X, Li J, et al. Extraction optimization and evaluation of the antioxidant and α-glucosidase inhibitory activity of polysaccharides from *Chrysanthemum morifolium* cv. Hangju. Antioxidants. 2020;9:59. https://doi.org/10.3390/antiox9010059.
Hromádková Z, Kostalova Z, Ebringerova A. Comparison of conventional and ultrasound-assisted extraction of phenolics-rich heteroxylans from wheat bran. Ultrason Sonochem. 2008;15:1062–68.

Hromádková Z, Paulsen BS, Polovka M, et al. Structural features of two heteroxylan polysaccharide fractions from wheat bran with anti-complementary and antioxidant activities. Carbohydr Polym. 2013;93:22–30.

Hu T, Liu D, Chen Y, Wu J, Wang S. Antioxidant activity of sulfated polysaccharide fractions extracted from *Undaria pinnitafida in vitro*. Int J Biol Macromol. 2010;46:193–8.

Ishii T. Structure and functions of feruloylated polysaccharides. Plant Sci. 1997;127:111–27.

Jun JY, Jung MJ, Jeong IH. Antimicrobial and antibiofilm activities of sulfated polysaccharides from marine algae against dental plaque bacteria. Mar Drugs. 2018;16:301. https://doi.org/10.3390/md16090301.

Jung HY, Bae IY, Lee S, et al. Effect of the degree of sulfation on the physicochemical and biological properties of *Pleurotus eryngii* polysaccharides. Food Hydrocoll. 2011;25:1291–5.

Kardošová A, Machová E. Antioxidant activity of medicinal plant polysaccharides. Fitoterapia. 2006;77:367–73.

Kim S. Competitive biological activities of Chitosan and its derivatives: antimicrobial, antioxidant, anticancer, and anti-inflammatory activities. Int J Polym Sci. 2018;2018:Article ID 1708172. https://doi.org/10.1155/2018/1708172.

Kim DH, Bae EA, Jang IS, et al. Anti-Helicobacter pylori activity of mushrooms. Arch Pharm Res. 1996;19:447–9.

Kim EA, Lee SH, Ko CI, et al. Protective effect of fucoidan against AAPH-induced oxidative stress in zebrafish model. Carbohydr Polym. 2014;102:185–91.

Kumar SK, Shankar S, Mohan SC, et al. *In vitro* antioxidant and antimicrobial activity of polysaccharides extracted from edible mushrooms *Pleurotus Florida* and *Agrocybe cylindracea*. Singapore J Chem Biol. 2017;6:17–22.

Lee KY, Jeong MR, Choi SM, et al. Synergistic effect of fucoidan with antibiotics against oral pathogenic bacteria. Arch Oral Biol. 2013;58:482–92.

Li XM, Li XL, Zhou AG, et al. Evaluation of antioxidant activity of the polysaccharides extracted from *Lycium barbarum* fruits *in vitro*. Eur Polym J. 2007;43:488–97.

Li LY, Li LQ, Guo CH, et al. Evaluation of in vitro antioxidant and antibacterial activities of *Laminaria japonica* polysaccharides. J Med Plants Res. 2010;4:2194–8.

Li H, Xu J, Liu Y. Antioxidant and moisture-retention activities of the polysaccharide from *Nostoc commune*. Carbohydr Polym. 2011;83:1821–7.

Li GY, Luo ZC, Yuan F, et al. Combined process of high-pressure homogenization and hydrothermalextraction for the extraction of fucoidan with good antioxidant properties from Nemacystus decipients. Food Bioprod Process. 2017;106:35–42.

Lin CL, Wang CC, Chang SC, et al. Antioxidative activity of polysaccharide fractions isolated from *Lycium barbarum* Linnaeus. Int J Biol Macromol. 2009;45:146–51.

Liu W, Wang H, Pang X, et al. Characterization and antioxidant activity of two low-molecular-weight polysaccharides purified from the fruiting bodies of *Ganoderma lucidum*. Int J Biol Macromol. 2010a;46:451–7.

Liu W, Wang H, Yao W, et al. Effects of sulfation on the physicochemical and functional properties of a water-insoluble polysaccharide preparation from *Ganoderma lucidum*. J Agric Food Chem. 2010b;58:3336–41.

Lo TCT, Chang CA, Chiu KH, et al. Correlation evaluation of antioxidant properties on the monosaccharide components and glycosyl linkages of polysaccharide with different measuring methods. Carbohydr Polym. 2011;86:320–7.

Ma CW, Feng M, Zhai X, et al. Optimization for the extraction of polysaccharides from *Ganoderma lucidum* and their antioxidant and antiproliferative activities. J Taiwan Inst Chem Eng. 2013;44:886–94.

Ma L, Chen H, Zhu W, et al. Effect of different drying methods on physicochemical properties and antioxidant activities of polysaccharides extracted from mushroom *Inonotus obliquus*. Food Res Int. 2013a;50:633–40.

Manivasagan P, Sivasankar P, Venkatesan J, et al. Production and characterization of an extracellular polysaccharide from *Streptomyces violaceus* MM72. Int J Biol Macromol. 2013;59:29–38.

Mao G, Zou Y, Feng W, et al. Extraction, preliminary characterization and antioxidant activity of Se-enriched Maitake polysaccharide. Carbohydr Polym. 2014;101:213–9.
Marudhupandi T, Kumar TTA. Antibacterial effect of fucoidan from *Sargassum wightii* against the chosen human bacterial pathogens. Int Curr Pharm J. 2013;2:156–8.
Meng L, Sun S, Li R, et al. Antioxidant activity of polysaccharides produced by *Hirsutella* sp. and relation with their chemical characteristics. Carbohydr Polym. 2015;117:452–7.
Mu H, Zhang A, Zhang W, et al. Antioxidative properties of crude polysaccharides from *Inonotus obliquus*. Int J Mol Sci. 2012;13:9194–206.
Mu S, Yang W, Huang G. Antioxidant activities and mechanisms of polysaccharides. Chem Biol Drug Design. 2020. https://doi.org/10.1111/cbdd.13798.
O'Sullivan AM, O'Callaghan YC, O'Grady MN, et al. An examination of the potential of seaweed extracts as functional ingredients in milk. Int J Dairy Technol. 2014;67:182–92.
Palanisamy S, Vinosha M, Manikandakrishnan M, et al. Investigation of antioxidant and anticancer potential of fucoidan from Sargassum polycystum. Int J Biol Macromol. 2018;116:151–61.
Paterson RR. *Ganoderma* – a therapeutic fungal biofactory. Phytochemistry. 2006;67:1985–2001.
Peasura N, Laohakunjit N, Kerdchoechuen O, et al. Assessment of biochemical and immunomodulatory activity of sulphated polysaccharides from *Ulva intestinalis*. Int J Biol Macromol. 2016;91:269–77.
Pierre G, Sopena V, Juin C, et al. Antibacterial activity of a sulfated galactan extracted from the marine alga *Chaetomorpha aerea* against *Staphylococcus aureus*. Biotechnol Bioprocess Eng. 2011;16:937–45.
Priyanka P, Arun AB, Young CC, et al. Prospecting exopolysaccharides produced by selected bacteria associated with marine organisms for biotechnological applications. Chin J Polym Sci. 2015;33:236–44.
Rjeibi I, Feriani A, Hentati F, et al. Structural characterization of water-soluble polysaccharides from *Nitraria retusa* fruits and their antioxidant and hypolipidemic activities. Int J Biol Macromol. 2019;129:422–32.
Rjeibi I, Zaabi R, Jouida W, et al. Characterization of Polysaccharides Extracted from Pulps and Seeds of *Crataegus azarolus L. var. aronia*: Preliminary Structure, Antioxidant, Antibacterial, α-Amylase, and Acetylcholinesterase Inhibition Properties. Oxid Med Cell Longev. 2020;2020:1903056, 11 pages. https://doi.org/10.1155/2020/1903056.
Rubavathi S, Ramya M. Invitro assessment of antimicrobial and antioxidant activity of bioactive compounds from marine algae. Int J Curr Microbiol Appl Sci. 2016;5:253–66.
Sabirin F, Kazi JA, Ibrahim IS, Rashit MMA. Screening of seaweeds potential against oral infections. J Appl Sci Res. 2015;11:1–6.
Sathishkumar R, Ananthan G, Senthil SL, et al. Structural characterization and anticancer activity of extracellular polysaccharides from ascidian symbiotic bacterium *Bacillus thuringiensis*. Carbohydr Polym. 2018;190:113–20.
Shanmugan M, Mody KH. Heparinoid-active sulphated polysaccharides from marine algae as potential blood anticoagulant agents. Curr Sci India. 2000;79:1672–83.
Shannon E, Ghannam NA. Antibacterial derivatives of marine algae: an overview of pharmacological mechanisms and applications. Mar Drugs. 2016;14(81):1–23.
Shao BM, Dai H, Xu W, et al. Immune receptors for polysaccharides from *Ganoderma lucidum*. Biochem Biophys Res Commun. 2004;323:133–41.
Shao P, Chen XX, Sun PL. *In vitro* antioxidant and antitumor activities of different sulphated polysaccharides isolated from three algae. Int J Biol Macromol. 2013;62:155–61.
Shen S, Cheng H, Li X, et al. Effects of extraction methods on antioxidant activities of polysaccharides from camellia seed cake. Eur Food Res Technol. 2014;238:1015–21.
Siu KC, Chen X, Wu JY. Constituents actually responsible for the antioxidant activities of crude polysaccharides isolated from mushrooms. J Funct Foods. 2014;11:548–56.
Skalicka-Woźniak K, Szypowski J, Łoś R, et al. Evaluation of polysaccharides content in fruit bodies and their antimicrobial activity of four *Ganoderma lucidum* (W Curt.: Fr.) P. Karst. strains cultivated on different wood type substrates. Acta Soc Bot Pol. 2012;81:17–21.

Somasundaram SN, Shanmugam S, Subramanian B, et al. Cytotoxic effect of fucoidan extracted from Sargassum cinereum on colon cancer cell line HCT-15. Int J Biol Macromol. 2016;91:1215–23.
Song Y, Yang Y, Zhang Y, et al. Effect of acetylation on antioxidant and cytoprotective activity of polysaccharides isolated from pumpkin (*Cucurbita pepo*, lady godiva). Carbohydr Polym. 2013;98:686–91.
Sousa WM, Silva RO, Bezerra FF, et al. Sulfated polysaccharide fraction from marine algae *Solieria filiformis*: structural characterization, gastroprotective and antioxidant effects. Carbohydr Polym. 2016;152:140–8.
Squillaci G, Finamore R, Diana P, et al. Production and properties of an exopolysaccharide synthesized by the extreme halophilic archaeon Haloterrigena turkmenica. Appl Microbiol Biotechnol. 2015;100:613–23.
Suay I, Arenal F, Asensio FJ, et al. Screening of basidiomycetes for antimicrobial activities. Antonie Van Leeuwenhoek. 2000;78:129–39.
Sun YY, Wang H, Guo GL, et al. The isolation and antioxidant activity of polysaccharides from the marine microalgae *Isochrysis galbana*. Carbohydr Polym. 2014a;113:22–31.
Sun LQ, Wang L, Li L, et al. Characterization and antioxidant activities of degraded polysaccharides from two marine *Chrysophyta*. Food Chem. 2014b;160:1–7.
Sun ML, Zhao F, Shi M, et al. Characterization and biotechnological potential analysis of a new exopolysaccharide from the arctic marine bacterium *Polaribacter sp*. Sci Rep. 2015;5:18435.
Tang Z, Zhou C, Cai Y, et al. Purification, characterization and antioxidant activities in vitro of polysaccharides from *Amaranthus hybridus L*. PeerJ. 2020;8:e9077. https://doi.org/10.7717/peerj.9077.
Trabelsi L, Chaieb O, Mnari A, et al. Partial characterization and antioxidant and antiproliferative activities of the aqueous extracellular polysaccharides from the thermophilic microalgae *Graesiella sp*. BMC Complement Altern Med. 2016;16:210.
Tsiapali E, Whaley A, Kalbfleisch J, et al. Glucans exhibit weak antioxidant activity, but stimulate macrophage free radical activity. Free Radic Biol Med. 2001;30:393–402.
Veenashri BR, Muralikrishna G. *In vitro* anti-oxidant activity of xylo-oligosaccharides derived from cereal and millet brans – a comparative study. Food Chem. 2011;126:1475–81.
Vishwakarma J, Vavilala SL. Evaluating the antibacterial and antibiofilm potential of sulphated polysaccharides extracted from green algae *Chlamydomonas reinhardtii*. J Appl Microbiol. 2019;127:1004–17.
Wang H, Gao XD, Zhou GC, et al. *In vitro* and *in vivo* antioxidant activity of aqueous extract from *Choerospondias axillaris* fruit. Food Chem. 2008;106:888–95.
Wang X, Wang J, Zhang J, et al. Structure- antioxidant relationships of sulfated galactomannan from guar gum. Int J of Biol Macromol. 2010;46:59–66.
Wang C, Chen Y, Hu M, et al. *In vitro* antioxidant activities of the polysaccharides from *Tricholoma lobayense*. Int J Biol Macromol. 2012a;50:534–9.
Wang Y, Yang Z, Wei X, et al. Antioxidant activities potential of tea polysaccharide fractions obtained by ultra filtration. Int J Biol Macromol. 2012b;50:558–64.
Wang J, Zhao B, Wang X, et al. Synthesis of selenium-containing polysaccharides and evaluation of antioxidant activity *in vitro*. Int J Biol Macromol. 2012c;51:987–91.
Wang CY, Mao WJ, Chen ZQ, et al. Purification, structural characterization and antioxidant property of an extracellular polysaccharide from *Aspergillus terreus*. Process Biochem. 2013a;48:1395–401.
Wang Y, Zhao Y, Andrae-Marobela K, et al. Tea polysaccharides as food antioxidants: an old woman's tale? Food Chem. 2013b;138:1923–7.
Wang X, Zhang Z, Yao Z, et al. Sulfation, anticoagulant and antioxidant activities of polysaccharide from green algae *Enteromorpha linza*. Int J Biol Macromol. 2013c;58:225–30.
Wang J, Yang T, Tian J, et al. Synthesis and characterization of phosphorylated galactomannan: the effect of DS on solution conformation and antioxidant activities. Carbohydr Polym. 2014a;113:325–35.

Wang QA, Song YF, He YH, et al. Structural characterization of algae Costaria costata fucoidan and its effects on CCl4-induced liver injury. Carbohydr Polym. 2014b;107:247–54.
Wang J, Hu S, Nie S, et al. Reviews on mechanisms of in vitro antioxidant activity of polysaccharides. Oxid Med Cell Long. 2016;2016:Article ID 5692852. https://doi.org/10.1155/2016/5692852.
Wang CL, Fan QP, Zhang XF, et al. Isolation, characterization, and pharmaceutical applications of an exopolysaccharide from *Aerococcus Uriaeequi*. Mar Drugs. 2018;16:337.
Wei M, Xiong SL, Jin H, et al. Isolation and antioxidant activity of water-soluble acidic polysaccharide from *Prunella vulgaris* Linn. Food Sci. 2010;1:22.
Wei D, Chen T, Yan M, et al. Synthesis, characterization, antioxidant activity and neuroprotective effects of selenium polysaccharide from *Radix hedysari*. Carbohydr Polym. 2015;125:161–8.
Wu Z, Ming J, Gao R, et al. Characterization and antioxidant activity of the complex of tea polyphenols and oat *β*-glucan. J Agric Food Chem. 2011;59:10737–46.
Xia S, Gao BY, Li AF, et al. Preliminary characterization, antioxidant properties and production of *chrysolaminarin* from marine Diatom *Odontella aurita*. Mar Drugs. 2014;12:4883–97.
Xie W, Xu P, Liu Q. Antioxidant activity of water-soluble chitosan derivatives. Bioorg Med Chem Lett. 2001;11:1699–701.
Xie J, Wang Z, Shen M, et al. Sulfated modification, characterization and antioxidant activities of polysaccharide from *Cyclocarya paliurus*. Food Hydrocoll. 2015;53:7–15.
Xie JH, Jin ML, Morris GA, Zha XQ, ChenHQ YY, LiJE WZJ, GaoJ NSP, Shang P, Xie MY. Advances on bioactive polysaccharides from medicinal plants. Crit Rev Food Sci Nutr. 2016;56(supp1):S60–84. https://doi.org/10.1080/10408398.2015.1069255.
Xu H, Huang K. Selenium: its chemistry, biochemistry and application in life science. Wuhan: Huazhong Science and Technology University Press; 1994.
Xu J, Liu W, Yao W, et al. Carboxymethylation of a polysaccharide extracted from *Ganoderma lucidum* enhances its antioxidant activities *in vitro*. Carbohydr Polym. 2009;78:227–34.
Yamashita S, Sugita-Konishi Y, Shimizu M, et al. In vitro bacteriostatic effects of dietary polysaccharides. Food Sci Technol Res. 2001;7:262–4.
Yamauchi R, Tatsumi Y, Asano M, et al. Effect of metal salts and fructose on the autoxidation of methyl linoleate in emulsions. Agric Biol Chem. 1998;52:849–50.
Yan JK, Wang WQ, Ma HL, et al. Sulfation and enhanced antioxidant capacity of an exopolysaccharide produced by the medicinal fungus *Cordyceps sinensis*. Molecules. 2012;18:167–77.
Yan MX, Mao WJ, Liu X, et al. Extracellular polysaccharide with novel structure and antioxidant property produced by the deep-sea fungus *Aspergillus versicolor* N(2)bC. Carbohydr Polym. 2016;147:272–81.
Yang Y, Liu D, Chen Y, Wang S. *In vitro* antioxidant activities of sulfated polysaccharide fractions extracted from *Corallina officinalis*. Int J Biol Macromol. 2011;49: 1031–7.
Yoon SY, Eo SK, Kim YS, et al. Antimicrobial activity of Ganoderma lucidum extract alone and in combination with some antibiotics. Arch Pharm Res. 1994;17:438–4420.
Younes I, Rinaudo M. Chitin and chitosan preparation from marine sources. Structure, properties and applications. Mar Drugs. 2015;13:1133–74.
Yu F, Sheng J, Xu J, et al. Antioxidant activities of crude tea polyphenols, polysaccharides and proteins of selenium-enriched tea and regular green tea. Eur Food Res Technol. 2007;225:843–8.
Zha XQ, Wang JH, Yang XF, et al. Antioxidant properties of polysaccharide fractions with different molecular mass extracted with hot-water from rice bran. Carbohydr Polym. 2009;78:570–5.
Zhao L, Zhao G, Du M, et al. Effect of selenium on increasing free radical scavenging activities of polysaccharide extracts from a Se-enriched mushroom species of the genus *Ganoderma*. Eur Food Res Technol. 2008;226:499–505.

Zhao B, Zhang J, Yao J, et al. Selenylation modification can enhance antioxidant activity of *Potentilla anserina* L. polysaccharide. Int J Biol Macromol. 2013;58:320–8.
Zhao Q, Dong B, Chen J, Zhao B, Wang X, Wang L, Zha S, Wang Y, Zhang J, Wang Y. Effect of drying methods on physicochemical properties and antioxidant activities of wolfberry (*Lycium barbarum*) polysaccharide. Carbohydr Polym. 2015;127:176–81.
Zhao D, Xu J, Xu X. Bioactivity of fucoidan extracted from *Laminaria japonica* using a novel procedure with high yield. Food Chem. 2018;245:911–8.
Zhu H, Sheng K, Yan E, Qiao J, Lv F. Extraction, purification and antibacterial activities of a polysaccharide from spent mushroom substrate. Int J Biol Macromol. 2012;50:840–3.

Gel Properties of Microbial Polysaccharides 26

Gizem Akan and Ebru Toksoy Oner

Contents

Abstract

Microbial polysaccharides are natural sources for intermolecular gel structures used in several fields such as food, pharmaceutical, cosmetics, and other industries owing to their advanced biological properties such as biocompatibility, biodegradability, and nontoxicity. They are produced by microbes such as bacteria, archaea and fungi as secondary metabolites for supporting cellular structure, sustaining cell metabolism, storing energy within the cell, host defense, cell-to-

G. Akan · E. T. Oner (✉)
IBSB Industrial Biotechnology and Systems Biology Research Group, Department of Bioengineering, Marmara University, Istanbul, Turkey
e-mail: ebru.toksoy@marmara.edu.tr

J. M. Oliveira et al. (eds.), *Polysaccharides of Microbial Origin*,
https://doi.org/10.1007/978-3-030-42215-8_34

cell signaling, etc. Some microbial polysaccharides show anticancer, antioxidant, antimicrobial, and immunomodulatory activities. Bioactivities of microbial polysaccharides are affected by molecular weight, structure, monosaccharide composition, and functional groups. To enhance bioactivities of microbial polysaccharides, several modification methods are used such as physical and chemical cross-linking reactions. Xanthan gum, dextran, bacterial alginate, gellan gum, hyaluronic acid, curdlan, pullulan, and levan are some well-known microbial polysaccharides. Gelation of these microbial polysaccharides brings them several mechanical and biological properties which allow their use in biomedical applications. In this chapter, general information about microbial polysaccharides and gels, types of gels, characterization of gels, and gelling properties of microbial polysaccharides such as conditions of gel formation, physical properties, stability of polysaccharide gels, and biological properties were covered.

Keywords

Bioactive polysaccharides · Biopolymers · Microbial polysaccharides · Gel · Hydrogel

1 Introduction

Polysaccharides are biological macromolecules that belong to the carbohydrate family and synthesized by many living organisms. They are significant and abundant polymers that consist of sugar monomers (Barcelos et al. 2020). According to the chemical properties of polysaccharides, they are classified into multiple subgroups of heteropolymers or homopolymers, branched or unbranched, repeating or non-repeating, and charged or noncharged. Polysaccharides are either produced and stored inside the cell as intracellular polysaccharides, or secreted to the outside of the cell as exopolysaccharides, or linked to the cell surface as structural polysaccharides (Moradali and Rehm 2020).

Depending on their origin, they are classified as algal, plant polysaccharides, and microbial polysaccharides (Kırtel et al. 2017). While the polysaccharide market is dominated by plant and algal polysaccharides, polysaccharides of microbial origin have several advantages like sustainable and fast production, yields are not affected by seasonal changes, processes are easily scaled-up, high yields and low batch-to-batch variability (Chaisuwan et al. 2020; Hu and Xu 2020). Although they have extraordinary properties, only a few of them are commercially available due to their expensive production and purification processes (Kırtel et al. 2017). Nevertheless, there are lots of research about biomedical applications of microbial polysaccharides such as three-dimensional (3D) cell-laden alginate or hyaluronate-based scaffold structures that are recognized as successful candidates for artificial extracellular matrix (ECM) (Moradali and Rehm 2020).

High portion of polysaccharides are hydrocolloids. They have properties of being water-loving and spontaneous dispersion in water. Hydrocolloids are used as

thickener, stabilizer, emulsifier and gelling agent because of their high molecular weights (Ahmad et al. 2015). Gels are soft, elastic, semisolid systems which do not flow. They consist of two constituents: abundant fluid constituent and a three-dimensional cross-linked network as a result of bonding in the medium of fluid solvent (Nayak and Das 2018).

Hydrogels are three-dimensional structures that can absorb large amount of liquids and biological fluids (Sharma and Tiwari 2020). They are extremely absorbent (water content above 99%), thanks to this property hydrogels have high similarity with natural tissue structure (Banerjee and Bhattacharya 2012). Hydrogels are classified in so many different ways. Generally, they are classified based on the method of cross-linking (chemical cross-linking or physical cross-linking) or physical state (solid, semi-solid, liquid) (Sharma and Tiwari 2020).

This chapter aims to give an overview about microbial polysaccharides, gels, sources of polysaccharides, modification methods of polysaccharides, types of gels, characterization of gels, and gelling properties of microbial polysaccharides such as conditions of gel formation, physical properties, stability of polysaccharide gels and biological properties.

2 Microbial Polysaccharides

Carbohydrates are abundant biological molecules that are essential for most of the living organisms and they are classified as monosaccharides, oligosaccharides and polysaccharides. Whereas monosaccharides consist of only a single polyhydroxyaldehyde or ketone unit, oligosaccharides are short-chains of monosaccharide units containing a small number of monomers linked by glycosidic bonds and polysaccharides (so-called glycans) contain more than 20 monomeric sugars (Porter and Martens 2017). These macromolecules of life show considerable diversity in their composition and structure. Depending on the composition of their monosaccharide units, polysaccharides are categorized as homopolysaccharides and heteropolysaccharides. Homopolysaccharides consist of a single type of monosaccharide like glucan-type polysaccharides such as cellulose or dextran that are made up of glucose units or fructan-type polysaccharides such as inulin and levan where the monomeric unit is fructose. On the other hand, heteropolysaccharides have two or more different monosaccharide units and the repeating unit mostly consists of three to eight sugar residues as in xanthan or gellan (Xie et al. 2016).

Biosynthesis of homopolysaccharides is carried out by the action of a single glycoside hydrolase (sucrase) enzyme that uses sucrose as substrate and catalyzes the transglycosylation reactions forming the polymer chain. However, biosynthetic pathways of heteropolysaccharides involve multiple steps catalyzed by several enzymes at different cellular locations and hence are more complex. It starts with the uptake of sugar subunits and their activation with a high-energy bond through their conversion into sugar-nucleotides and followed by the assembly of the repeating monosaccharide unit on an isoprenoid lipid carrier by sequential transfer of monosaccharides from sugar nucleotides by glycosyltransferases; addition of any

acyl groups; polymerization of the repeating unit and secretion of the polysaccharide from the cell membrane into the extracellular environment (Kırtel et al. 2017).

Polysaccharides have several functions in nature that are linked to their cellular location as well. Some polysaccharides are produced and stored inside the cell as intracellular polysaccharides like glycogen or starch that serve as energy storage while others are present at the outer membrane as lipopolysaccharides (LPS) that mainly determine the immunogenic properties or serve as structural support like chitin or cellulose. Capsular polysaccharides (CPS) form a discrete surface layer (capsule) associated with the cell surface and they are responsible for virulence factors and pathogenicity like resistance to specific and nonspecific host immunity and adherence. Some polysaccharides are excreted to the outside of the cell as exopolysaccharides (EPS) which are related with cellular adhesion, cell to-cell interactions, biofilm formation and cell protection against environmental extremes. Motility is also an important phenomena since EPS like cellulose, alginate, or hyaluronic acid are produced when pathogens enter sessile, non-motile lifestyle where biofilm is formed that shields the pathogen from the attack of immune cells and antibiotic drugs (Moradali and Rehm 2020; Kırtel et al. 2017).

Microbial polysaccharides stand out from the other polysaccharides owing to their wide range of usability in industries such as food, pharmaceutical, cosmetics, and advanced biological properties such as biocompatibility, biodegradability, and non-toxicity (Ahmad et al. 2015; Hu and Xu 2020). They are produced through sugar fermentation from various microorganisms such as *Pseudomonas elodea, Alcaligenes faecais, Xanthomonas campestris, Halomonas smyrnensis, Zymomonas mobilis*, and *Acetobacter xylinum* for supporting cellular structure, sustaining cell metabolism, storing energy within the cell, host defense, cell-to-cell signaling, etc. (Wu et al. 2020). Xanthan gum, dextran, bacterial alginate, gellan gum, bacterial cellulose, hyaluronic acid, curdlan, pullulan, and levan are some of the well-known microbial polysaccharides (Tabernero and Cardea 2020; Yang et al. 2020). Polysaccharides from microbial origin have several advantages like sustainable and fast production processes that are easily scaled-up, high yields that are not affected by seasonal changes and low batch-to-batch variability of shorter production time when compared to polysaccharides of plant or algal origins (Yang et al. 2020). However, despite these features, they highly suffer from expensive production and downstream processes and hence do not have a solid share in the market yet (Kırtel et al. 2017).

3 Gels

Gels are soft, elastic, semisolid systems which do not flow. They consist of two constituents: abundant fluid constituent and a three-dimensional cross-linked network as a result of bondings in the medium of fluid solvent (Nayak and Das 2018). Gels are categorized according to the type of solvent entrapped in that three-dimensional network (Mondal et al. 2020). Various fluids can be used to obtain

gels such as water, oil, and air. According to the solvent material used in the gelation process, gels are categorized as organogels, xerogels, aerogels, and hydrogels. In organogels, the organic phase is entrapped in the three-dimensional mesh. They are thermo-reversible, non-glassy, non-crystalline materials. Xerogels are produced by drying a gel with unhindered shrinkage. It has a high porous structure and high surface area in addition to its small pore size. Aerogels are materials in which entrapped fluid in structuring network is replaced with gas. In aerogels, solvent removal under super critical conditions gives a gel structure with low density and high porosity. Hydrogels are materials in which water is entrapped in the three-dimensional network (Banerjee and Bhattacharya 2012).

Polymer gels have a large market spanning diverse industries from health care to oil and gas mainly dominated by the Asia Pacific region and the market size is expected to increase from 38 billion USD in 2016 to more than 55 billion USD with over 6% CAGR by 2024. Especially, an exponential growth is expected in the medical & healthcare applications where hydrogels have a solid share for applications in tissue engineering, bone and cardiac regeneration, drug delivery systems, contact lenses, wound care, cosmetic surgeries (breast implants), spinal cord repair, etc. Whereas almost 50% of the raw materials are poly acrylic acid based, there is a rising demand for biobased gels like polysaccharides and proteins. Over 40% of the market is dominated by particulate gels that include granules, micro-particles and powders, widely used in various applications such as personal care and agriculture. 3M, BASF, KAO Corporation, Hydrogel Healthcare Ltd., B. Braun Melsungen AG, Medtronic Inc., LG Chem Ltd., Sanyo, Alliqua Biomedical Inc., Medline Industries Inc. and many other are major players in hydrogel market that constitute over 50% of total polymer gel market share (Global Market Insights 2020).

4 Hydrogels

Hydrogels are three-dimensional structures that can absorb large amount of liquids and biological fluids (Sharma and Tiwari 2020). They are extremely absorbent (water content above 99%), thanks to this property hydrogels have high similarity with natural tissue structure. They are also sensitive gels to the changes in environment, they are able to change their structure as a response for changes in temperature, pH, and concentration (Banerjee and Bhattacharya 2012). As a result of these advantages of hydrogels, they are widely used in many biological applications such as drug and protein delivery, tissue engineering, wound dressings and enzyme immobilization (Nayak and Das 2018). Hydrogels are effectively used in biomedical applications by reason of biocompatibility, flexible synthesis methods, and suitable physical properties. Polysaccharide-based hydrogels are favorably used materials especially for wound dressings and they have features of high biodegradability, non-toxicity, high water absorbing capacity, being renewable and biocompatible (Hu and Xu 2020).

4.1 Classification of Hydrogels

Hydrogels are classified in so many different ways. Generally, they are classified based on the method of cross-linking (chemical cross-linking or physical cross-linking) or physical state (solid, semi-solid, liquid) (Sharma and Tiwari 2020).

Since hydrogels are mainly constructed with cross-linking, they fall into two main categories based on cross-linking: physically cross-linked hydrogels and chemically cross-linked hydrogels (Sharma and Tiwari 2020). These two cross-linking methods vary from each other with respect to the utilization of cross-linking agents. Cross-linking agents are not very desirable for biological applications due to toxicity of the agents. These agents have to be completely removed before use in vivo. Cross-linking also affects the integrity of the substances to be captured and cross-linked. On the other hand, chemical cross-linking leads to an enhancement in gel system's mechanical stability as a consequence of covalent bonds and inter/intra molecular linkages formed (Ahmad et al. 2015).

Physically cross-linked hydrogels are protected from dissolving by physical interactions between polymer chains. Physically cross-linked hydrogels are gaining attention owing to their relatively simple production and not including any use of chemical agents in the production method. There are several methods to produce physically cross-linked hydrogels such as ionic interaction, hydrogen bonding, freeze-thawing, and stereo complex formation (Sharma and Tiwari 2020).

Ionic cross-linking is carried out by adding divalent and trivalent counter ions to the structural network. Polymers with repeating ionizable groups interact with multivalent oppositely charged ions and form ionically cross-linked gel structures. As an example for this type of cross-linking, alginate with mannuronic and glucuronic acid residues is able to be cross-linked by calcium ions (Ebara et al. 2014). Hydrogen bonding can also be used to obtain physically cross-linked gel networks. However, they are weak structures and may be dilute and be disrupted by flow of water in a few hours. Because of this, their use is limited, they are generally used for short-acting drug release systems (Ebara et al. 2014; Hu et al. 2019).

Repeating freeze-thawing cycles can cause formation of physically cross-linked hydrogels. They are obtained by microcrystal formation in gel structure as a result of freeze-thawing. This method is widely applied in biological applications owing to enhanced mechanical structure produced compared to the other methods (Sharma and Tiwari 2020). In stereo complex formation, racemic crystallization occurs between two polymeric enantiomers formed by stereo complex interactions which can be used to produce physically cross-linked hydrogels (Hu et al. 2019).

In chemical cross-linking, polymer chains are covalently bonded to form strong hydrogel structure. For this reason, chemically cross-linked hydrogels are stable and not easily being dissolved by any solvent (Chung and Park 2009). Physical cross-linking is not a desirable method compared to the chemically cross-linked hydrogels, due to the some properties such as pore size of the internal gel network, time of gelation and degradation (Slaughter et al. 2009).

There are several methods to produce chemically cross-linked hydrogels such as free radical polymerization, oxime reaction, enzymatic cross-linking, Diels–Alder

reaction, Schiff base formation, and Thiol-Michael addition (Hu et al. 2019; Xiang et al. 2020).

In medical applications, hydrogels with specific physical properties are gaining attention because of their incomparable characteristics of swelling and diffusion. These properties are generally related with the composition of the hydrogel. Depending on the variety of compositions, hydrogels are classified as solid, semi-solid and liquid hydrogels (Sharma and Tiwari 2020).

5 Gel Properties of Microbial Polysaccharides

Polysaccharide gels have different unique properties related to the having different features such as cross-linking type, constituent polymers, size, etc. Polysaccharide gels are judged by some significant properties, which are swelling, aging, rheological behavior, syneresis, aging, electrostatic potential distribution, electrical oscillation, mechanoelectric effect, electrical contraction, interaction with oppositely charged surfactants, etc. For the most part, these gels show sensitive behavior against external stimuli such as pH, pressure, temperature, and electric fields (Nayak and Das 2018).

5.1 Conditions of Gel Formation

Commercially available polysaccharides are generally sold and stored in powder form. Polysaccharide powder must be dispersed into water before any application. Thus, the interaction of polysaccharide with water can happen immediately, causing the powder to accumulate and then get wet, dispersed, and dissolve. A sol with various rheological properties will be achieved, meanwhile the polysaccharide powder is fully hydrated. These rheological properties depend on some factors such as the polymer concentration, polysaccharide types (internal structural properties), in addition to the temperature, pH and ionic environment (external environmental factors). Fundamentally, these factors show their effects by changing polysaccharides' binding structures.

Some polysaccharides are able to form gel structure easily, while the other ones have poor gelling property. They are categorized as gelling polysaccharides and non-gelling polysaccharides. Gelling microbial polysaccharides have a tendency to form stable intermolecularly related gel structures. Hence, they can easily form a gel when their external conditions are changed to cause gelation. On the other hand, mild intermolecular entanglements can be formed in some polysaccharides. When exposed to shear stress, entanglements may easily deteriorate, and this ensures that the aqueous solutions of polysaccharides show significant fluidity. These type of polysaccharides are typically classified as non-gelling polysaccharides (Yang et al. 2020).

In addition, some polysaccharides may have weak gel structures that act like elastic gels under low deformation conditions but can break down under high shear

or large deformation, although they are soft and flexible. Structures of weak gels are in between solutions (composed of polysaccharides) and true strong gels. Xanthan gum and gellan gum are typical examples having weak gel properties (Mohammed et al. 2007).

Gel formation conditions generally depend on various physicochemical factors. The formation of gels can be generally induced by two factors such as temperature (thermal-induced gels) and ionic strength (ionic induced gels) (Banerjee and Bhattacharya 2012).

For polysaccharides having high negative charges, alginate as an example, the formation of linking regions is related to the ionic cross-links between dissociated carboxylic groups (-COOH) in contiguous polysaccharide chains. The mechanism of gelling is named as "egg-box" model (Mondal et al. 2020).

In addition, several gelling polysaccharides (e.g., gellan) are able to form a gel in the condition lack of divalent cations (Morris et al. 2012). Such a gelation mechanism generally includes the conformational transition from the irregular coil geometry to the double helix during cooling, and then the double helix aggregations during further cooling (Ventura et al. 2013). Under the circumstances, the presence of cations may suppress intermolecular electrostatic repulsions which leads to support for gelation process.

Internal molecular properties have an important part of role of influencing the gelling behavior of polysaccharides. For instance, polysaccharides with the high molecular mass of polysaccharides can easily form intermolecular interactions. Likewise, the ratio of α-L-glucuronate in alginate affects the gelling property of alginate. Favorability of reacting with bivalent cations is significantly increased by the content of increased amounts of glucuronate residues in the alginate (Yang et al. 2020).

Thermal-induced polysaccharide gels are classified as cold-set gels and heat-set gels. Heat-set gels are formed by heating and causing conformational changes in polymer's structure which allow to the intermolecular interactions and water binding. Gels formed from microbial polysaccharides curdlan are typical examples for heat-set gels (Zhang et al. 2020). In case of cold-set gels, they are formed by cooling down the hot polysaccharide solutions. Generally, they gelate by the help of hydrogen bondings caused by conformational changes in polysaccharide's molecular structure. These gels often show thermo-reversible properties. Although cold-set gels have strong properties at low temperatures, they generally tend to disintegrate above critical temperatures. Typical examples for cold-set gels are gellan and carrageenans (Jones and McClements 2010).

5.2 Physical Properties

Although high portion of polysaccharides have gelling capacity, gel properties and gelling conditions are distinctive. These distinctive features are related with the sources of polysaccharides and setting conditions of gel. Because of this, various

polysaccharide gels with different physical and textural properties are able to be produced (Yang et al. 2020).

Physical morphological characterization of hydrogels usually involves tests for water content, water vapor transmission rate, and mechanical property. Water content is one of the most significant characteristics in hydrogel dressings (Mousavi et al. 2019). The porosity and three-dimensional structure of the hydrogel have significant effect on the moisture content (Field and Kerstein 1994). To analyze the water content of hydrogels, there are two different methods. One of them is thermogravimetric analysis (TGA) and the other one is swelling rate test (SR). In addition, swelling rate and kinetic degradation are able to be determined with a swelling equilibrium test (Xiang et al. 2020).

The mechanical properties of the polysaccharide gels are highly related to their range of applications. There are several ways or tools to determine the mechanical properties. Rheometry, textural analysis, and dynamic mechanical analysis are some of them (Lin et al. 2020).

Rheological properties are related to the existing molecular network. For a gel under compression, relationship between tension (force per unit area) and stress (deformation as a result of applied force) can be determined. Young's modulus or elastic modulus is the rate of the stress to strain of a material during testing in the linear elastic limit. Maximum stress condition that gel can resist is the rupture strength. Change in volume with respect to original volume during application of force from all directions can be determined and this gives the bulk modulus (Kim et al. 2006).

5.3 Stability of Polysaccharide Gels

Polysaccharides when used in gel structures, they can be responsive to the various external stimuli factors. Because of this property, they generally categorized as pH responsive polysaccharides, thermo-responsive polysaccharides, and multi-responsive polysaccharides.

Polysaccharides have large number of polar hydroxyl groups; they can form a broad structural network of hydrogen bonds between different polymer molecules and water molecules in aqueous solutions. Overhydration significantly affects the stability and physiochemical properties of the polysaccharide gels. The environmental pH is very important for degradation properties of polysaccharide gels. For example, alginate shows different degradation characteristics in different pH conditions.

In biological applications, polysaccharides in the gels may interact with the enzymes present in human body and disintegrate. For instance, alginate cannot be broken down in mammals due to the deficiency of alginase enzyme that can break down the polymer chains. Nevertheless, degradation by unbounding of the divalent ions from the polysaccharide to the environment in consequence of reaction with monovalent ions (e.g., sodium ions) can be achieved in ionically induced alginate gels. In the case of gellan gum hydrogels, they are stable and not disintegrate in

acidic environment. Under high pH conditions, gellan gum hydrogels swell. In another case, carrageenan is known to degrade in acidic stomach conditions. Furthermore, β-(1-4) bond present in carraegeenan can be cleaved by intestinal enzyme lactases in human body.

5.4 Biological Properties

Polysaccharides from different origins have varying biological activities. Their activities varies according to the molecular weight, structural conformation (type of linkage and degree of branching), functional groups, and composition (Chaisuwan et al. 2020).

For biological applications, mechanical properties are significant criteria in assessing the feasibility of a hydrogel (Baby 2020). For instance, the gel must be stiff enough to maintain its form as a scaffold for cell growth because mechanical integrity is essential for the cell adhesion, cell phenotyping, and cell gene expression (Waffle et al. 2016). Suitable flow properties of hydrogels allow them to be perfect candidates for injectable therapeutic carriers. In this context, solid pre-formed gels can be delivered to a targeted in vivo region by simple syringe injection (Kopeček 2007). In removing injection shear, the immediate recovery of gel hardness ensures that the encapsulated loads (such as therapeutics) remain localized against biological forces in vivo during the formation of the hydrogel (Baby 2020).

Microbial polysaccharide-based hydrogels have similar properties with the natural extracellular matrix. Thus, they are biocompatible and biodegradable in nature (Upadhyay 2017). In addition, they exhibit incomparable properties like being stimuli-sensitive and bio-sensitive, which make them desirable for various tissue engineering and regenerative medicine applications (Gentilini et al. 2017).

Modifications of polysaccharide molecules by the addition of chemical parts can facilitate cell adhesion, because chemical cross-linking usually involves toxic chemical agent and results in harsh chemical conditions. Comprehensive cleaning is required before the materials are used for biomedical applications. As alternative for toxic chemical agents, there are also some physical modification methods are being applied. Direct mixing of bioactive molecules into the network of hydrogels is offered as a simple method for biological modification.

Gellan gum hydrogels have desirable properties such as biocompatibility, mild gelation conditions, structural similarity with natural glycosaminoglycans present in human body, and adjustable mechanical properties. A mild gelling state facilitates the inclusion of cells that makes gellan gum hydrogels good candidate for several tissue engineering and regenerative medicine applications. However, absence of specific cell adhesion sites in gellan gum restricts their applications for anchorage-dependent cell cultures (Ng et al. 2020).

Conventionally, xanthan gum has a significant position in pharmaceutical applications as binding, thickening, and emulsion stabilizing agent (Katzbauer 1998).

Nowadays, hydrogels from xanthan gum are used as injectable scaffolds for cartilage tissue engineering purposes due to its non-toxic nature and shear thinning properties (Kumar et al. 2018). Even though biocompatibility of xanthan gum hydrogels is high, they have disadvantages like severe gelling conditions, weak mechanical performance, and absence of cell attachment parts and these disadvantages restricts their use in biological applications (Bueno et al. 2014).

Dextran is an important homopolysaccharide used as antithrombotic and volume expander (Sun and Mao 2012). Since dextran is not able to form hydrogel naturally, composite dextran-based hydrogels are being researched (Ng et al. 2020).

Polysaccharide gels designed for tissue engineering and regenerative medicine should ensure cell adhesion, proliferation, and differentiation, like the natural extracellular matrix. Interaction between the cell and scaffold mostly related to the presence of ligands.

Unfortunately, microbial polysaccharides are mostly extracellular polysaccharides that microbes produce for structural purposes, so they naturally lack of these ligands and not able to provide a biological response from cells. As a solution for this problem, bioactive materials are being used as an addition to the matrices made by microbial polysaccharides (Huettner et al. 2018).

On the other hand, microbial polysaccharides have extraordinary biological properties such as anticancer activity, antioxidant activity, anti-inflammatory activity and antimicrobial activity.

Microbial polysaccharides are used for the development of micro or nanoparticles that have significant potential to be applied in biomedical, environmental, food and agricultural industries. Moreover, microbial polysaccharides are responsive to several external stimuli. As a result of this property, stimulus sensitive smart polysaccharides are being investigated for further biotechnological applications (Chaisuwan et al. 2020).

5.5 Swelling Properties

The polymeric gels can swell by absorbing an increased amount of liquid (solvent). Generally, the solvents diffuse into the gel matrix, thus gel interactions are created through gel-solvent interactions. Poor swelling behavior is often the result of some cross-linking in gel matrices that prevent matrix dissolution. In these gel systems, they swell significantly when the solvent mixtures have a solubility parameter compared to the gelling agent. According to the swelling parameter, various polymeric gels which have high swelling property can be produced using polyacrylates. These gels are named as superabsorbent polymers. When in contact with the aqueous medium, superabsorbent polymers swell and a hydrogel structure is obtained. The most important characteristics of these superabsorbent polymers is that they can hold up to 1000 times moisture of their dry weight and they still keep holding the absorbed aqueous medium even under pressure conditions (Nayak and Das 2018).

5.6 Rheological Behavior

Solutions composed of gel materials and dispersions of flocculating materials are often pseudoplastic in nature, that means they show non-Newtonian flow property. When the shear stress applied, the poor configuration of the inorganic particles in the aqueous medium is damaged due to the collapse of the intermolecular network and it shows a better flow. Similarly, the application of shear stress promotes the molecules toward stress and reduces the flow resistance of macromolecules (Nayak and Das 2018).

5.7 Structure

Stiffness of the gel structure results from the emergence of the intermolecular network developed by connection of the gelling components to each other. The character and force type of the gelling components are responsible for the relationships that determine the structure of the network and gel properties (Nayak and Das 2018).

5.8 Syneresis

Many gel systems are often exposed to contractions in their posture. Intermolecular fluid is externalized by accumulating on the gel surface. This behavior is called as syneresis. As the concentrations of polymer components decrease, the syneresis of the gel usually becomes more apparent. This happens because of the loosening of the elastic stresses that are created during the setting process of the gel networks. When the stresses decrease, the intermolecular space reachable for the solvent(s) decreases, which forces the externalization of the liquids (Nayak and Das 2018).

5.9 Aging

Typically, colloidal gel networks show a slow spontaneous accumulation as known as the aging of the gel network. Aging in gel systems leads to the development of relatively dense networks of the gelling agent (Nayak and Das 2018).

5.10 Common Microbial Polysaccharides and Their Gels

5.10.1 Xanthan Gum

Among all microbial polysaccharides, xanthan gum is the mostly used in industry and studied in literature, and is one of the rare microbial polysaccharides that is universally approved for utilization in food products free from any limitation of quantity (Wu et al. 2020). Xanthan is a viscous microbial EPS consisting of

D-glucose, D-mannose and D-glucuronic acid repeating units and mainly produced by the plant-pathogen *Xanthomonas* spp. and play a significant role in pharmaceutical and food industry as stabilizer, emulsifier, thickener, binder. Related to the temperature and ionic strength, xanthan shows various molecular configurations due to its anionic character. It is generally used for increasing viscosity or as coating agent for carriers (Ng et al. 2020). Due to its desired characteristics, xanthan is recently commercialized by many companies such as Pfizer, Sanofi, Merck. Estimated annual consumption of xanthan is about US$23 million, with universal growth of 6–7%. In recent papers, injectable xanthan gum hydrogels for cartilage tissue engineering have been investigated (Barcelos et al. 2020).

Xanthan gum is subjected to a one-stage temperature-dependent gelling process. Dispersion of xanthan gum in water at room temperature causes a formation of colloidal heterogenous suspension. Homogenous solution is obtained after heating the heterogeneous suspension to a temperature above the sol-gel transition temperature. Finally, hard hydrogels are produced after cooling the homogenous solution (Ng et al. 2020).

5.10.2 Gellan Gum

Gellan gum is linear exopolysaccharide generally obtained from *Sphingomonas elodea* and composed of repeating D-glucose, L-rhamnose, and D-glucuronic acid units (Ng et al. 2020). Gellan gum has good gelling property and it can be used to form coatings. Gellan gum has a property of high-temperature stability (Tabernero and Cardea 2020). It is suitable to be used as a modifier for viscosity, or as a stabilizing agent. Gellan gum is significantly used in food industry as a food additive carrier, antimicrobial agent, gelling, and texturisation agent. Gellan can be found commercially in two forms, one of them is acetylated form and the other is deacetylated form. All have gelling properties, but the acetylated form can produce semitransparent elastic gels, on the contrary of deacetylated form which can generate transparent stiff gels. Because of this, deacetylated form is more suitable to form gels for applications in tissue engineering and regenerative medicine (Ng et al. 2020). Gellan gum are also utilized for paper cups, pH monitoring, drug release, gelling agent in dental care, wound healing, paper cleaning, etc. (Barcelos et al. 2020).

The gelation of gellan gum includes a separate two-stage mechanism. At first, a temperature-dependent process is carried out. Randomly coiled chains in the aqueous gellan solution is transformed into highly ordered double helixes by heating the solution above 80 °C for 20–30 min and then cooling it. The next step is cross-linking the solution with cations to obtain a stable hydrogel. In this step, divalent cations are preferred instead of monovalent cations for cross-linking due to the reasons that divalent cations form a strong electrostatic bridge between the backbone of the gellan gum and carboxylates and the monovalent cations are not effective enough for forming strong electrostatic interactions (Tabernero and Cardea 2020).

5.10.3 Dextran

Dextran is an homopolysaccharide composed primarily of linear α-1,6 glycosidic linkages (between glucose monomers) with α-(1,3) glycosidic branches, and

secreted extracellularly by *Leuconostoc, Streptococcus, Weissella* and *Lactobacillus* spp. Molecular weight of the dextran ranges from 10 to 2000 kDa (Barcelos et al. 2020). Dextran has a neutral charge and because of this property, gelling process of the dextran is generally done after modifications in surface (Tabernero and Cardea 2020). Dextran is biocompatible and having a good potential for biomedical applications. Moreover, dextran is able to reduce blood viscosity and avoids the formation of blood clots. Furthermore, dextran hydrogels have been reported that they can enable to neovascularization and skin regeneration in vivo applications (Hu and Xu 2020). Dextran have several industrial applications such as stabilizing and viscosity adjusting agent, and food additive, as well as plasma expander and blood flow adjuvant. In addition, there are commercialized dextran products such as the dextran derivative gel SephadexVR which is produced by Pharmacia and used for gel filtration and purification. In addition to the other features of dextran, it is reported that dextran has antiviral and prebiotic activities. There are various commercialized dextran products in industry, and these are owned by companies such as Pharmacosmos (the leading company among the others, and focused on dextran production and distribution), Pharmachem corporation, Sigma-Aldrich, and Amersham Biosciences (Barcelos et al. 2020).

5.10.4 Hyaluronic Acid

Hyaluronic acid, so-called hyaluronan, is well known linear EPS consisting of repeating disaccharide units of β-(1,3)-*N*-acetyl glucosamine and β-(1,4)-glucuronic acid, and naturally secreted by *Streptococcus* spp. (Barcelos et al. 2020). Besides being produced microbially, hyaluronic acid is found in the soft connective tissue of all living organism as a mucopolysaccharide. Hyluronic acid is highly known due to its applications in cosmeceutical and pharmaceutical industries, and its characteristics of high water-binding capacity, viscous, biocompatible, biodegradable, non-toxic, non-inflammatory, and anti-aging properties (Barcelos et al. 2020). Another feature of the hyaluronic acid is that it can readily form intra- and inter-molecular bonds in aqueous solution because of its structure with great numbers of hydroxyl and carboxyl groups. Hyaluronic acid having numerous advantages, is used for so many applications such as tissue engineering, cancer diagnosis, cell-based and regenerative therapies, drug delivery, etc. Recently in literature, it is reported that tissue tapes from hyaluronan based hydrogels are developed. Hyaluronic acid based hydrogels are multipurpose materials in biomedical applications, since they can be used in many ways especially for tissue engineering purposes (Hu and Xu 2020).

5.10.5 Levan

Levan is a homopolysaccharide composed of β-2,6 glycosidic linked fructose units with branches at β-2,1 positions, and are synthesized generally by levansucrase enzyme in bacteria such as *Bacillus, Halomonas, Zymomonas, Pseudomonas, Streptococcus, Aerobacter, Rahnella, Erwinia, Microbacterium*, but not only produced by bacteria but also by some fungi, yeasts, and some halophilic Archaea. The source of levan is important for its structural-functional features. While high molecular weight (more than 20,000 fructose monomers) with occasional branching is produced by

microbes, plants produce very short linear levan chains of a few hundred fructose residues. This situation makes levan produced from different sources having different industrial applications. Levan has general properties of low viscosity, neutral charge, high water solubility without swelling, adhesive strength, film-forming capacity, as well as bioactivities of anti-tumor, antioxidant, and anti-inflammatory (Öner et al. 2016; Versluys et al. 2018).

Levan polysaccharide has been part of traditional healthy foods consumed by various cultures like the fermented beans of Japanese food Natto or products of sourdough fermentation by lactic acid bacteria. Structurally, levan is very similar to the well-known inulin, a fructan of β-2,1 linked fructose residues that is the most widely used prebiotics in food and feed market. Therefore, health promoting and especially, prebiotic properties of levan have attracted both scientific and industrial attention recently. Moreover, in nature, levan biosynthesis is associated with diverse biological roles like a protective and structural shield in biofilms, as an energy source by bacteria under starvation conditions, as an oxygen barrier helping create the microaerobic conditions required for nitrogen fixation for the endophytes, as a strong adhesive, it helps for cellular attachment to surfaces and it is also involved in several (a)biotic stress resistance mechanisms and signaling which are believed to give an adaptive advantage for plants and microbes to survive under water-limiting conditions. Also, and in fact a very important feature for biomedical applications, levan is known to stabilize biological membranes by maintaining the structural integrity of the lipid bilayers through penetrating the membranes with its small and flexible 5-membered ring in a superior way than a more rigid 6-membered ring does. This feature may become important in designing gels for cryotechnological applications like cell preservation or biobanking.

Since fructans do not participate in the physiological functions of animals, their use as part of gels for drug delivery or tissue engineering purposes become advantageous over glucans like starch and cellulose. Especially for diabetic patients, since the hydrolysis products of levan contains majorly fructose rather than glucose. Hence because all these advantageous features, levan has been gaining attention in a wide variety of applications such as wound healing materials, prebiotics, packaging films, food additives, controlled drug release, and so on. Injectable levan-based hydrogel was developed by Choi et al. for soft tissue engineering purposes (Choi et al. 2018). Moreover, a novel *Halomonas* levan based hydrogel is created with biocompatible, viscoelastic, and porous properties in addition to the highest swelling ratio for levan hydrogels of all time (Demirci et al. 2020). Levan is being used in forms of hydrogels, films, nanofibers, and 3D printed scaffolds (De Siqueira et al. 2020).

5.10.6 Alginate

Alginate is a linear polysaccharide composed of β-D-mannuronic and α-L-guluronic acids generally extracted from brown algae but can also be produced microbially by soil bacteria *Pseudomonas* and *Azotobacter* spp. Alginates are widely used polysaccharides with so many applications such as encapsulation material in pharmaceuticals, stabilizer and thickener for food, and adhesive agent, filler, and color pigment in paper industry. Cell entrapment and immunologic applications of

alginates are being investigated for biomedical purposes. Alginate hydrogels are produced by cross-linking with divalent cations and used for cell proliferation as well as encapsulation purposes in drugs, cells, vaccines. In addition, alginate hydrogels are used as bioactive materials for wound dressings. These several applications of alginate generally arise from its significant properties of having high viscosity, thermal stability, sol-gel transition. Physical characteristics of alginate hydrogels are affected by the ratio of mannuronic acid and guluronic acid with respect to each other as well as molecular weight, sequence and guluronic acid chain length. On the other hand, bacterial alginates do not have GRAS (Generally Recognized As Safe) approval although they are highly investigated in literature (Barcelos et al. 2020; Moradali and Rehm 2020; Tabernero and Cardea 2020).

5.10.7 Curdlan

Curdlan is a linear microbial exopolysaccharide consisting of linear β-(1,3) glucose residues. While it is not soluble in water, it is soluble in many organic solvents and dimethylsulfoxide (DMSO).

Curdlan has several gel types, one of them is the reversible gel form which is obtained by heating the polysaccharide solution to the temperature of 60 °C, the other one is the irreversible gel form which is produced by heating to the temperature level of 80 °C. Gel formation depends on the helical structure of curdlan at these temperatures. Curdlan has both single and triple helices structure at room temperature and condensed triple helices at higher temperatures. In fact, curdlan is named after this "curdle" competence when heated.

These unusual thermal-dependent gelation properties as well as its immunostimulatory effect make curdlan an important polysaccharide in biomedical industry. In case of applications, curdlan is used for scaffolds, composite gels, and drug carrying systems (Tabernero and Cardea 2020).

5.10.8 Pullulan

Pullulan is a homopolysaccharide of maltotriose units interlinked by α-1,6 glycosidic units and secreted by *Aureobasidium pullulans*. Several applications of pullulan in medical, pharmaceutical, and food industries involves drug-carrier for cancer treatment, drug delivery, wound healing, tissue engineering, granulation and coating of tablets, edible films, and food packaging material. Pullulan is an approved food ingredient in Europe and USA, and generally applied as prebiotic or dietary fiber. It has various properties such as solubility in water, low viscosity, pH stability in wide range, good oxygen barrier, and strong adhesive properties. In polysaccharide market, pullulan have large market value than dextran and xanthan, but it is a very expensive polymer due to the production and purification processes involved (Barcelos et al. 2020; Tabernero and Cardea 2020; Wu et al. 2020).

Unfortunately, pullulan can form viscous solution when dissolved in water, but it cannot form a gel structure. In the presence of metal ions, these ions interact with hydroxyl groups in pullulan which results in an increase in viscosity. But even under these conditions, pullulan is not able to form a gel structure (Jindal and Singh Khattar 2018).

6 Conclusions

This chapter described the general aspects about gelling properties of microbial polysaccharides as well as the knowledge about types of polysaccharide, gels, modification methods, biological properties, mechanical properties, and general market overview. Gels with natural origin like microbial polysaccharide gels, have unique properties of biocompatibility, good cell adhesion, proliferation, biodegradability, and so on. Some microbial polysaccharides show anticancer, antioxidant, antimicrobial, and immunomodulatory activities. Including biomedical applications, microbial polysaccharide gels have so many applications in various industries such as food, pharmaceutical, cosmetics. These applications include drug delivery, coatings, food additives, stabilizers, thickeners, emulsifying agents, and others. However, majority of these applications is generally used in researches, because some of the microbially produced polysaccharides are concerned about having endotoxins residues remaining from the production processes. Although polysaccharides from microbial origins have an advantage of shorter production time when compared to the polysaccharides from other origins, plant and algal originated polysaccharides outshined them because of expensive production and purification processes of microbial polysaccharides. Xanthan gum, dextran, bacterial alginate, gellan gum, hyaluronic acid, curdlan, pullulan, and levan are some well-known microbial polysaccharides. Gelation of these microbial polysaccharides brings them several mechanical and biological properties which allow their use in biomedical applications. However, there are several issues that need to be addressed.

While designing gel systems involving microbial polysaccharides, especially for medical purposes, safety of the cellular factories becomes an important issue. Since EPS is associated with virulence, most of the producer organisms are pathogens and hence their products are also usually contaminated with endotoxins that in turn requires extra steps for downstream processing and also increasing the overall production costs. Hence it is very important to ensure the GRAS status of the producer system and the biopolymer itself. For the homopolysaccharides like dextran or levan of gram-negative pathogens, enzymatic production systems could be advantageous as long as the enzyme is recombinantly produced in an endotoxin free system or purified extensively. For microbial heteropolysaccharides whose biosynthesis involves multiple steps and associated enzymes, GRAS status of the producer system should be known otherwise, the biological responses in in vivo studies could be misleading.

Some of the microbial polysaccharides naturally lack ligands thus, they are not able to provide a biological response from cells. To overcome this, physical and chemical modifications are done to improve their characteristics. Polymer gels have a large market spanning diverse industries from health care to oil and gas, and microbial polysaccharide gels are covering a part of this market. Despite the drawbacks and challenges of microbial polysaccharides and microbial polysaccharide-based gel systems which restricts their commercial use, they have numerous advantages with respect to the other gel systems. Hopefully, drawbacks of microbial polysaccharide gels will be solved by biotechnological improvements, and their commercial use will become much wider.

References

Ahmad NH, Mustafa S, Man YBC. Microbial polysaccharides and their modification approaches: a review. Int J Food Prop. 2015;18:332–47. https://doi.org/10.1080/10942912.2012.693561.

Baby DK. Chapter 9: Rheology of hydrogels. In: Thomas S, Sarathchandran C, Chandran N, editors. Rheology of polymer blends and nanocomposites. Micro and nano technologies. Amsterdam: Elsevier; 2020. p. 193–204.

Banerjee S, Bhattacharya S. Food gels: gelling process and new applications. Crit Rev Food Sci Nutr. 2012;52:334–46. https://doi.org/10.1080/10408398.2010.500234.

Barcelos MCS, Vespermann KAC, Pelissari FM, Molina G. Current status of biotechnological production and applications of microbial exopolysaccharides. Crit Rev Food Sci Nutr. 2020;60:1475–95. https://doi.org/10.1080/10408398.2019.1575791.

Bueno VB, Bentini R, Catalani LH, et al. Synthesis and characterization of xanthan–hydroxyapatite nanocomposites for cellular uptake. Mater Sci Eng C. 2014;37:195–203. https://doi.org/10.1016/j.msec.2014.01.002.

Chaisuwan W, Jantanasakulwong K, Wangtueai S, et al. Microbial exopolysaccharides for immune enhancement: fermentation, modifications and bioactivities. Food Biosci. 2020;35:100564. https://doi.org/10.1016/j.fbio.2020.100564.

Choi W II, Hwang Y, Sahu A, et al. An injectable and physical Levan-based hydrogel as a dermal filler for soft tissue augmentation. Biomater Sci. 2018;6:2627–38.

Chung HJ, Park TG. Self-assembled and nanostructured hydrogels for drug delivery and tissue engineering. Nano Today. 2009;4:429–37.

De Siqueira EC, de Souza RJ, Pinheiro IO, Formiga FR. Levan-based nanostructured systems: an overview. Int J Pharm. 2020;580:119242.

Demirci T, Hasköylü ME, Eroğlu MS, et al. Levan-based hydrogels for controlled release of Amphotericin B for dermal local antifungal therapy of Candidiasis. Eur J Pharm Sci. 2020;145:105255.

Ebara M, Kotsuchibashi Y, Uto K, et al. Smart hydrogels. Tokyo: Springer; 2014. p. 9–65.

Field CK, Kerstein MD. Overview of wound healing in a moist environment. Am J Surg. 1994;167:S2–6. https://doi.org/10.1016/0002-9610(94)90002-7.

Gentilini R, Munarin F, Bloise N, et al. Polysaccharide-based hydrogels with tunable composition as 3D cell culture systems. Int J Artif Organs. 2017;41:213–22. https://doi.org/10.5301/ijao.5000667.

Global Market Insights. Polymer gel market growth – industry size outlook report 2017–2024. Global Market Insights, Inc. 2020. https://www.gminsights.com/industry-analysis/polymer-gel-market. Accessed 17 July 2020.

Hu H, Xu FJ. Rational design and latest advances of polysaccharide-based hydrogels for wound healing. Biomater Sci. 2020;8:2084–101. https://doi.org/10.1039/d0bm00055h.

Hu W, Wang Z, Xiao Y, et al. Advances in crosslinking strategies of biomedical hydrogels. Biomater Sci. 2019;7:843–55. https://doi.org/10.1039/c8bm01246f.

Huettner N, Dargaville TR, Forget A. Discovering cell-adhesion peptides in tissue engineering: beyond RGD. Trends Biotechnol. 2018;36:372–83. https://doi.org/10.1016/j.tibtech.2018.01.008.

Jindal N, Singh Khattar J. Chapter 4: Microbial polysaccharides in food industry. In: Grumezescu AM, Holban AM (eds) Biopolymers for food design. Handbook of food bioengineering. London Academic; 2018. p. 95–123.

Jones OG, McClements DJ. Functional biopolymer particles: design, fabrication, and applications. Compr Rev Food Sci Food Saf. 2010;9:374–97. https://doi.org/10.1111/j.1541-4337.2010.00118.x.

Katzbauer B. Properties and applications of xanthan gum. Polym Degrad Stab. 1998;59:81–4. https://doi.org/10.1016/S0141-3910(97)00180-8.

Kim H-J, Decker EA, Julian McClements D. Preparation of multiple emulsions based on thermodynamic incompatibility of heat-denatured whey protein and pectin solutions. Food Hydrocoll. 2006;20:586–95. https://doi.org/10.1016/j.foodhyd.2005.06.007.
Kırtel O, Avşar G, Erkorkmaz BA, Öner ET. Microbial polysaccharides as food ingredients. In: Microbial production of food ingredients and additives. Elsevier; 2017. p. 347–83.
Kopeček J. Hydrogel biomaterials: a smart future? Biomaterials. 2007;28:5185–92. https://doi.org/10.1016/j.biomaterials.2007.07.044.
Kumar A, Rao KM, Han SS. Application of xanthan gum as polysaccharide in tissue engineering: a review. Carbohydr Polym. 2018;180:128–44. https://doi.org/10.1016/j.carbpol.2017.10.009.
Lin D, Kelly AL, Miao S. Preparation, structure-property relationships and applications of different emulsion gels: bulk emulsion gels, emulsion gel particles, and fluid emulsion gels. Trends Food Sci Technol. 2020;102:123–37. https://doi.org/10.1016/j.tifs.2020.05.024.
Mohammed ZH, Haque A, Richardson RK, Morris ER. Promotion and inhibition of xanthan "weak-gel" rheology by calcium ions. Carbohydr Polym. 2007;70:38–45. https://doi.org/10.1016/j.carbpol.2007.02.026.
Mondal S, Das S, Nandi AK. A review on recent advances in polymer and peptide hydrogels. Soft Matter. 2020;16:1404–54. https://doi.org/10.1039/c9sm02127b.
Moradali MF, Rehm BHA. Bacterial biopolymers: from pathogenesis to advanced materials. Nat Rev Microbiol. 2020;18:195–210. https://doi.org/10.1038/s41579-019-0313-3.
Morris ER, Nishinari K, Rinaudo M. Gelation of gellan – a review. Food Hydrocoll. 2012;28:373–411.
Mousavi S, Khoshfetrat AB, Khatami N, et al. Comparative study of collagen and gelatin in chitosan-based hydrogels for effective wound dressing: physical properties and fibroblastic cell behavior. Biochem Biophys Res Commun. 2019;518:625–31. https://doi.org/10.1016/j.bbrc.2019.08.102.
Nayak AK, Das B. Introduction to polymeric gels. In: Polymeric gels. Elsevier; 2018. p. 3–27.
Ng JY, Obuobi S, Chua ML, et al. Biomimicry of microbial polysaccharide hydrogels for tissue engineering and regenerative medicine – a review. Elsevier; 2020.
Öner ET, Hernández L, Combie J. Review of Levan polysaccharide: from a century of past experiences to future prospects. Biotechnol Adv. 2016;34:827–44. https://doi.org/10.1016/j.biotechadv.2016.05.002.
Porter NT, Martens EC. The critical roles of polysaccharides in gut microbial ecology and physiology. Annu Rev Microbiol. 2017;71:349–69.
Sharma S, Tiwari S. A review on biomacromolecular hydrogel classification and its applications. Int J Biol Macromol. 2020;162:737–47. https://doi.org/10.1016/j.ijbiomac.2020.06.110.
Slaughter BV, Khurshid SS, Fisher OZ, et al. Hydrogels in regenerative medicine. Adv Mater. 2009;21:3307–29.
Sun G, Mao JJ. Engineering dextran-based scaffolds for drug delivery and tissue repair. Nanomedicine. 2012;7:1771–84.
Tabernero A, Cardea S. Supercritical carbon dioxide techniques for processing microbial exopolysaccharides used in biomedical applications. Mater Sci Eng C. 2020;112:110940. https://doi.org/10.1016/j.msec.2020.110940.
Upadhyay R. Use of polysaccharide hydrogels in drug delivery and tissue engineering. Adv Tissue Eng Regen Med Open Access. 2017;2:145.
Ventura I, Jammal J, Bianco-Peled H. Insights into the nanostructure of low-methoxyl pectin–calcium gels. Carbohydr Polym. 2013;97:650–8. https://doi.org/10.1016/j.carbpol.2013.05.055.
Versluys M, Kirtel O, Toksoy Öner E, Van den Ende W. The Fructan syndrome: evolutionary aspects and common themes among plants and microbes. Plant Cell Environ. 2018;41:16–38.
Waffle L, Godin L, Harris LB, Kontopoulou M. Rheological and physical characteristics of crustal-scaled materials for centrifuge analogue modelling. J Struct Geol. 2016;86:181–99. https://doi.org/10.1016/j.jsg.2016.02.014.
Wu X, Zha J, Koffas MA. Microbial production of bioactive chemicals for human health. Curr Opin Food Sci. 2020;32:9–16. https://doi.org/10.1016/j.cofs.2019.12.007.

Xiang J, Shen L, Hong Y. Status and future scope of hydrogels in wound healing: synthesis, materials and evaluation. Eur Polym J. 2020;130:109609. https://doi.org/10.1016/j.eurpolymj.2020.109609.

Xie J-H, Jin M-L, Morris GA, et al. Advances on bioactive polysaccharides from medicinal plants. Crit Rev Food Sci Nutr. 2016;56:S60–84.

Yang X, Li A, Li X, et al. An overview of classifications, properties of food polysaccharides and their links to applications in improving food textures. Trends Food Sci Technol. 2020;102:1–15. https://doi.org/10.1016/j.tifs.2020.05.020.

Zhang H, Zhang F, Yuan R. Chapter 13: Applications of natural polymer-based hydrogels in the food industry. In: Chen Y, editor. Hydrogels based on natural polymers. Amsterdam: Elsevier; 2020. p. 357–410.

Polysaccharides in Bacterial Biofilm

27

Understanding Oral Biofilm

Bárbara Emanoele Costa Oliveira, Ana Carolina dos Santos Ré, Carolina Patricia Aires, and Antônio Pedro Ricomini Filho

Contents

Abstract

Biofilms are necessary for the development of several oral diseases, and the biofilm matrix is an important virulence trait in these microbial communities. In oral biofilms, the presence of extracellular polysaccharides (EPS) in the matrix,

B. E. Costa Oliveira
Graduate Program in Dentistry, University Ceuma, São Luís, Maranhão, Brazil

A. C. dos Santos Ré · C. P. Aires
Department of BioMolecular Sciences, School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo (USP), Ribeirão Preto, São Paulo, Brazil
e-mail: ana3.santos@usp.br; airescp@fcfrp.usp.br

A. P. Ricomini Filho (✉)
Department of Biosciences, Piracicaba Dental School, University of Campinas (UNICAMP), Piracicaba, São Paulo, Brazil
e-mail: ricomini@unicamp.br

J. M. Oliveira et al. (eds.), *Polysaccharides of Microbial Origin*,
https://doi.org/10.1007/978-3-030-42215-8_35

formed through sucrose metabolism, plays a relevant role in biofilm accumulation. Depending on their composition and structure, the glucose- and fructose-based extracellular polymers are involved in microbial adhesion, biofilm structure, antimicrobial resistance, and also, energy reserve. Furthermore, another type of polymer, intracellularly accumulated when high concentrations of carbohydrate are available, is involved in energy storage and microbial metabolism maintenance in periods of nutrient lack and may also provide an adaptive advantage. Therefore, to explore the diverse functions of extra- and intracellular polysaccharides in oral biofilms, as well as their complex structures, is a crucial step towards understanding oral diseases and developing strategies for their control.

Keywords

Biofilm · Polysaccharides · Sucrose · Carbohydrates · Metabolism

1 Introduction

Biofilms are formed on biotic (living) and abiotic (nonliving) surfaces, which are present in different environments in nature. Sources of nutrients, temperature, pH, and gas concentrations (O_2, CO_2, etc.) are different under different environmental conditions, thus, the microbial population, and consequently, the matrix composition of biofilms will be different too. In addition to these factors, the flow of fluids over the biofilms will also contribute to the growth of certain microbial species. Considering all these factors, it is possible to understand why the biofilm formed on rocks in a river differs completely from a biofilm formed in a person's mouth. However, it is not necessary to have such completely different environments to have biofilms with different compositions. In the human body itself, the biofilm formed on the skin, urinary tract, and digestive tract differs from the biofilm formed in the mouth, and even inside the mouth, there are distinct microenvironments that in turn contribute to biofilm growth with different compositions. Thus, the understanding on biofilms is quite broad, and this is also valid for when we study the composition of polysaccharides in bacterial biofilms.

Biofilms are defined by a sessile microbial community characterized by cells attached to a surface and to each other, being incorporated into a matrix of extracellular polymeric substances. Thus, the study of biofilms encompasses both the microbial population and the composition of the extracellular matrix and how these two components are three-dimensionally organized. The extracellular matrix is generally composed by products of microbial origin, such as polysaccharides, proteins, lipids, and nucleic acids. The composition of the matrix is closely related to the microorganisms present in the biofilm and to the nutrients available in the environment. The offered nutrients contribute to modulate the population of microorganisms, due to the microbial metabolism, as well as to enable the development of extracellular matrix with different constituents, which are substrates for the synthesis of different polymers. In this way, it is possible to understand how dynamic biofilms

are and how the nutritional source provided by the environment contributes to changes in their composition. Therefore, the understanding on microorganisms and nutritional sources is necessary when we study the composition of polysaccharides in bacterial biofilms.

Concerning the polysaccharides present in biofilms, they can be classified into three major groups based on their function, which are closely related to their occurrence in nature: structural, storage, and gel formation. These polysaccharides can be stored inside bacterial cells, be part of the cell membrane, and/or be a product of extracellular synthesis, which will be dispersed in the biofilm matrix, intermingling bacterial cells. Among the different biofilms formed in nature, oral biofilm has been widely studied with great interest in the polysaccharide content, dispersed in the matrix, or stored inside the microbial cells. When formed on dental surface, it is commonly referred as dental biofilm, being also described as dental plaque, but they can be formed over other substrata present in oral environment, such as dental implants, and prostheses. A peculiarity of this biofilm is that the nutrients in the human diet are responsible for changes in the biofilm, contributing to the development of oral diseases, mainly related to dental caries disease. In view of the complexity of the different types of biofilms present in nature and the particularity that polysaccharides have in oral biofilms, which are related with the health and disease process, this chapter aims to explore the role of polysaccharides with emphasis on oral biofilms.

2 Polysaccharides in Oral Biofilms

Biofilms play a central role in the onset and development of oral diseases, including dental caries, periodontal disease, peri-implantitis, and candidoses (Bowen et al. 2018; Hoare et al. 2017). The biofilm structure favors microbial maintenance and protection against shear forces in oral cavity, and the matrix composition is relevant for that process. The biofilm matrix is composed of diverse polymeric substances, such as polysaccharides, proteins, lipids, and nucleic acids (DNA, RNA). Among them, polysaccharides seem to be necessary to provide a well-stable biofilm structure. Extracellular polysaccharides (EPS) may be one of the main components of the biofilm matrix and determine its physical and biochemical properties (Bowen et al. 2018). In caries disease, the rich-EPS content in the matrix is responsible for providing a unique feature to the biofilm that contributes to the disease development (Bowen et al. 2018).

The composition and structure of EPS are very variable and depend on several factors, such as type of microorganisms, availability of carbon substrata (both inside and outside the cell), and environmental conditions. EPS macromolecule is present throughout the biofilm formation, especially during the adhesion of microorganisms, being also fundamental to promote bacterial accumulation on the tooth surface and to provide protection against antimicrobials (Paes Leme et al. 2006; Klein et al. 2015). In addition, EPS provide reservoirs for nutrient acquisition and stratification

of the bacterial community, establishing gradients of nutrients that further potentiate a suitable environment for bacteria to persist (Bowen et al. 2018).

2.1 Intracellular and Extracellular Polysaccharides

Many oral microorganisms accumulate energy reserves to deal with starvation conditions temporarily present in oral environment (Wilson et al. 2010). In oral bacteria, the main energy-storage products are: (1) intracellular polysaccharide (IPS), stored into the cell, and (2) soluble extracellular polysaccharide (EPS), dispersed throughout the biofilm matrix (Costa Oliveira et al. 2017). Although the latter is recognized for their role in facilitating the adherence of the growing biofilm, evidence suggested that oral streptococci secrete extracellular enzymes able to degrade soluble EPS and metabolize the resultant monomers (Lemos et al. 2019), using it as sole carbon source. Thus, oral bacteria are capable of storing IPS for long periods of time but can also produce an extracellular source of nutrients. Therefore, IPS and soluble EPS play critical roles in bacteria survival since they are source of nutrients when exogenous carbohydrate is not sufficient, or it is absent, providing a critical advantage to select oral biofilm members capable of surviving.

IPS and soluble EPS differ in the type of carbohydrates and glycosidic linkages, type and degree of branching, length of the glycan, and conformation of the polymers (Fig. 1). While IPS is a glycogen-like glucan with α-(1→4) and α-(1→6) linkages, water-soluble EPS are composed by α-glucans (dextran) and β-fructans (Busuioc et al. 2009). Dextran is mainly composed of α-(1→6) linked glucose

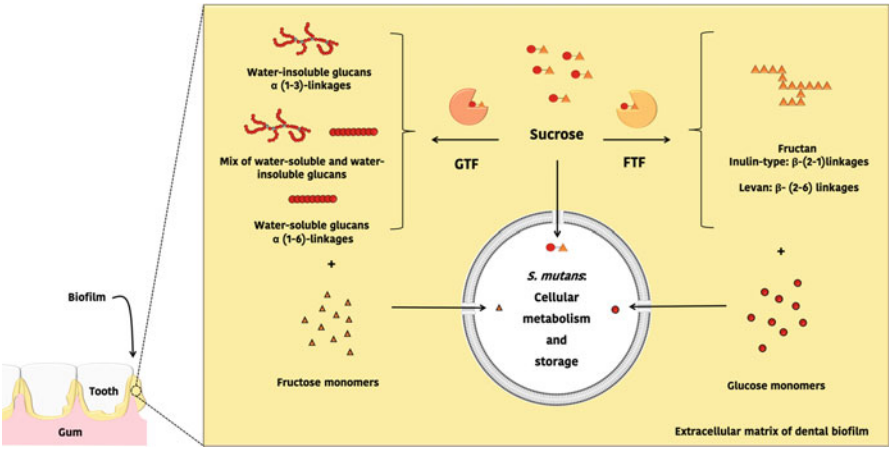


Fig. 1 Glucans (chains in red) and fructans (chains in orange) are synthetized by glucosyltransferases (GTF) and fructosyltransferases (FTF), respectively, when sucrose is available in the environment. Free fructose and glucose monomers released by the hydrolysis of sucrose are used in cellular metabolism and can be stored as energy as well. *Streptococcus mutans* bacterium was used as a representative model to illustrate the dynamic of polysaccharide synthesis

residues that may display a variable degree of branching at position 3 (resulting in α-(1→3) linked branches). The presence of α-(1→6) linkages in polysaccharides is related to their solubility property due to the capability of binding to a great number of water molecules via hydrogen bonds. In turn, fructans present water solubility and are distinguished by their fructosyl linkages: levan type with β-(2→6) linked fructosyl units and inulin type with β-(2→1) linked fructosyl units.

Although the degradation of stored EPS seems to be relevant for bacterial metabolism, the main EPS fraction of a biofilm matrix is not degraded by oral microorganisms: the water-insoluble α-glucan content, usually referred as α-(1→3) glucan, and also as mutan (Klein et al. 2015; Lemos et al. 2019). The term mutan was proposed by Prof. Bernhard Guggenheim in 1970 to distinguish the water-insoluble glucan from the soluble one. As the biofilm develops, α-(1→3) glucans form an insoluble and cohesive matrix while dextrans can be metabolized and used by bacteria as an energy source (Klein et al. 2015; Costa Oliveira et al. 2017). Thus, while insoluble EPS create a cohesive structure into the matrix, the soluble EPS provide a source of nutrients when the starvation persists for a certain period of time (Lemos et al. 2019). This dynamic organization can rapidly modulate the formation of pathogenic biofilms and provide a functional versatility for bacteria survival (Bowen et al. 2018).

2.2 Understanding the Role of Sucrose in Oral Biofilms

Sucrose is a disaccharide formed from condensation of a molecule of glucose and a molecule of fructose (α-glucose-(1↔2)-β-fructose; Glc(α1↔2β)Fru; Fig. 2). Sucrose, commonly referred as table sugar, is obtained from beet and cane and is added in food, candies, beverages, soft drinks, and juices to provide sweet taste. It is considered the most cariogenic dietary carbohydrate and is unequivocally implicated in the cause of dental caries (Bowen et al. 2018; Cury et al. 2000). Previous studies showed that biofilm formed in the presence of sucrose presents great amounts of extracellular polysaccharides (EPS) when compared to biofilms formed in the presence of lactose (Aires et al. 2002), starch (Souza et al. 2018), or its constituent monomers, glucose and fructose (Cury et al. 2000; Tenuta et al. 2006; Costa Oliveira et al. 2017). Thus, sucrose is the only substrate from human diet that can be used for EPS synthesis (Paes Leme et al. 2006), in either the soluble or insoluble form. That is only possible due to chemical composition and structure of sucrose molecule.

Sucrose is unique among the carbohydrates from human diet. Different from other disaccharides, as maltose (α-glucose-(1→4)-β-glucose; Glc(α1→4)Glc) and lactose (β-galactose-(1→4)-β-glucose; Gal(β1→4)Glc), sucrose contains no free anomeric carbon atom. Therefore, the α-glucose and β-fructose are linked at their anomeric carbons by a glycosidic bond (Fig. 2), a molecule configuration that provides an extremely high free energy when hydrolyzed (Schmid et al. 2019). When the glycosidic bond is cleaved by sucrolytic enzymes, such as glucosiltransferases (GTFs), frutosiltransferases (FTFs), and sucrases (invertases), they can

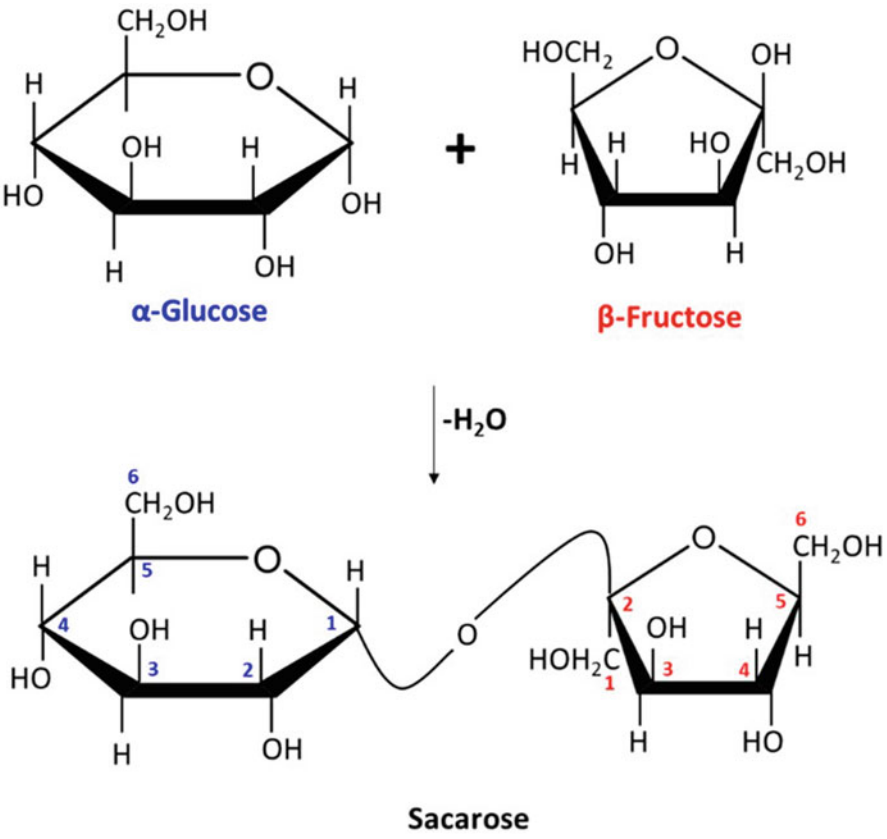


Fig. 2 Structure of sucrose (α-glucose-(1↔2)-β-fructose; Glc(α1↔2β)Fru), the most cariogenic carbohydrate from human diet. The energy necessary to catalyze synthesis of polysaccharides comes from the cleavage of the glycosidic bond of sucrose

release the monomers and provide the energy necessary to catalyze other reactions, such as EPS synthesis (van Hijum et al. 2006; Bowen et al. 2018).

The frequent consumption of sucrose-containing products is linked to dental caries development, because, in addition of being fermented to acids that provoke dental demineralization, sucrose is used to synthesize EPS that modify the biofilm matrix (Fig. 3), contributing to increase the dental demineralization process (Cury et al. 2000; Paes Leme et al. 2006). Insoluble EPS synthesized from sucrose is able to enhance the diffusion pattern of acids throughout the biofilm matrix by increasing the porosity of the extracellular matrix, permitting deeper penetration of dietary sugars into the biofilm and greater acid production nearby the tooth surface (Bowen et al. 2018).

Insoluble EPS acts as a "biological glue" that enhances the adherence of microorganisms to each other and to the tooth surface, providing faster biofilm formation (Paes Leme et al. 2006). In addition, sucrose and its constituent monomers (glucose and fructose) released during the enzymatic cleavage can be internalized by sugar-

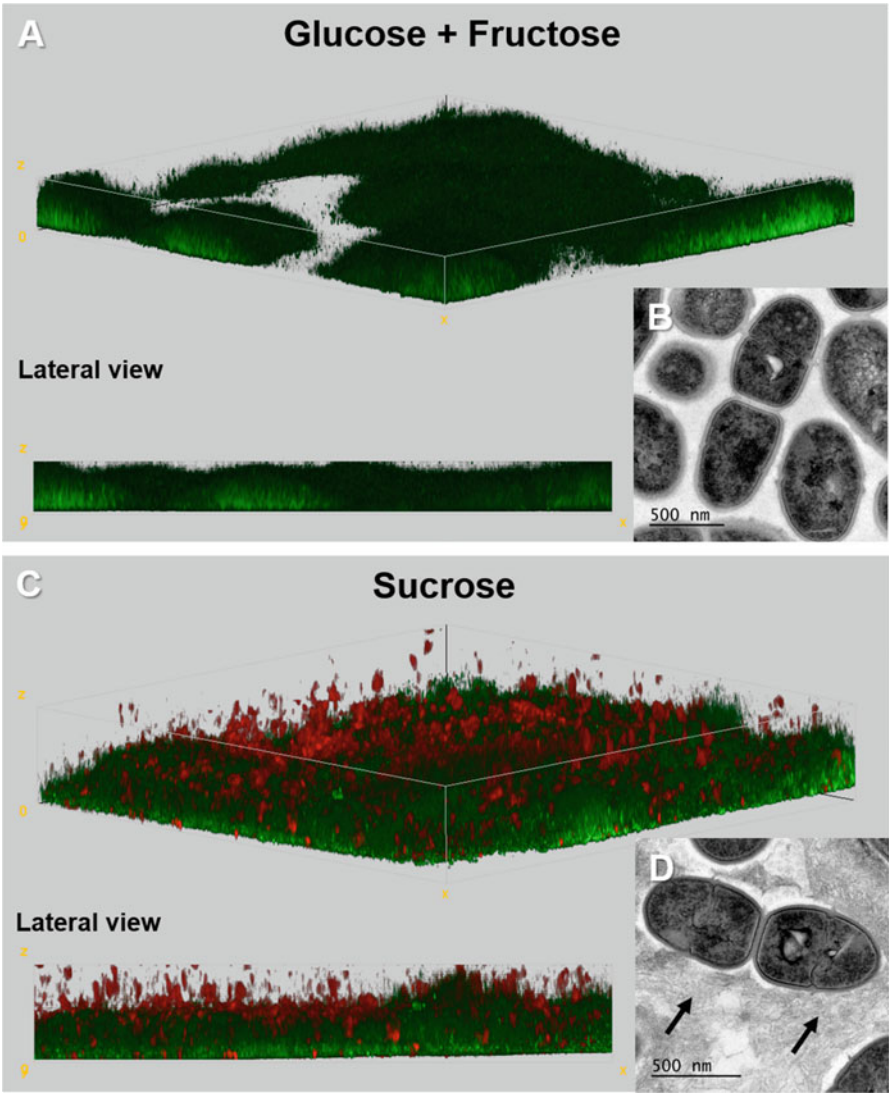


Fig. 3 Images from *Streptococcus mutans* biofilms grown in medium containing sucrose, or its constituent monosaccharides, glucose and fructose, obtained by confocal laser scanning microscopy (CLSM) or transmission electron microscopy (TEM). For CLSM (**a** and **c**), bacterial cells are stained in green (Syto 9) and EPS labeled in red (Dextran conjugate – Alexa Fluor 647). For TEM (**b** and **d**), EPS was contrasted to provide better visualization (black arrow) among the cells. It is possible to observe that EPS was only formed when sucrose was present in the medium

phosphotransferase system (PTS) from bacteria, resulting in acids that can lead to dental demineralization. When in excess, the sugars are stored as intracellular polysaccharide (IPS) (Fig. 4). This shows that sucrose presents a particular role in dental caries development.

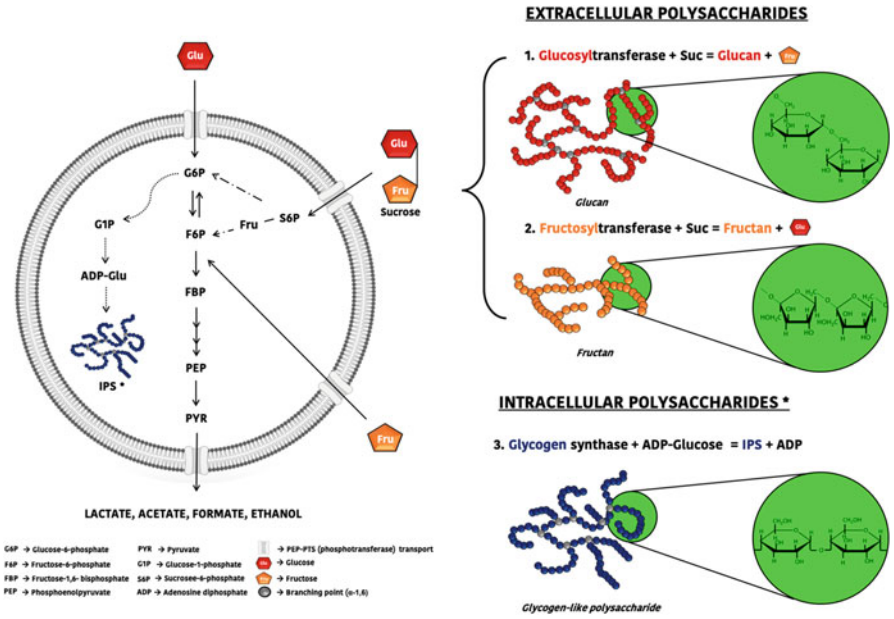


Fig. 4 Sucrose is essential for extracellular polysaccharides synthesis, in addition to its role as energy source and intracellular polysaccharide synthesis (Partially created by BioRender®)

Although sucrose plays a remarkable role in changing the matrix, other carbohydrates may contribute to this process. When the biofilm is exposed to sucrose and starch, the cariogenic potential of this combination is considered more cariogenic than sucrose alone (Souza et al. 2018). Starch is frequently consumed simultaneously with sucrose and could influence the biofilm composition, modulating dental caries disease (Souza et al. 2018). It is suggested that a novel glucan is formed in the presence of starch hydrolysates. Thus, the combination of starch hydrolysates and sucrose could modify the polysaccharides formed, having a distinct glucan structure, mainly in linkage composition and arrangement of linkages. The modified structure could offer distinct binding sites to bacteria, which may have implications in adhesion of bacteria to surfaces. However, the exact chemical structure of this modified glucan in complex biofilms remains unclear and should be considered in future studies.

The insoluble EPS content of biofilms formed on dental surface and its role in caries disease has been extensively investigated (Bowen et al. 2018). However, due to the insoluble EPS contribute to a stable structure, they are certainly relevant for the biofilm formed over other substrata that may be present in the oral cavity, such as dental implants, prostheses, and dentures. The undisturbed biofilm near gingival and peri-implant margins is the first step to trigger an inflammatory process that could lead to the development of periodontal and peri-implant disease, respectively. Although the site rich in crevicular fluid has been highlighted as the first reason for the bacterial population changes in the biofilm, an in situ study showed that the

increased biofilm volume due to EPS accumulation allowed greater bacterial growth, including strict anaerobic species (Souza et al. 2019), showing the importance of the biofilm structure on the microbial changes. EPS-rich matrix has also been evaluated in biofilms formed over dentures that are associated with oral candidiasis, evaluating the role of bacteria and *Candida* spp. on the disease development (Hoare et al. 2017). Therefore, further studies are needed to explore how polysaccharides in the biofilm matrix interfere with the development of other oral diseases.

3 Extracellular Polysaccharides in Oral Biofilms

In natural conditions, biofilms are composed of mixtures of microorganisms. Thus, the enormous number of microbial species capable of forming polysaccharides with a great range of enzymes produced gives rise to an infinite number of permutations. However, most information related to glucans and fructans, as well as their respective enzymes and genes, is concentrated in lactic acid bacteria, which in mouth environment includes *Streptococcus* spp. and *Lactobacillus* species (van Hijum et al. 2006).

3.1 Glucosyltransferase (GTF) and Fructosyltransferase (FTF): Genes and Enzymes

Glucosyltransferase (GTF) enzymes have an average molecular mass of 160,000 Da, being part of the glycoside hydrolase family 70 (GH70) (van Hijum et al. 2006; Hoshino et al. 2012; Xu et al. 2018). The structure of GTFs involves a conserved portion with a central catalytic GH70 domain (for sucrose binding and splitting) and a variable region with a C-terminal repeat region (for potential glucan binding) (Xu et al. 2018). Interestingly, GTF enzymes and their genes have not been reported outside the lactic acid bacteria (van Hijum et al. 2006).

Streptococcus mutans, a bacterium associated with caries disease, presents at least three GTFs that are encoded by *gtfB*, *gtfC*, and *gtfD* genes (Hoshino et al. 2012; Xu et al. 2018). The *gtfB* and *gtfC* genes are in an operon-like arrangement and encode GTFB, which synthesize mostly water-insoluble, α-(1→3)-rich glucan, and GTFC synthesizes a mixture of water-insoluble and water-soluble glucans. GTFD enzyme, encoded by *gtfD*, is responsible for water-soluble α-(1→6)-rich glucan synthesis and it is not linked to the *gtfBC* locus (Bowen and Koo 2011). The coding sequences of *gtfB* and *gtfC* can be cotranscribed and subjected to the same regulatory mechanisms (Bowen and Koo 2011). The gene *gtfD* is located upstream of *gtfBC* loci, presenting an independent promoter, and may be regulated in a manner opposite that of *gtfB* and *gtfC* (Bowen and Koo 2011). Several factors can influence the *gtf* gene transcription, translation, and secretion, like carbohydrate availability and source, environmental pH, and growth rate or phase (Bowen and Koo 2011).

Like *S. mutans*, other streptococci also rely on certain glucan synthesizing GTFs to influence the biofilm structures. *Streptococcus gordonii*, *Streptococcus oralis*, and

Streptococcus sanguinis are able to produce only soluble glucans by a GTF encoded by genes *gtfG*, *gtfR*, and *gtfP,* respectively (Xu et al. 2018). Four GTFs active that synthesizes glucans are present in *Streptococcus sobrinus* (*gtfI, gtfS, gtfT,* and *gtfU*) and *Streptococcus salivarius* (*gtfJ, dtfK, gtfL,* and *gtfM*) (Xu et al. 2018). Other oral lactic acid bacteria, especially genera *Lactobacillus,* also have GTFs that are able to produce α-glucan (Hoshino et al. 2012).

It is interesting to note that *S. mutans* have three different enzymes to act on the same substrate to form polysaccharides. Bowen and Koo (2011) hypothesize that each one plays a distinct role in the formation of dental biofilm and it highlights the greater influence that *S. mutans* has on the formation and composition of dental biofilm (Bowen and Koo 2011). For example, the authors proposed a revised model related to the interaction of the three GTFs in dental biofilm formation. Briefly, GTFC is incorporated into dental pellicle while GTFB (mainly) is adsorbed on bacterial surfaces, including those that do not produce GTFs (Bowen and Koo 2011). Surface-adsorbed GTFB and GTFC rapidly utilize dietary sucrose to synthesize water-insoluble and water-soluble glucans in situ. In turn, GTFD could serve as primers for GTFB enhancing the synthesis of water-insoluble polysaccharides.

Fructosyltransferase (FTF) enzymes belong to the hydrolase family 68 and their structures are divided into four regions, based on deduced amino acid sequences (van Hijum et al. 2006). The structure has a signal peptide, an N-terminal stretch that varies in length, a conserved catalytic core for sucrose cleavage, and a C-terminal stretch of various lengths. In relation to fructans, limited information about their synthesis is available, at least for oral bacteria. Most data on FTFs are from other microbial species that are not present in the mouth, such as *Bacillus* spp. and *Zymomonas* spp. (van Hijum et al. 2006). In oral environment, FTFs that produce inulin are exclusively present in a *S. mutans*, while FTFs that synthesize levan are widely distributed (van Hijum et al. 2006).

Although *S. mutans ftf* gene coding for FTF enzyme has been isolated and characterized, information about its regulation and expression is scarce. The addition of sucrose to steady state cultures resulted in significant increase in *ftf* expression. The intact *ftf* gene seems to code for a 797-amino-acid protein with a predicted molecular weight of 87,600 Da and an inverted repeat region (positions 565–593), which may function as a regulatory region.

Even though fructans may influence the pathogenesis of dental caries acting as extracellular carbohydrate storage, many important gaps remain to be filled in the understanding of fructan regulation in oral bacteria. Whereas some authors suggest that fructans may play an important role in the adhesion and colonization of several cariogenic bacteria to hard surfaces (Rozen et al. 2001), others show that the disruption of the *ftf* gene in *S. mutans* does not affect bacterial adhesion capacity (Nagasawa et al. 2017). On the other hand, it was hypothesized that the binding of FTF to glucan could also increase *S. mutans* retention to glucan-coated surfaces by bridging between glucans, increasing biofilm pathogenicity (Rozen et al. 2001). Therefore, fructans and glucans would be an integral part of the polysaccharide matrix of oral biofilms. Thus, the role of genes that encode FTFs from oral bacteria remains to be clarified and should be considered for future investigation.

3.2 Glucosyltransferase (GTF) and Fructosyltransferase (FTF): Mechanism of Action

A transferase is a class of enzymes that catalyze the transfer of a specific functional group from one compound (the donor) to another compound (the acceptor). As part of this major class, glycosyltransferases are a large family of enzymes that catalyze the transfer of a sugar residue from a glycosyl donor to a variety of acceptor substrates. Glucosyltransferase (GTF) and fructosyltransferase (FTF) are glycosyltransferase enzymes, and their activity refers to catalyze the transfer of glucosyl and fructosyl sugar units, respectively (Fig. 1). As previously mentioned, both enzymes use sucrose as substrate to synthesize the respective homopolysaccharide product. Sucrose (sugar obtained from beet and cane) is a disaccharide composed of a glucose and fructose residues linked by a glycosidic bond.

In contrast to other disaccharides that contain a free anomeric carbon, as maltose (Glc(α1→4)Glc) and lactose (Gal(β1→4)Glc), sucrose contains no free anomeric carbon (Glc(α↔2β)Fru), being both of them involved in the glycosidic bond. Therefore, sucrose possesses an energy-rich glycosidic bond that when hydrolyzed by GTF and FTF provides sufficient energy for the transferase activity during the glucan and fructan synthesis, respectively. The free-energy change for hydrolysis of the glycosidic bond in sucrose molecule is approximately −6.6 kcal/mol (Arnold 1968). This value is much lower for maltose, about −3.0 kcal/mol, which refers to the energy of the glycosidic bond between the two glucose residues (Arnold 1968). Given that sucrose is a high energy compound (−6.6 kcal/mol) and the enzymes (GTF and FTF) have high specificity to this substrate, the hydrolysis of each sucrose molecule will provide sufficient energy for a new glycosidic bond (~ −3.0 kcal/mol) of the sugar residue that will be incorporated in the polysaccharide under formation during the transferase activity. Therefore, when GTF and FTF enzymes are exposed to sucrose, the reaction to synthesize the polysaccharides will naturally occur.

The transferase mechanism during the synthesis of glucans and fructans has been extensively investigated; however, it is still not fully understood (van Hijum et al. 2006). There is a wide variety of GTF and FTF enzymes, and although some of them may only hydrolyze sucrose releasing glucose and fructose molecules to the environment, here it will be shown the relevant mechanisms for EPS synthesizes in oral biofilms.

Glucosyltransferases (GTFs) – The enzymes responsible for the synthesis of glucans are usually referred to as glucosyltransferases. GTFs catalyze the transfer of glucose residues from sucrose cleavage to form glucans mainly by two types of reactions (Fig. 5):

1. **Glucan synthesis**: This reaction occurs by successive transfer of glucose residues to the polymer (Monchois et al. 1999). Glucan synthesis occurs by autopolymerization and is commonly divided into two broad categories: dextrans (water-soluble EPS) and mutans (water-insoluble EPS). Glucan is considered a determinant factor for biofilm pathogenicity (Bowen and Koo 2011).
2. **Carbohydrate synthesis by acceptor reaction**: Numerous sugars can act as acceptors, including starch hydrolysates (maltose and oligosaccharides) that occur in the

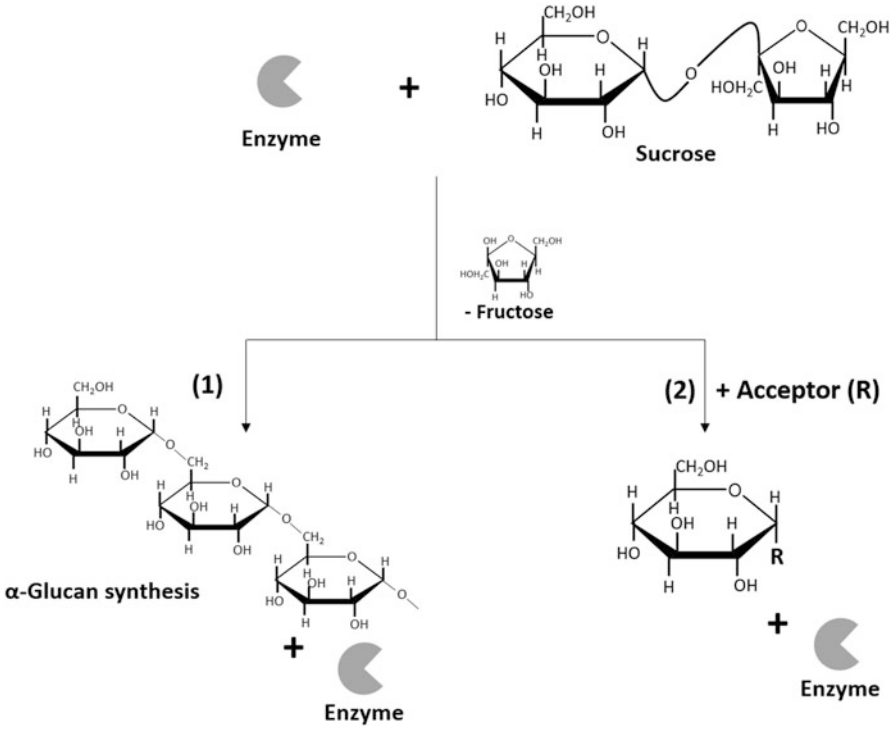


Fig. 5 Reactions catalyzed by glucosyltransferases (GTFs). After sucrose cleavage by GTF, fructose monomer is released to the environment, while glucose monomer is used to synthesize glucan chains. (**1**) Glucan is synthesized by the successive transfer of glucose units; (**2**) carbohydrate synthesis by acceptor reaction

mouth from the action of salivary α-amylase on food starch. The resulting acceptor products of subsequent interactions of the starch hydrolysates with GTFs could contribute to the formation of a cariogenic biofilm (Bowen and Koo 2011). Glucans can also be regarded as an acceptor and α-(1→3) branch point formation may be the result of acceptor reaction (Monchois et al. 1999). The phenomenon of insolubilization of exogenous glucan by formation of α-(1→3) linkages has also been noticed with different glucansucrases (Monchois et al. 1999).

Fructosyltransferases (FTFs) – The enzymes responsible for the synthesis of fructans are usually referred to as fructosyltransferases (FTFs) or, more specifically, levansucrase (in the case of levan synthesis) and inulosucrase (in the case of inulin synthesis). FTFs catalyze the transfer of fructose residues from sucrose cleavage to form fructans mainly by two types of reactions (Fig. 6):

1. **Fructan synthesis** (levan or inulin), which occurs fructosyl transfer (transferase reaction) by polymerization (transfructosylation) to the growing fructan chain. As sucrose is used as acceptor in this initial priming reaction, bacterial fructans

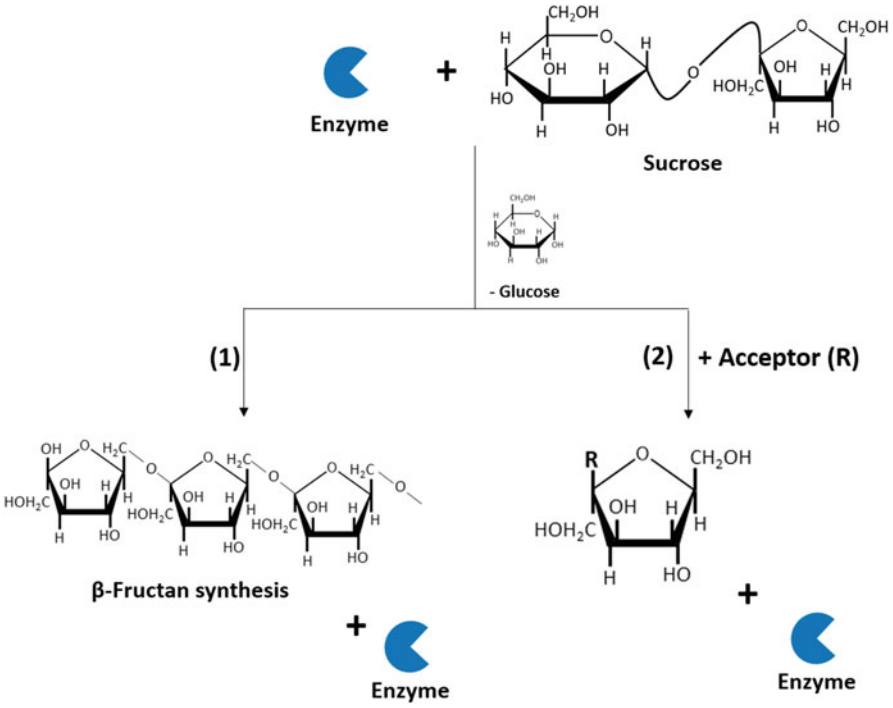


Fig. 6 Reactions catalyzed by fructosyltransferases (FTFs). After sucrose cleavage by FTF, glucose monomer is released to the environment, while fructose monomer is used to synthesize fructan chains. (**1**) Fructan synthesis by successive transfer of fructose units; (**2**) carbohydrate synthesis by acceptor reaction

contain a nonreducing glucose unit at the end of the fructan chain. In subsequent steps, the fructansucrase elongates the primer to produce fructans (Meyer 2015).

2. **Carbohydrate synthesis by acceptor reaction**: The acceptor molecule (R) can be another carbohydrate, as raffinose, and oligosaccharide is the final product, which can also be used for bacterial metabolism (Raga-Carbajal et al. 2018; Lemos et al. 2019).

3.3 Types of Extracellular Polysaccharides (EPS): Insoluble and Soluble

3.3.1 Insoluble EPS

α-(1→3) Glucan (Mutan)

Glucans with different types of glycosidic linkages and anomeric configurations are the most widespread polysaccharides in oral biofilm. In terms of pathogenicity, the

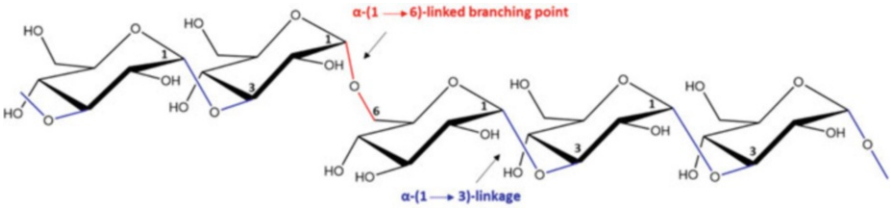


Fig. 7 Conformational structure of the mutan. This polysaccharide contains mainly α-(1→3) linkages (highlighted in blue) with some α-(1→6)-linked branching points (highlighted in red). The ordered structure of this polysaccharide forms interconnected helices, favoring more intramolecular interactions between polymer segments than with water molecules

insoluble α-(1→3) glucan is directly related to biofilm accumulation. Mutans have a highly branched structure and are composed of glucose molecules linked with α-(1→3) glycosidic linkages in main chain, with α-(1→6) bonds in side chains (Aires et al. 2011) (Fig. 7). The distribution of α-(1→3) linkages in a glucan is suggested as a solubility determinant and the backbone composition of sequences of α-(1→3) linkages may confer a rigid and less deformable structure. Thus, the large proportion of these bonds in α-(1→3) glucans explains the insoluble nature of this polymer.

The proportions of these bonds may vary significantly depending on the type of glucans and the conditions of synthesis. The ordered structure of α-(1→3), α-(1→6) glucans has a form of interconnected helices, providing intramolecular interactions between polymer segments. In turn, the presence of α-(1→6) linkages is related to the adhesive properties of mutans. Thus, these EPS are essential to the assembly of the three-dimensional structure of the EPS matrix by contributing to accumulation and substantially enhance the cohesive and adhesive properties of biofilm (Klein et al. 2015).

In presence of insoluble glucans, this dynamic structure forms a highly compartmentalized architecture that limits the substances diffusion into and out of the biofilm, which could facilitate the formation of acidic microenvironments, where acid-tolerant microorganisms are able to grow, survive, and produce more acid, increasing enamel demineralization (Klein et al. 2015). Moreover, this compartmentalized architecture also acts as a physical barrier, conferring protection to bacteria by limiting the diffusion of antimicrobial agents (Klein et al. 2015).

While the hydrolysis products of soluble EPS and IPS could be useful for bacterial metabolism, an insoluble glucan produced into matrix could not provide nutrients for oral biofilm since no bacteria can degrade it, which ensures dental biofilm accumulation. Thus, strategies to reduce the disease potential of dental biofilm have included the possibility of using mutan-degrading enzymes to disrupt the molecular architecture of biofilms. Some of these enzymes, termed as α-1,3-glucanases, or mutanases, have shown high potential as a strategy to control biofilm formation. This could be a feasible approach to control the main virulence factor of oral pathogenic biofilms (Koo et al. 2013).

3.3.2 Soluble EPS

α-(1→6) Glucan (Dextran)

Dextran is a generic term for a family of glucans presenting preponderance of α-(1→6) linked units made by polymerization of the α-glucose moiety of sucrose in a reaction catalyzed by the enzyme dextransucrase, a glucosyltransferase (Schmid et al. 2019). This dextran also contains a relatively high degree of α-(1→3) branch linkages attached to the main chain and the average of molecular weight is in a range between 6.2 and 7.1 × 10^6 Da (Aires et al. 2011) (Fig. 8). For this polymer, the intramolecular interactions between polymer and water dominate, leading to a strong molecule-water interaction via hydrogen bonding, which promotes high water solubility of those carbohydrates.

The structural conformation of this polysaccharide favors the interactions with water molecules and the solvent creates a solvating envelope around the polymer chain, which keeps the polysaccharide molecules away from each other. This arrangement can also bind ions and other macromolecules, such as DNA, lipids, and proteins, which are important to biofilm development (Klahan et al. 2018).

It has been proposed that dextrans are an important feature in the colonization by microorganism on the teeth. Initial attraction of oral bacteria to surfaces in the oral

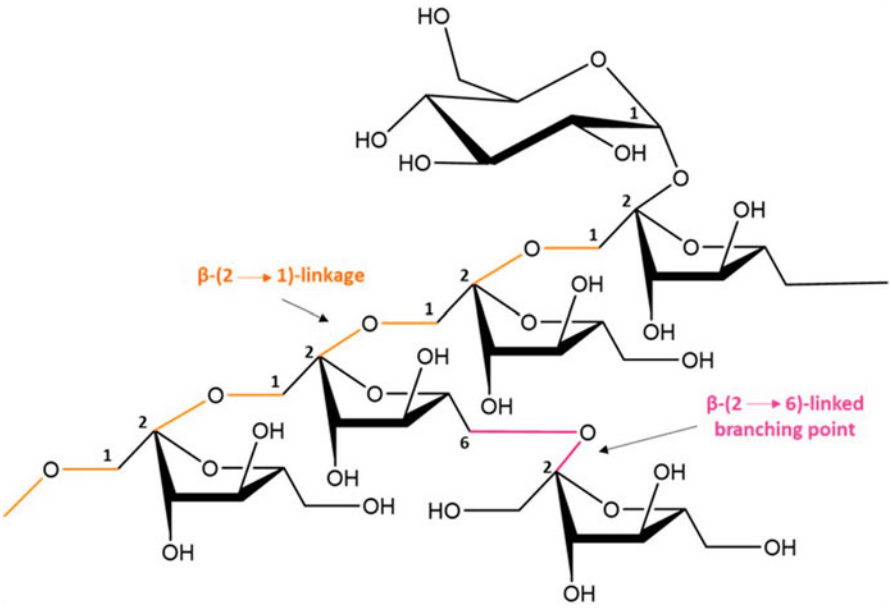


Fig. 8 Conformational structure of the dextran. This polysaccharide contains mainly α-(1→6) linkages (highlighted in red) with some α-(1→3)-linked branching points (highlighted in blue). This arrange favors the interactions with water molecules and its hydroxyl groups, allowing the creation of a solvating envelope around the polymer chain, which keeps the polysaccharide molecules away from each other

cavity is mediated by physicochemical forces that lead to a primary association of the bacteria with the surface. For more permanent adhesion, bacterial adhesins, a cell wall-anchored surface protein, bind to specific salivary glycoproteins adsorbed on the tooth surface as part of the acquired enamel pellicle (Sato et al. 2002). One of these adhesins produced by mutans group streptococci is the glucan-binding protein C (GBPc), one of the most important adhesins for bacterial adhesion (Jenkinson and Lamont 1997; Sato et al. 2002). GBPs attach to glucans, especially dextran, on the surfaces of other microorganisms. For example, before *S. mutans* establishment in biofilm, dextran synthesized by the early-colonizer *S. sanguinis* and its respective GBPc allows *S. mutans* adhesion (Sato et al. 2002). Once adhered, *S. mutans* produces GTFs that will synthesize their own dextrans, providing binding sites for attachment of late colonizers.

Dextran can also act as source of energy for the bacterial biofilm (Costa Oliveira et al. 2017). In the mouth environment, *S. mutans* expresses dextran-degrading enzymes, an extracellular dextranase (DexA) and an intracellular glucan α-(1→6) glucosidase, which provides a source of energy for bacteria via the decomposition of α-(1→6) glycosidic bond (Hayacibara et al. 2004; Klahan et al. 2018). DexA produces isomaltooligosaccharides from dextran, and these are transported by bacteria and then metabolized to glucose by glucan α-(1→6) glucosidase (Klahan et al. 2018). Specifically, DexA may modify glucans by altering the ratio of α-(1→6) to α-(1→3)-linked chains, reducing solubility in water, and may also provide α-(1→6)-rich fragments to prime further glucan synthesis (Hayacibara et al. 2004).

β-(2→6) Fructan (Levan)

Levans are high molecular mass polysaccharides, composed of β-(2→6)-linked fructose monomers with variable degrees of β-(2→1)-linked side chains with a terminal glucose residue (Fig. 9). These polysaccharides are relatively soluble and are mainly produced by *Streptococcus mutans*, *Streptococcus salivarius, Actinomyces viscosus, Rothia dentocariosa,* and some species of *Lactobacillus* spp., as *Lactobacillus reuteri* (Rozen et al. 2001). Levan biosynthesis is carried out extracellularly via levansucrase enzyme in the presence of the sole carbon source, sucrose (Zhang et al. 2019). Upon exposure to sucrose, levan accumulates on dental biofilm and is fermented to acid when the environmental sugar is depleted.

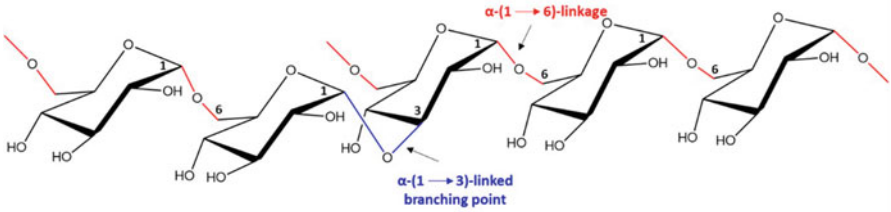


Fig. 9 Conformational structure of the levan-type polysaccharide. This polysaccharide has one glucose residue and fructose molecules bonded with mainly β-(2→6) linkages (highlighted in pink) with some β-(2→1)-linked branching points (highlighted in orange)

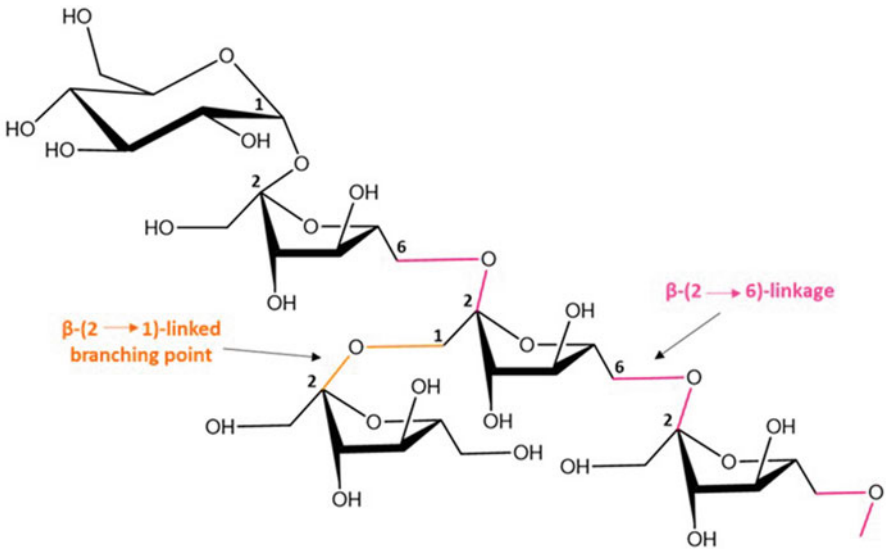


Fig. 10 Conformational structure of the inulin-type polysaccharide. This polysaccharide has one glucose residue and fructose molecules bonded with mainly β-(2→1) linkages (highlighted in orange) with some β-(2→6)-linked branching points (highlighted in pink)

Like *S. mutans*, *Actinomyces viscosus* produces an FTF and a fructan-hydrolyzing enzyme. *Actinomyces* spp. are typically major constituents of the subgingival microbiota and contribute to periodontal diseases development. In this environment, the production of levans can trigger inflammatory reactions and act as mitogens for B cells.

β-(2→1) Fructan (Inulin)

Inulin type fructans have linear chain of fructose polymer with β-(2→1) linked and contain no more than 5% β-(2→6)-linked branches, usually presenting a glucose end group (Mensink et al. 2015) (Fig. 10). This polymer is less soluble in water, with decreasing solubility for higher molecular weight fractions (Mensink et al. 2015). This is due to a rather elongated helical structure in solution, which results in low accessibility of the polymer to water molecules reducing hydration of the fructose unites.

Inulin can act as short-term storage reservoirs in the dental biofilm and may also play a role in bacterial adhesion to the tooth surface (Rozen et al. 2001). Notably, the only bacterial species that produces an inulin type fructan, known so far, is *S. mutans* (Burne and Penders 1992), one of the most cariogenic bacteria.

4 Intracellular Polysaccharides (IPS)

During oral biofilm growth, microorganisms are subject to intermittent carbohydrate exposure as a consequence of the host's diet frequency, known as "feast and famine" episodes. Due to the hostile environment found into the mouth, oral bacteria must

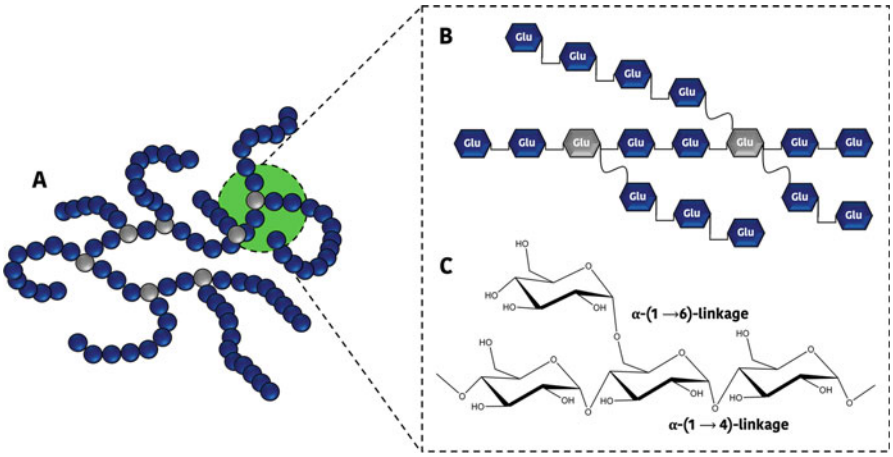


Fig. 11 Schematic representation of an intracellular glycogen-like polysaccharide. (**a**) granular structure, (**b**) glucose residues, and (**c**) structural conformation are showed. In blue, α-(1→4) linked glucose monomers, and in gray, the α-(1→6) branching points

quickly adapt to the sudden nutrient changes, controlling their growth, metabolism, and physiology. In this scenario, the oral microbiota has developed survival mechanisms that involve from the ability to metabolize several carbohydrates (Moye et al. 2014), to the accumulation of their excess as an intracellular glycogen-like polysaccharide (Preiss 2009; Wilson et al. 2010) (Fig. 4). This energy source is also known as intracellular polysaccharide (IPS), a glucose-based polymer that is involved in microorganism persistence skills (Busuioc et al. 2009; Sekar et al. 2020), which seem to contribute also to biofilm biomass (Komiyama et al. 1988; Takahashi and Yamada 2000; Fung et al. 2013) (Fig. 11).

IPS accumulation has been reported in several studies (Roger et al. 2011; Sekar et al. 2020), including in oral microorganisms involved in dental caries (Spatafora et al. 1995; Busuioc et al. 2009; Costa Oliveira et al. 2021), periodontal disease (Takahashi and Yamada 2000), and oral candidoses (Fu et al. 2008). Since IPS might represent an adaptative advantage compared to cells unable to synthesize them (Klotz and Forchhammer 2017), it is important to approach the role of IPS in oral biofilms. There are differences regarding the steps for glycogen-like polysaccharides synthesis. However, the classical mechanism suggested is described as follows.

4.1 IPS Metabolism

During feast periods, when carbohydrate concentration dramatically increases into the mouth, the excess of internalized sugars is converted into glucose-6-phosphate (G6P). Then, the phosphoglucomutase (PGM) enzyme converts G6P into glucose-1-phosphate (G1P), the primary step for IPS synthesis in bacteria. Afterward, G1P is

used by adenosine diphosphate-glucose pyrophosphorylase enzyme (ADP-Glu-PP, GlgC&D) to initiate the synthesis of IPS through ADP-glucose (ADP-Glu) formation, that is, the sugar-nucleotide donor of glucose (Birkhed and Tanzer 1979; Preiss 1984; Asención Diez et al. 2013). In this step, glycogen synthase enzyme (GlgA) uses ADP-Glu, transferring glucose units to the main chain joined by α-(1→4) bonds.

Afterward, IPS branchings are formed by the glycogen branching enzyme (GlgB) that break α-(1→4) linkages from the main chain end and transfer oligosaccharide sequences (Preiss 2006; Wilson et al. 2010), attaching them to inner glucose residues, forming about 8–12% of α-(1→6)-linked ramifications (Preiss 2006). Analysis of 169 bacterial GlgB has pointed out that GlgB enzymes could be classified into Types I or II, depending on the length of their N-terminal domain (Wang et al. 2019). Thus, the former is longer and mostly found into gram-negative species, meanwhile the latter is shorter and is often present into Gram-positive species (Wang et al. 2019). Additionally, there is evidence of a Type III enzyme found in Actinobacteria phylum, presenting an extended N-terminus (Wang et al. 2019). The process of IPS synthesis is illustrated in Fig. 12.

Different from most bacteria, yeasts, on the other hand, present extra steps for glycogen biosynthesis, because glycogen synthases from eukaryotic cells need a precursor protein for initiating the process, known as glycogenin (Cheng et al. 1995). This protein works catalyzing the glucose transfer from uridine diphosphate glucose (UDP-Glu), rather than ADP-Glu, to a tyrosine residue in the glycogenin (Wilson et al. 2010). After forming a glucose 1-O-tyrosyl linkage, glycogenin catalyzes the oligosaccharide chain elongation in which glucose residues from UDP-Glu are linked to each other by α-(1→4) bonds and this complex (oligosaccharide + glycogenin) can be used as substrate for glycogen synthase enzyme. Then, GlgB is apt to build up the ramifications in the molecule, transferring oligosaccharides from the main chain and polymerizing them through α (1→6) bonds. The use of UDP-Glu as sugar nucleotide donor is not restrict to yeasts, being found also in *Actinomyces naeslundii*, and *Prevotella intermedia* and *P. nigrescens* (Takahashi and Yamada 2000).

These steps might occur either during cell growth or after reaching a stationary phase, when anabolic reactions stop (Klotz and Forchhammer 2017). In *E. Coli*, the glycogen biosynthetic enzymes were induced in the stationary phase (Preiss and Romeo 1994). Evaluation of ADP-Glu-PP and glycogen synthase from *S. mutans* showed the peak of enzyme activity did not occur at the same time as the peak of glycogen synthesis (Birkhed and Tanzer 1979). Additionally, studies have suggested that biosynthetic reactions depend on the environmental pH (Asención Diez et al. 2013). Moreover, it was demonstrated that during acidic pH reached due to sugar fermentation, the activity of ADP-Glu-PP would be disfavored (Asención Diez et al. 2013). Therefore, the higher formation of IPS in bacteria might be expected after interrupting nutrient consumption, allowing the pH recovery and the optimal functioning of the enzyme.

Besides that, these enzymes are activated or inhibited by allosteric regulators, so that the IPS metabolism can be controlled in accordance to the cellular metabolic

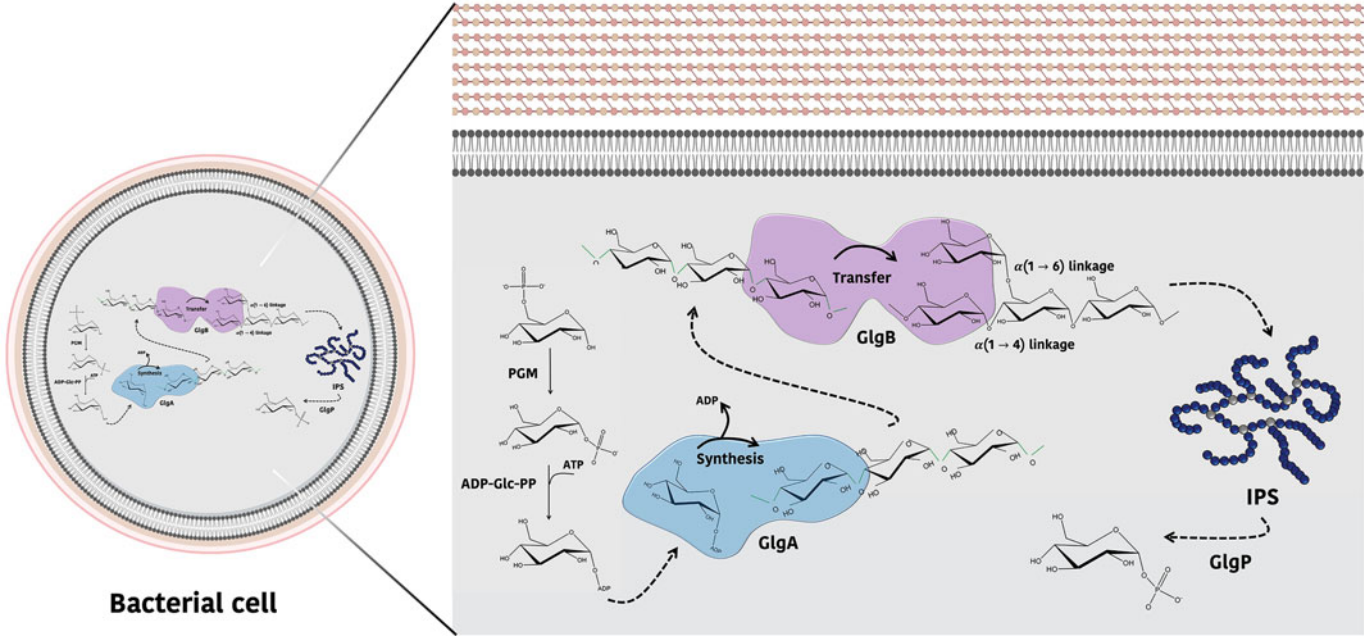


Fig. 12 Intracellular metabolism in bacteria, from synthesis to degradation. Illustration showing the synthesis of G1P by PGM enzyme and the subsequent formation of ADP-Glu by ADP-Glu-PP (GlgC&D), that is, the substrate for glycogen synthesis by GlgA (glycogen synthase enzyme) and GlgB (branching enzyme). Upon a nutrient lack condition, the granule of IPS can be degraded and G1P is again released (Partially created by BioRender®)

need, and the glycolytic pathway by-products are part of it (Preiss 2006). Under carbohydrate exposure, the excess of phosphorylated sugars accumulated inside the cells, like fructose-6-phosphate and fructose-1,6-bisphosphate, works as activators for IPS synthesis, meanwhile AMP, ADP, GTP, or orthophosphate would work as IPS allosteric inhibitors (Preiss 2006; Montero et al. 2009). In turn, the cellular energy status and the glycogen accumulation seem to be determined by extracellular Mg^{2+} levels (Montero et al. 2009). Furthermore, the conversion of glycolytic pathway intermediates into substrates for IPS synthesis is a way to avoid reaching toxic levels that may lead to cellular death, thereby being an important strategy for survival.

On the other hand, when the extracellular concentration of carbohydrates dramatically decreases, as occurred between the meals and in the overnight period (famine), the degradation of IPS might occur (Spatafora et al. 1995). In this case, IPS are consumed through the break of α-(1→4) linkages from the nonreducing ends by the glycogen phosphorylase enzyme (GlgP/phsG) and/or α (1→6) break by the glycogen debranching enzyme (GlgX), when present in the microorganism (Preiss 2006; Wilson et al. 2010). Hence, G1P is released from the molecule and the now free monosaccharide can reentry into the glycolytic pathway (Wilson et al. 2010) and be used by the microorganism to exert different functions.

RNAseq analysis evidence glycogen-related genes are usually organized as an operon (*glgBACDP – glg* operon) encoding biosynthetic and degradative enzymes, as found in *S. mutans* (SMU.1535–1539) (Harris et al. 1992; Spatafora et al. 1995) and *Bacillus subtilis*. However, there are other possible arrangements allowing *glg* genes being clustered in more than one operon, as occurred in *E. coli* (*glgBX* and *glgCAP*) (Preiss 2009; Wilson et al. 2010). Literature shows differences in transcription of *S. mutans glg* operon according to nutrient availability (Moye et al. 2014) and type of carbohydrate (Busuioc et al. 2009). Moreover, a difference in IPS synthesis (Costa Oliveira et al. 2017) and degradation (Busuioc et al. 2009), depending on the available carbohydrate source, might occur, highlighting the mechanisms regulating gene expression, synthesis, and consumption of IPS still need investigation. In fact, the role of IPS in microorganisms is diverse and seems to go beyond being an energy source (Costa Oliveira et al. 2021). However, the main attributes of oral bacteria are summarized in the next topic.

4.2 The Role of IPS and Its Importance in Oral Biofilms

To be considered an energy reserve, a molecule should be used for microorganism viability maintenance when lacking exogenous carbon sources. In this regard, glycogen-like polysaccharides have been considered important energy resources, responsible to keep the metabolism of microorganisms by providing glucose through their degradation. Albeit related to energy storage and survival functions, the role of IPS is not completely understood and its synthesis seem to vary according to the carbohydrate source (Robrish et al. 1991; Busuioc et al. 2009; Costa Oliveira et al. 2017, 2021) and type of microorganism (Preiss 2006; Wilson et al. 2010). Studies

have reported IPS as a durable energy source (Wang et al. 2019), mainly due to the number of branchings that interfere with the rate of IPS degradation occurred during nutrient lack periods. Conversely, IPS seems to be quickly used upon reaching a nutrient-limiting condition in *E. coli*, suggested as important attributes for the planktonic-biofilm lifestyle transition in those bacteria (Sekar et al. 2020).

Clinically speaking, the glucose released from IPS degradation might contribute to acid production and microorganism metabolism maintenance during fasting periods. Thus, consumption of IPS is mainly related to dental caries development because the acidic environment can favor tooth demineralization in periods of absence of exogenous carbohydrate thereby contributing to increase biofilm virulence. Previous studies performed in vitro have demonstrated that acids continued to be produced by *S. mutans* even during fasting periods (Costa Oliveira et al. 2017). Interestingly, it was recently suggested a relationship between extra- and intracellular polysaccharides synthesis when S. mutans are grown under sucrose exposure. Thus, the presence of an EPS-rich matrix seemed to delay not only the IPS utilization under starvation, but also interfere with glg operon expression in comparison to glucose and fructose exposure (Costa Oliveira et al. 2021). Therefore, these virulence traits might be able to enhance S. mutans persistence and survival, directly interfering with dental caries. Also, in situ studies have found a more acidic pH in resting cariogenic biofilms after an overnight period without carbohydrate exposition; meanwhile Spatafora et al. (1995) have found that *S. mutans* strains able to overproduce glycogen were also hypercariogenic in vivo. *Actinomyces naeslundii* and *Actinomyces viscosus*, oral microorganisms associated with subgingival biofilms and to root caries lesions, were able to synthesize IPS in greater amount (Komiyama et al. 1988) and to degrade them under nutrient lack condition. In consequence, an acidic environment was found, indicating IPS metabolism contributes to tooth demineralization (Komiyama et al. 1988). Interestingly, the clinical isolates from carious sites were able to incorporate more glucose into glycogen in lower pH, fact not observed in isolates from noncarious sites.

Maintenance of acid production via glycogen degradation was reported in bacteria related not only to dental caries but also to periodontal disease. Takahashi and Yamada (2000) have demonstrated that the *P. intermedia* and *P. nigrescens* could maintain their survival abilities and acid production for about 7 h, besides leading to higher energy efficiency through the rescue of the high-energy phosphoryl bond of pyrophosphate. Furthermore, *Fusobacterium nucleatum* also showed to be able to storage glycogen granules, being galactose the carbohydrate that favors this storage (Robrish et al. 1991).

Recently, IPS was related to the ability of a microrganism to inactivate a dormancy status (Klotz and Forchhammer 2017), playing an important role for bacterial survival and persistence. IPS are linked to many cellular functions, such as iron and nitrogen metabolisms, osmotic stress, stringent response, nucleotide metabolism (Montero et al. 2009), besides representing a varied percentage of biofilm dry weight (5–80%) (Komiyama et al. 1988; Takahashi and Yamada 2000; Fung et al. 2013), evidencing its influence also on biofilm biomass. Therefore, not only extracellular but also intracellular polysaccharides should be considered when virulence traits of oral biofilms are explored.

5 Conclusion

The role of polysaccharides in oral biofilms has been extensively investigated. In the chapter, the composition, function, and structure of intracellular and extracellular polysaccharides were described, being relevant for understanding oral diseases and developing strategies for their control. Although there is great evidence about extracellular polysaccharides (EPS) and caries development, more studies are necessary to explore how polysaccharides in the biofilm matrix contribute to the development of other oral diseases. In addition, the knowledge about soluble EPS, which are produced by species of *Streptococcus* and *Lactobacillus*, must be expanded. Although intracellular polysaccharides (IPS) are a relevant source of energy reserve, their role in microorganisms is diverse and seems to go beyond being an energy source. Moreover, the mechanisms that regulate gene expression for IPS synthesis and degradation need to be further investigated.

References

Aires CP, Tabchoury CPM, Del Bel Cury AA, Cury JA. Effect of a lactose-containing sweetener on root dentine demineralization in situ. Caries Res. 2002;36:167–9. https://doi.org/10.1159/000059331.

Aires CP, Tenuta LM, Carbonero ER, et al. Structural characterization of exopolysaccharides from biofilm of a cariogenic streptococci. Carbohydr Polym. 2011;84:1215–20. https://doi.org/10.1016/j.carbpol.2010.12.076.

Arnold WN. The selection of sucrose as the translocate of higher plants. J Theor Biol. 1968;21:13–20. https://doi.org/10.1016/0022-5193(68)90056-8.

Asención Diez MD, Demonte AM, Guerrero SA, et al. The ADP-glucose pyrophosphorylase from *Streptococcus mutans* provides evidence for the regulation of polysaccharide biosynthesis in Firmicutes. Mol Microbiol. 2013;90:1011–27. https://doi.org/10.1111/mmi.12413.

Birkhed D, Tanzer JM. Glycogen synthesis pathway in *Streptococcus mutans* strain NCTC 10449S and its glycogen synthesis-defective mutant 805. Arch Oral Biol. 1979;24 (1):67–73. https://doi.org/10.1016/0003-9969(79)90177-8.

Bowen WH, Koo H. Biology of Streptococcus mutans-derived glucosyltransferases: role in extracellular matrix formation of cariogenic biofilms. Caries Res. 2011;45:69–86. https://doi.org/10.1159/000324598.

Bowen WH, Burne RA, Wu H, Koo H. Oral biofilms: pathogens, matrix, and polymicrobial interactions in microenvironments. Trends Microbiol. 2018;26:229–42. https://doi.org/10.1016/j.tim.2017.09.008.

Burne RA, Penders JE. Characterization of the *Streptococcus mutans* GS-5 fruA gene encoding exo-beta-D-fructosidase. Infect Immun. 1992;60:4621–32. https://doi.org/10.1128/IAI.60.11.4621-4632.1992.

Busuioc M, Mackiewicz K, Buttaro BA, Piggot PJ. Role of intracellular polysaccharide in persistence of Streptococcus mutans. J Bacteriol. 2009;191:7315–22. https://doi.org/10.1128/JB.00425-09.

Cheng C, Mu J, Farkas I, et al. Requirement of the self-glucosylating initiator proteins Glg1p and Glg2p for glycogen accumulation in *Saccharomyces cerevisiae*. Mol Cell Biol. 1995;15:6632–40. https://doi.org/10.1128/mcb.15.12.6632.

Costa Oliveira BE, Cury JA, Ricomini Filho AP. Biofilm extracellular polysaccharides degradation during starvation and enamel demineralization. PLoS One. 2017;12:e0181168. https://doi.org/10.1371/journal.pone.0181168.

Costa Oliveira BE, Ricomini Filho AP, Burne RA, Zeng L. The route of sucrose utilization by streptococcus mutans affects intracellular polysaccharide metabolism. Front Microbiol. 2021;12:636684. https://doi.org/10.3389/fmicb.2021.636684. PMID: 33603728; PMCID: PMC7884614.

Cury JA, Rebelo MA, Del Bel Cury AA, et al. Biochemical composition and cariogenicity of dental plaque formed in the presence of sucrose or glucose and fructose. Caries Res. 2000;34:491–7. https://doi.org/10.1159/000016629.

Fu XH, Meng FL, Hu Y, Zhou JQ. *Candida albicans*, a distinctive fungal model for cellular aging study. Aging Cell. 2008;7:746–57. https://doi.org/10.1111/j.1474-9726.2008.00424.x.

Fung T, Kwong N, Zwan T, Wu M. Residual glycogen metabolism in Escherichia coli is specific to the limiting macronutrient and varies during stationary phase. J Exp Microbiol Immunol. 2013;17:83–7.

Harris GS, Michalek SM, Curtiss R 3rd. Cloning of a locus involved in Streptococcus mutans intracellular polysaccharide accumulation and virulence testing of an intracellular polysaccharide-deficient mutant. Infect Immun. 1992;60(8):3175–85. https://doi.org/10.1128/iai.60.8.3175-3185.1992.

Hayacibara MF, Koo H, Vacca Smith AM, et al. The influence of mutanase and dextranase on the production and structure of glucans synthesized by streptococcal glucosyltransferases. Carbohydr Res. 2004;339:2127–37. https://doi.org/10.1016/j.carres.2004.05.031.

Hoare A, Marsh PD, Diaz PI. Ecological therapeutic opportunities for oral diseases. Microbiol Spectr. 2017;5(4). https://doi.org/10.1128/microbiolspec.BAD-0006-2016.

Hoshino T, Fujiwara T, Kawabata S. Evolution of cariogenic character in Streptococcus mutans: horizontal transmission of glycosyl hydrolase family 70 genes. Sci Rep. 2012;2:518. https://doi.org/10.1038/srep00518.

Jenkinson HF, Lamont RJ. Streptococcal adhesion and colonization. Crit Rev Oral Biol Med. 1997;8:175–200. https://doi.org/10.1177/10454411970080020601.

Klahan P, Okuyama M, Jinnai K, et al. Engineered dextranase from Streptococcus mutans enhances the production of longer isomaltooligosaccharides. Biosci Biotechnol Biochem. 2018;82:1480–7. https://doi.org/10.1080/09168451.2018.1473026.

Klein MI, Hwang G, Santos PHS, et al. *Streptococcus mutans*-derived extracellular matrix in cariogenic oral biofilms. Front Cell Infect Microbiol. 2015;5:10. https://doi.org/10.3389/fcimb.2015.00010.

Klotz A, Forchhammer K. Glycogen, a major player for bacterial survival and awakening from dormancy. Future Microbiol. 2017;12:101–4. https://doi.org/10.2217/fmb-2016-0218.

Komiyama K, Khandelwal RL, Heinrich SE. Glycogen synthetic and degradative activities by *Actinomyces viscosus* and *Actinomyces naeslundii* of root surface caries and noncaries sites. Caries Res. 1988;22:217–25. https://doi.org/10.1159/000261109.

Koo H, Falsetta ML, Klein MI. The exopolysaccharide matrix. J Dent Res. 2013;92:1065–73. https://doi.org/10.1177/0022034513504218.

Lemos J, Palmer S, Zeng L, et al. The biology of *Streptococcus mutans*. Microbiol Spectr. 2019;7. https://doi.org/10.1128/microbiolspec.GPP3-0051-2018.

Mensink MA, Frijlink HW, van der Voort Maarschalk K, Hinrichs WLJ. Inulin, a flexible oligosaccharide I: review of its physicochemical characteristics. Carbohydr Polym. 2015;130:405–19. https://doi.org/10.1016/j.carbpol.2015.05.026.

Meyer D. Health benefits of prebiotic fibers. Adv Food Nutr Res. 2015;74:47–91. https://doi.org/10.1016/bs.afnr.2014.11.002.

Monchois V, Willemot RM, Monsan P. Glucansucrases: mechanism of action and structure-function relationships. FEMS Microbiol Rev. 1999;23:131–51. https://doi.org/10.1111/j.1574-6976.1999.tb00394.x.

Montero M, Eydallin G, Viale AM, et al. *Escherichia coli* glycogen metabolism is controlled by the PhoP-PhoQ regulatory system at submillimolar environmental Mg2+ concentrations, and is highly interconnected with a wide variety of cellular processes. Biochem J. 2009;424:129–41. https://doi.org/10.1042/BJ20090980.

Moye ZD, Zeng L, Burne RA. Fueling the caries process: carbohydrate metabolism and gene regulation by *Streptococcus mutans*. J Oral Microbiol. 2014;5:6. https://doi.org/10.3402/jom.v6.24878.

Nagasawa R, Sato T, Senpuku H. Raffinose induces biofilm formation by *Streptococcus mutans* in low concentrations of sucrose by increasing production of extracellular DNA and fructan. Appl Environ Microbiol. 2017;83. https://doi.org/10.1128/AEM.00869-17.

Paes Leme AF, Koo H, Bellato CM, et al. The role of sucrose in cariogenic dental biofilm formation-new insight. J Dent Res. 2006;85:878–87. https://doi.org/10.1177/154405910608501002.

Preiss J. Bacterial glycogen synthesis and its regulation. Annu Rev Microbiol. 1984;38:419–58. https://doi.org/10.1146/annurev.mi.38.100184.002223.

Preiss J. Bacterial glycogen inclusions: enzymology and regulation of synthesis. In: Shively JM, editor. Inclusions in prokaryotes. Berlin: Springer; 2006. p. 71–108.

Preiss J. Glycogen biosynthesis. In: Schaechter M, editor. Encyclopedia of microbiology. Cambridge, MA: Academic Press (Elsevier); 2009. p. 145–58.

Preiss J, Romeo T. Molecular biology and regulatory aspects of glycogen biosynthesis in bacteria. Prog Nucleic Acid Res Mol Biol. 1994;47:299–329. https://doi.org/10.1016/s0079-6603(08)60255-x.

Raga-Carbajal E, López-Munguía A, Alvarez L, Olvera C. Understanding the transfer reaction network behind the non-processive synthesis of low molecular weight levan catalyzed by *Bacillus subtilis* levansucrase. Sci Rep. 2018;8:15035 . https://doi.org/10.1038/s41598-018-32872-7.

Robrish SA, Oliver C, Thompson J. Sugar metabolism by fusobacteria: regulation of transport, phosphorylation, and polymer formation by *Fusobacterium mortiferum* ATCC 25557. Infect Immun. 1991;59:4547–54. https://doi.org/10.1128/IAI.59.12.4547-4554.1991.

Roger P, Delettre J, Bouix M, Béal C. Characterization of *Streptococcus salivarius* growth and maintenance in artificial saliva. J Appl Microbiol. 2011;111:631–41. https://doi.org/10.1111/j.1365-2672.2011.05077.x.

Rozen R, Bachrach G, Bronshteyn M, et al. The role of fructans on dental biofilm formation by *Streptococcus sobrinus, Streptococcus mutans,* Streptococcus gordonii and Actinomyces viscosus. FEMS Microbiol Lett. 2001;195:205–10. https://doi.org/10.1111/j.1574-6968.2001.tb10522.x.

Sato Y, Senpuku H, Okamoto K, et al. *Streptococcus mutans* binding to solid phase dextran mediated by the glucan-binding protein C. Oral Microbiol Immunol. 2002;17:252–6. https://doi.org/10.1034/j.1399-302X.2002.170408.x.

Schmid J, Bechtner J, Vogel RF, Jakob F. A systematic approach to study the pH-dependent release, productivity and product specificity of dextransucrases. Microb Cell Factories. 2019;18:153. https://doi.org/10.1186/s12934-019-1208-8.

Sekar K, Linker SM, Nguyen J, et al. Bacterial glycogen provides short-term benefits in changing environments. Appl Environ Microbiol. 2020;86(9):e00049-20. https://doi.org/10.1128/AEM.00049-20.

Souza SE, Sampaio AA, Del Bel Cury AA, et al. Starch combined with sucrose provokes greater root dentine demineralization than sucrose alone. Caries Res. 2018;52:323–30. https://doi.org/10.1159/000486427.

Souza JGS, Cury JA, Ricomini Filho AP, et al. Effect of sucrose on biofilm formed in situ on titanium material. J Periodontol. 2019;90:141–8. https://doi.org/10.1002/JPER.18-0219.

Spatafora G, Rohrer K, Barnard D, Michalek S. A *Streptococcus mutans* mutant that synthesizes elevated levels of intracellular polysaccharide is hypercariogenic in vivo. Infect Immun. 1995;63:2556–63. https://doi.org/10.1128/IAI.63.7.2556-2563.1995.

Takahashi N, Yamada T. Glucose metabolism by Prevotella intermedia and Prevotella nigrescens. Oral Microbiol Immunol. 2000;15:188–95. https://doi.org/10.1034/j.1399-302x.2000.150307.x.

Tenuta LM, Ricomini Filho AP, Del Bel Cury AA, Cury JA. Effect of sucrose on the selection of mutans streptococci and lactobacilli in dental biofilm formed in situ. Caries Res. 2006;40(6):546–9.

van Hijum SAFT, Kralj S, Ozimek LK, et al. Structure-function relationships of glucansucrase and fructansucrase enzymes from lactic acid bacteria. Microbiol Mol Biol Rev. 2006;70:157–76. https://doi.org/10.1128/MMBR.70.1.157-176.2006.

Wang L, Liu Q, Hu J, et al. Structure and evolution of glycogen branching enzyme N-termini from bacteria. Front Microbiol. 2019;14:3354. https://doi.org/10.3389/fmicb.2018.03354.

Wilson WA, Roach PJ, Montero M, et al. Regulation of glycogen metabolism in yeast and bacteria. FEMS Microbiol Rev. 2010;34:952–85. https://doi.org/10.1111/j.1574-6976.2010.00220.x.

Xu R-R, Yang W-D, Niu K-X, et al. An update on the evolution of glucosyltransferase (Gtf) genes in *Streptococcus*. Front Microbiol. 2018;9:2979. https://doi.org/10.3389/fmicb.2018.02979.

Zhang W, Xu W, Ni D, et al. An overview of levan-degrading enzyme from microbes. Appl Microbiol Biotechnol. 2019;103:7891–902. https://doi.org/10.1007/s00253-019-10037-4.

Bioactive Polysaccharides from Microalgae

28

Schonna R. Manning, Katherine A. Perri, and Karlin Blackwell

Contents

Abstract

Microalgae represent an untapped resource of mixed polysaccharides with potential applications in biotechnology, therapeutics, pharmaceuticals, and drug delivery. There are presently ~160,000 described species of algae and approximately 80% of these are classified as microalgae, including the cyanobacteria. Numerous reviews and research articles can be found on the structural characterization and bioactivity of a variety of polysaccharides derived from seaweeds and kelps, i.e., macroalgae, and many of these compounds are widely used in industry and clinical applications. By comparison, there are relatively few studies on the bioactivity of polysaccharides from microalgae or their potential applications. This chapter will provide an overview of structural and bioactive polysaccharides and related case studies for major groups of microalgae, including the Cyanophyta (cyanobacteria), Chlorophyta (green algae), Rhodophyta (red algae), and selected golden microalgae in the Chromista, e.g., Bacillariophyta, Haptophyta, and Ochrophyta. Many groups of microalgae produce bioactive polysaccharides, including extracellular polymeric substances (EPS) and sulfated polysaccharides (SPS) that have demonstrated antibacterial, antiviral, antioxidant, and anticancer potential. Microalgal

S. R. Manning (✉) · K. A. Perri · K. Blackwell
Department of Molecular Biosciences, The University of Texas at Austin, Austin, TX, USA
e-mail: schonna.manning@utexas.edu

J. M. Oliveira et al. (eds.), *Polysaccharides of Microbial Origin*,
https://doi.org/10.1007/978-3-030-42215-8_37

glycans are composed predominantly of pentose, hexose, and deoxyhexose monosaccharide subunits with various glycosidic linkages and degrees of branching. Their bioactivity can often be attributed to the monosaccharide composition, content of uronic acids, molecular weight fraction, and/or degree of modification, e.g., sulfation, amination, acylation, phosphorylation, and methylation. While current studies have been limited to a few species, there are many more microalgae to be explored, providing immense opportunities for metabolite discovery in the field of microalgal polysaccharides and natural products.

Keywords

Antimicrobial · Antiviral · Extracellular polymeric substances (EPS) · Microalgae · Sulfated polysaccharides (SPS)

1 Introduction

Algae are broadly classified as primarily photosynthetic organisms that fix atmospheric carbon dioxide into organic sugars for the synthesis of macromolecules, including reserve carbohydrates and a diverse array of mixed polysaccharides with structural and bioactive characteristics. The groups recognized as algae include both prokaryotic and eukaryotic members, the latter of which were derived from multiple endosymbiotic events. As a result, the relationships of the algae are very complicated, creating tremendous genetic and biochemical diversity (Fig. 1). Blue-green algae, also known as cyanobacteria (Cyanophyta), are a highly diverse group of oxygenic bacteria and the predecessor of modern chloroplasts in eukaryotic algae. The Glaucophyta, Rhodophyta (red algae), and Chlorophyta (green algae) in the Kingdom Plantae are eukaryotic lineages that arose from the primary endosymbiosis of a cyanobacterium, and ultimately a green algal ancestor led to the evolution of land plants. The secondary endosymbiosis of a green alga by nonphotosynthetic protists in two separate events gave rise to euglenoids and the chlorarachniophytes (Cercozoa). And, the secondary endosymbiosis of a red alga gave rise to golden-brown algal lineages of the Chromista, including the Miozoa, Bacillariophyta, Haptophyta, Cryptophyta, and Ochrophyta. Adding to this genetic complexity, tertiary endosymbiotic events have been documented with dinoflagellates (Miozoa) engulfing other dinoflagellates, haptophytes, and bacillariophytes. Thus, the relationships of the algae are constantly evolving as new taxa and genetic data are considered. So far, more than 160,000 species of algae have been documented (algaebase.org), and it is predicted there may be hundreds of thousands of species of microalgae, including millions of ecotypes (strains), that have yet to be discovered.

Groups of algae can often be distinguished by their polysaccharides, including the type and location of storage carbohydrates (Table 1) as well as their cell wall composition, which may contain cellulose, modified polysaccharides, and/or glycoproteins. Macrophyte algae, i.e., seaweeds and kelps, are well known for the production of sulfated and nonsulfated polysaccharide-based hydrocolloids that are

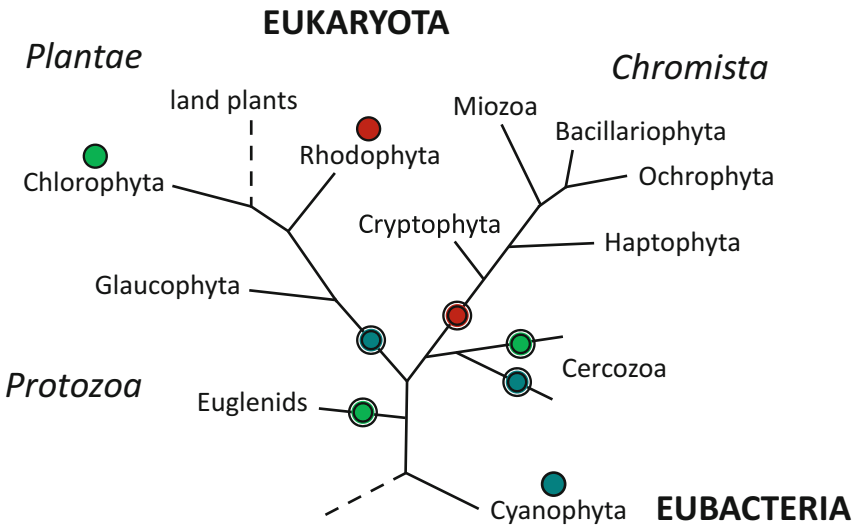


Fig. 1 Tree illustrating the complex relationships and diversity of the algae. Cyanophyta, i.e., blue-green algae, are the foundation of oxygenic photosynthesis. The major eukaryotic taxa arose through primary and secondary endosymbiotic events involving the uptake of a cyanobacterium (●), green alga (●), or red alga (●). This tree was developed based on taxonomic data available at the time of publication (algaebase.org)

Table 1 Algal storage polysaccharides and subcellular location

Phylum	Storage carbohydrate (location)	Glycosidic linkage
Cyanophyta	Cyanophycean starch (Cytoplasm)	α1→4(α1→6)
Glaucophyta	Floridean starch (Cytoplasm)	α1→4(α1→6)
Chlorophyta	Amylose or amylopectin (Chloroplast)	α1→4(α1→6)
Rhodophyta	Floridean starch (Periplastidial space)	α1→4(α1→6)
Euglenids	Paramylon (Cytoplasm)	β1→3
Cercozoa	Glucan (Cytoplasm, chloroplast)	β1→3
Miozoa	Amylose (Cytoplasm)	α1→4(α1→6)
Cryptophyta	Amylose (Periplastidial space)	α1→4(α1→6)
Bacillariophyta	Chrysolaminarin (Vacuoles)	β1→3(β1→6)
Haptophyta	Chrysolaminarin (Vacuoles)	β1→3(β1→6)
Ochrophyta	Laminarin, chrysolaminarin (Vacuoles)	β1→3(β1→6)

commonly found as cell wall components and associated extracellular polymeric substances (EPS), consisting of complex polysaccharide-protein matrices. These include gelling agents such as alginic acid, carrageenan, agar, and agarose (Fig. 2), which have been used in laboratories and clinical environments for decades. Polysaccharides and EPS from algae can be structurally complex and difficult to produce synthetically. Thus, natural resources of algal polysaccharides are preferred and are quite valuable. Potential applications using macroalgal polysaccharides have included encapsulating cells for research applications, biogels, slow-release drug

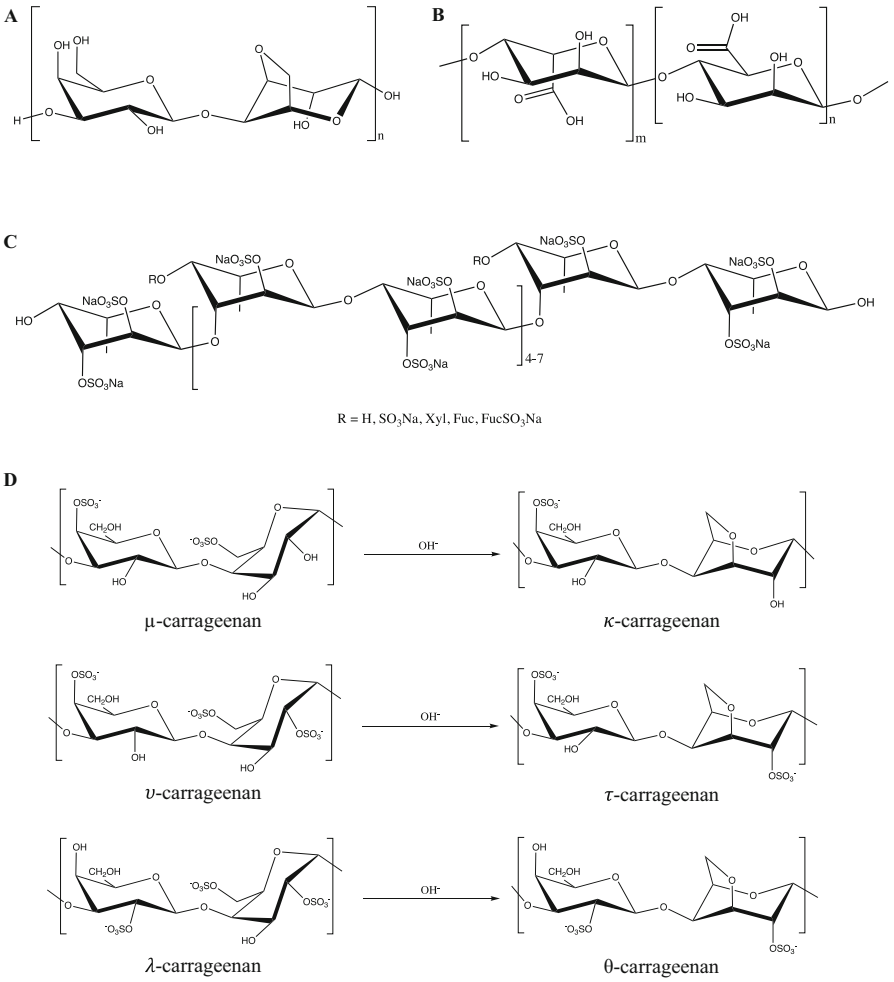


Fig. 2 Structures of macroalgal polysaccharides: (**a**) agarose, (**b**) alginic acid, (**c**) fucoidan, and (**d**) six major forms of carrageenan

delivery systems, and therapeutics. Furthermore, there is growing potential for the development of macroalgal polysaccharides for use in biotechnology, including nutraceutical and pharmaceutical applications.

The Chlorophyta include both microalgal and macroalgal forms, i.e., seaweeds. Green seaweeds, e.g., *Ulva*, *Acetabularia*, *Codium*, and *Caulerpa*, are known to produce numerous sulfated polysaccharides (SPS), including ulvans, rhamnans, arabinogalactans, galactans, and mannans. These SPS are structurally diverse and many studies have reported antiviral, anticoagulant, antimicrobial, antioxidant, antitumor, and anti-inflammatory properties (see Wang et al. 2014 for review). Ulvans are a common family of SPS found in *Ulva* and *Enteromorpha* that can represent up to 30% of the biomass dry weight. The main repeating disaccharide is

β-D-glucoronic acid-(1→4)-α-L-rhamnose, known as aldobiouronic acid; sulfation often occurs on the second carbon of the glucuronic acid and the third carbon of the rhamnose subunits. Purified ulvan is semicrystalline and highly hygroscopic, and similar types of sulfated glycans can be found in related taxa.

Brown algae, i.e., kelps (Phaeophyceae, Ochrophyta), produce cell wall polysaccharides and derivatives that form viscous gels that are used extensively in industry, including food, textiles, hygiene, and medical products. Kelps like *Ascophyllum*, *Ecklonia*, *Fucus*, and *Laminaria* are commonly seeded and grown or wild harvested for the production of fucans (fucoidan), alginic acids (algin), and alginates (Fig. 2). Fucans are sulfated 1,3-α-fucose residues that have documented anticoagulant, antioxidant, and antitumorigenic activities. Alginic acid is a polysaccharide gelling agent composed of linear 1,4-linked β-D-mannuronic and α-L-guluronic acids with the ratios of these monosaccharides differing among strains; a higher ratio of mannuronic acid to guluronic acid produces a softer gel, whereas a higher guluronic acid content produces a stiffer gel. Alginates are extracted as metal salts of alginic acids, e.g., calcium and sodium alginate, which can comprise up to 40% of the dry weight. Kelp biomass is often digested with dilute acid to leave insoluble alginic acid which is then reconstituted with alkali, dried, milled, and stored. These compounds are highly hygroscopic and commonly used as thickeners in a variety of products, including ice cream and cosmetics. Note that aside from gametes and juvenile sporophytes, there are no true microscopic members within the Phaeophyceae. Although, there are numerous microalgal representatives in other groups of the Chromista.

Rhodophyta include both macroalgal and microalgal members, although red seaweeds are perhaps best known for their widespread use in cuisine, food additives, and laboratory applications. Rhodophyte seaweeds are used for the commercial-scale production of several gelling agents, including carrageenan, agar, and agarose (Fig. 2). Carrageenan and agars are linear polysaccharides composed of alternating 1,3-β-galactose and 1,4-α-galactose. These compounds are very similar in composition, with the exception that agar biosynthesis incorporates L-galactose and carrageenan biosynthesis incorporates D-galactose. Carrageenan is mass produced and harvested from species of *Chondrus*, *Euceuma*, *Gigartina*, *Hypnea*, and *Kappaphycus*, which is traditionally extracted from dried algal biomass with cold water and filtration, followed by acetone, alcohol, and water washes. Agar is a mixed polysaccharide produced by genera in the *Gelidiaceae* and *Gracilariaceae* that is composed of agaropectin and agarose with varying degrees of sulfation, methylation, and pyruvylation. Agaropectin is an acidic SPS primarily composed of D-glucuronic and pyruvic acid that can be separated by filtration under pressure. Additional purification is needed to generate high-purity agarose, a linear disaccharide with alternating 1,3-linked β-D-pyranose and 1,4-linked 3,6-anhydro-α-L-pyranose residues. While moderate quantities of phycocolloids can be obtained from many seaweeds, cultivars of *Gelidium*, *Pterocladia,* and *Gracilaria* are the primary resources of high-quality agar and agarose, wherein agars from *Gracilaria* have a higher degree of sulfation, methylation, and pyruvylation than *Gelidium* and *Pterocladia*.

While there are many reviews on the characterization and utility of macroalgal polysaccharides, relatively few studies exist on the bioactive potential of polysaccharides obtained from microalgal sources (see examples in Fig. 3). Examinations

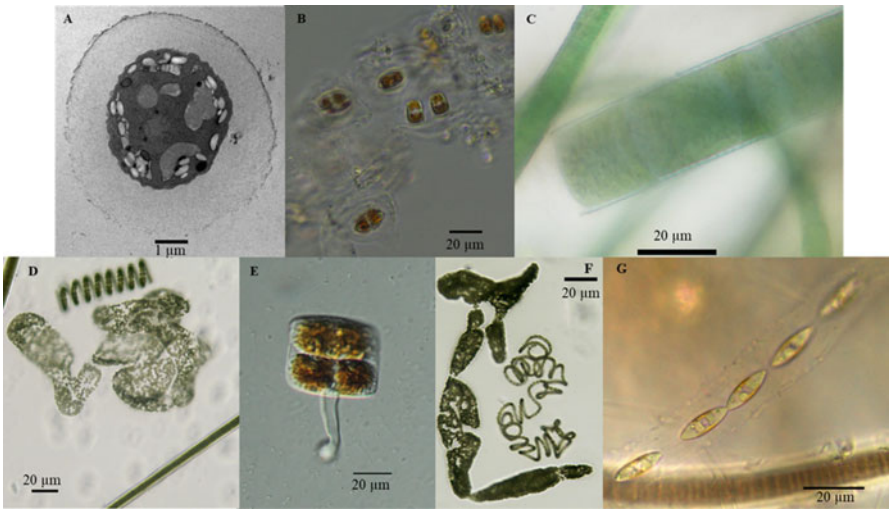


Fig. 3 Polysaccharide sheathes and mucilage in various microalgae: (**a**) scanning electron micrograph of *Porphyridium cruentum* UTEX 161 stained with Ruthenium Red. (Credit: Dr. Romanovich); (**b**) light micrograph of *Mastogloia* sp. (Credit: Dr. Ashworth); (**c**) light micrograph of *Limnothrix* sp.; (**d**) light micrograph of *Microcystis wesenbergii*, *Dolichospermum crassa*, and *Phormidium* sp.; (**e**) light micrograph of *Mastogloia* sp. (Credit: Dr. Ashworth); (**f**) light micrograph of *Microcystis aeruginosa* and *Dolichospermum lemmermanii*; and (**g**) light micrograph of *Navicula* sp. (Credit: Dr. Ashworth)

thus far have revealed that microalgal polysaccharides possess a wide range of chemical structures and bioactivities, although research has been restricted to a selected number of species/strains. Similar to their seaweed and kelp relatives, microalgal glycans are composed predominantly of pentose, hexose, and deoxyhexose monosaccharide subunits with various glycosidic linkages, degrees of branching, and structural modifications, including sulfation, amination, acylation, phosphorylation, and methylation. Given the vast genetic and biochemical differences among the microalgae, it is predicted numerous bioactive polysaccharides will be discovered with potential applications in the areas of biomedical engineering and therapeutics. Thus, the rest of this chapter will serve as a guide to case studies that demonstrate the wide range of structural and bioactive potential of polysaccharides from microalgae in the Cyanobacteria, Chlorophyta, Rhodophyta, and selected members of the Chromista, i.e., bacillariophytes, haptophytes, and dinoflagellates.

2 Polysaccharides from Microalgae

2.1 Cyanophyta

Cyanobacteria are photosynthetic gram-negative prokaryotes that occur in marine, freshwater, and terrestrial systems. These organisms exist as single cells, colonies, or filaments and they have been documented on every continent. Many genera can

proliferate rapidly, or bloom, forming turbid surface scums in lakes and in the ocean that can be seen from space. Similar to other prokaryotes, individual cyanobacterial cells have no internal compartmentalization (i.e., organelles). And they have complex cell walls with an inner membrane, a peptidoglycan layer, an outer membrane, a mucoid sheath, a proteinaceous capsule, and finally a polymeric outer sheath. Colonies and filaments of cyanobacterial cells are typically encased in a larger mucilaginous sheath.

Storage polysaccharides in cyanobacteria, referred to as cyanophycean starch, are composed of ~95% glucose with complex branching, consisting of α(1→4) and α(1→6) linkages. Although, not all cyanophycean starch is produced identically among cyanobacteria. Species of the genus *Oscillatoria* have been shown to produce starch that is similar in structure to amylopectin and polymers are 23–26 glucose units long, while species of the genus *Nostoc* produce a highly branched storage starch that is a consistent 13 glucose units (Bertocchi et al. 1990).

The cytoplasmic membrane is a lipid bilayer, typical of other prokaryotes to allow for the transport of cellular metabolites and other substances into and out of the cell. Cell walls consist mostly of peptidoglycan with some proteins and lipopolysaccharides; these are amide-linked β-hydroxy fatty acids that give individual cells their identifying morphological characteristics to help with quorum sensing and detection of other environmental parameters. Sheath or capsular polysaccharides are distinctly different from cell wall polysaccharides, and the amount of carbohydrate dedicated to the sheath is extremely variable and dependent on the species (and even strain) – capsular polysaccharides can range from 6% to 38% of the total cellular dry weight (see Bertocchi et al. 1990 for review). Many of the cell wall and sheath polysaccharides are comprised of a mixture of hexopyranose and pentopyranose sugars with multiple α- and β-linkages, while uronic acid sugars are a small, but consistent component of these structures. Capsular polysaccharides from several species of *Anabaena* contained mostly xylose (~70%) and glucose (~10%), while species of *Oscillatoria* contained glucose, galactose, fucose, arabinose, and xylose in equal parts (Nicolaus et al. 1999). *Microcoleus vaginatus* was shown to have a high glucose content in its capsular polysaccharide (49–64%) with galactose (19–32%), mannose (3–5%), rhamnose (3–8%), and galacturonic acid (3–7%) as minor components (Ge et al. 2014).

Cyanobacteria possess biosynthetic pathways to produce a wide variety of complex polysaccharides. Mucilaginous polysaccharides and EPS are well described for some genera of cyanobacteria, particularly within the orders of Nostocales and Oscillatoriales. The most common hexopyranose sugars found in EPS are glucose (2–100%), galactose (5–26%), and mannose (5–22%); deoxyhexopyranose sugars, rhamnose (1–30%) and fucose (1–21%), and pentopyranose sugars, arabinose (0.5–22%), and xylose (0.5–73%) were also detected (Panoff et al. 1988; Garozzo et al. 1998; Nicolaus et al. 1999; Brüll et al. 2000; De Philippis et al. 2000; Singh and Das 2011; Ge et al. 2014). In addition to the neutral sugar forms, aminated, acetylated (e.g., uronic acids), and methylated versions of these common sugars have also been detected, but in quantities below 10% of the total carbohydrate fraction.

EPS from species of *Anabaena* (Fig. 4a) have been characterized to contain ~70% xylose (Nicolaus et al. 1999), and minor galactose, glucose, mannose, arabinose,

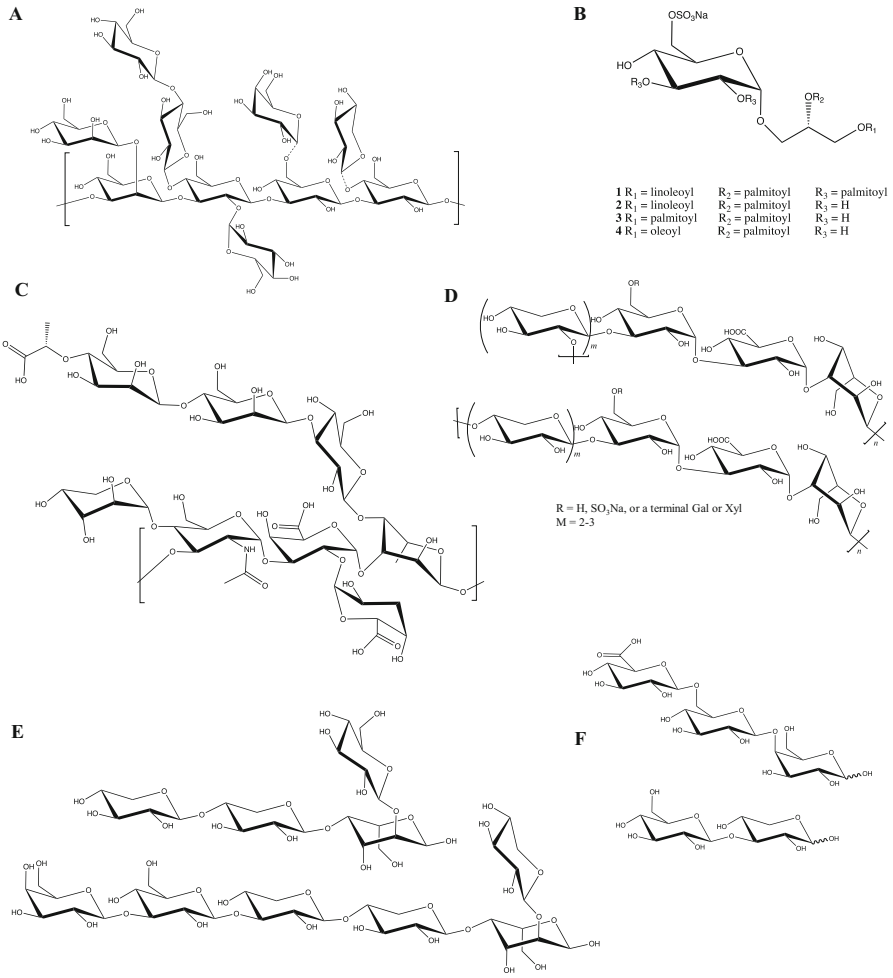


Fig. 4 Structures of carbohydrates and mixed polysaccharides from diverse groups of microalgae, including cyanobacteria: (**a**) polysaccharides from the cell envelope of heterocysts and akinetes of *Anabaena cylindrica* (Cardemil and Wolk 1979), (**b**) sulfoglucolipids from *Scytonema* sp. (Reshef et al. 1997), (**c**) exopolysaccharide from *Cyanospira capsulata* (Garozzo et al. 1998), (**d**) two different linkage possibilities (1→2 and 1→4) for the backbone of a sulfated polysaccharide from *Porphyridium* sp., (**e**) oligosaccharides from *Porphyridium* sp., and (**f**) components of nostoflan from *Nostoc flagelliforme* (Kanekiyo et al. 2005)

fucose, rhamnose, and glucosamine components were also detected (<5%). The EPS from a strain of *Cyanospira capsulata* was characterized (Fig. 4c), which contained a unique monosaccharide, 4-*O*-(1-carboxyethyl)mannose, as well as two uronic acids and glucosamine (Garozzo et al. 1998). De Philippis et al. (2000) characterized the EPS from 25 strains of *Nostoc* from the Pasteur Culture Collection and found, in almost all cases, glucose to be the most abundant monosaccharide. All but one strain

(PCC 7937) had either glucuronic or galacturonic acid present, and only one strain (PCC 7706) had higher concentrations of galactose, mannose, and xylose that were higher than glucose. EPS from field samples of *Nostoc commune* showed 50% glucose with 27% xylose, 22% galactose, and trace amounts of arabinose, glucuronic acid, and 2-*O*-methylglucose (Brüll et al. 2000). In another study, EPS from a strain of *Nostoc calciola* was characterized to have ribose as the main sugar component (36%) followed by xylose (34%) and rhamnose (30%), with only trace amounts of glucose (Singh and Das 2011).

A common modification of polysaccharides with significant medical interest is the sulfation of hydroxyl groups. The first report of SPS in cyanobacteria was documented by Panoff et al. (1988) while investigating EPS produced by two strains of *Synechococcus* (PCC 6803 and 6714). Their study showed these unicellular cyanobacteria produced an EPS containing 64–83% neutral sugars (glucose, mannose, galactose, arabinose, xylose, fucose, and rhamnose), 5–16% amine derivatives (glucosamine and galactosamine), 6–20% uronic acid derivatives (undetermined), and 0.6–1.2% sulfate. It was also determined that the age of the culture affected the concentration of sugars and the degree of sulfation. They confirmed that 13 out of 15 strains of the filamentous cyanobacterium *Cyanothece* contained at least trace amounts of sulfate. Although, to our knowledge, the SPS from strains of *Synechococcus* or *Cyanothece* have not been tested for their potential antiviral or antibacterial activity.

A bioactive SPS was isolated from *Arthrospira* (formerly, *Spirulina*) *platensis* as a calcium salt and named calcium spirulan. This SPS was characterized as containing mostly rhamnose (44.3%), followed by fructose (33.9%), glucuronic acid (7.1%), glucose (4.7%), galacturonic acid (4.0%), galactose (2.4%), ribose (2.2%), mannose (0.8%), and xylose (0.6%) with 3.24% sulfur content. Calcium spirulan was tested against herpes simplex virus type 1 (HSV-1), human cytomegalovirus (HCMV), Measles morbillivirus, Mumps orthorubulavirus, influenza-A virus, and human immunodeficiency virus type 1 (HIV-1) using multiple assays to determine antiviral activity (see Hayashi et al. 1996 and related studies). This SPS inhibited viral replication in a dose-dependent manner and calcium spirulan was shown to be effective against HCMV, HIV-1, measles, mumps, and influenza-A, but it was less effective against poliovirus and Coxsackievirus. When used to treat HIV-1 and HSV-1 in mice, calcium spirulan was shown to have a lower anticoagulant activity and a longer half-life in blood than dextran sulfate, a commercially produced SPS (Hayashi et al. 1996). It was determined that a dose of 1 mg/mL was ideal for inhibiting viral replication without suppressing host cell protein synthesis; although, infected hamsters showed prolonged survival at even higher doses of 100 and 500 mg/kg/day (Hayashi et al. 1993). In vitro studies showed that calcium spirulan inhibited HSV-1 infection by blocking the attachment of the virus to the host cell. Moreover, calcium spirulan had a potency comparable to that of a marketed drug (acyclovir), inhibiting the penetration of Kaposi's sarcoma-associated herpesvirus/human herpes virus 8, and prevent further infection of several herpes viruses (Mader et al. 2016). In addition to antiviral activity, calcium spirulan has also been shown to inhibit tumor invasion and metastasis of B16-BL6 melanoma, colon 26 M3.1

carcinoma, and HT-1080 fibrosarcoma cells though reconstituted basement membrane filters and laminin (Mishima et al. 1998).

Since, several other antiviral polysaccharides have been discovered from cyanobacteria. An uncharacterized SPS was isolated from another species of *Arthrospira* (formerly *Spirulina maxima*) and shown to be a potent inhibitor of HSV-1, HSV-2, pseudorabies virus, and HCMV. The effective dose that inhibited 50% of the virus particles from attaching was 0.333, 0.069, 0.103, and 0.142 mg/mL, respectively. However, it was not effective at concentrations of 2 mg/mL against adenovirus and not effective at all against measles, subacute sclerosing panencephalitis virus, vesicular stomatitis virus, poliovirus 1, or rotavirus SA-11 (Hernández-Corona et al. 2002). Nostoflan is yet another antiviral polysaccharide (Fig. 4f) that has been isolated from a terrestrial cyanobacterium, *Nostoc flagelliforme*, and tested against six common viruses. Nostoflan showed potent activity against HSV-1, HSV-2, HCMV, and influenza-A but was ineffective against adenovirus and Coxsackievirus (Kanekiyo et al. 2005). In another cyanobacterium, 4 of 11 sulfoglycolipids (Fig. 4b) isolated from *Scytonema* sp. (TAU strain SL-30-1-4) were shown to be potent DNA polymerase inhibitors for HIV-1 reverse transcriptase with nearly 100% inhibition at a concentration of 10 μM (Reshef et al. 1997).

Cyanobacterial polysaccharides have been shown to possess anti-inflammatory and immunostimulatory effects. Extracts of 12 intertidal filamentous cyanobacteria (11 *Phormidium* spp. and *Nostoc microscopicum*) were tested for their effectiveness in treating croton oil-induced edema in mice ear skin (Garbacki et al. 2000). Eight of the twelve extracts had significant effects ($P < 0.01$ or $P < 0.001$) with decreases in edema, resulting in a 33–56% reduction in mass. Three of the most potent extracts were characterized for their monosaccharide and sulfate composition, and all extracts contained mostly glucose (40–46%) and mannose (14–16%) with at least five other minor monosaccharides, including galacturonic and glucuronic acids. The sulfate composition ranged from 12% to 20% in the most potent extracts, but there was no direct correlation between increased sulfate concentration and the reduction of edema mass.

In another study, an uncharacterized polysaccharide extract from *Nostoc commune* showed an increase in macrophage activation/differentiation and inhibition of leukemic cell growth (Liao et al. 2015). This extract inhibited the growth of human leukemia U937 cells, triggered differentiation of U937 monoblast cells into monocytic/macrophagic lines, and increased nitric oxide and superoxide secretion of the macrophages. These responses would indicate this polysaccharide extract is stimulating and activating macrophage immunity.

There has been some success using polysaccharides extracted from *Cyanothece* spp. and *Cyanospira capsulata* to prevent the adhesion of *Helicobacter pylori* strain 51,932 to Kato III human gastric cells (Ascencio et al. 2004). Six strains of *Cyanothece* sp. and one strain of *Cyanospira capsulata* were tested, and all EPS extracts inhibited adhesion of *H. pylori* cells by 30–65%. One *Cyanothece* strain and the *Cyanospira capsulata* strain contained no sulfate, while two *Cyanthece* strains contained trace amounts of sulfate, and the remaining three *Cyanothece* strains contained abundant sulfate moieties. Results were compared to macroalgal

polysaccharide extracts where carrageenan showed 53% inhibition of adhesion while fucoidan had no affect at all. In another study, a sulfated glycoprotein complex was isolated and partially characterized from *Arthrospira platensis*. This mixed glycan contained 39% carbohydrate, 21% sulfate, and 7% protein, and assays showed antibiotic activity against *Vibrio vulnificus* at a concentration of 100 μg/mL (Rajasekar et al. 2019). Thus, it is anticipated many other antibiotic compounds exist in other cyanobacteria.

Polysaccharides of cyanobacterial origin have been heavily investigated, but for a limited number of genera and applications. There have been preliminary examinations on the use of SPS for antibiotics, and extensive research has been performed on the use of cyanobacterial SPS as potential antiviral agents. Cyanobacterial polysaccharides have also been proposed for antioxidants, the removal of heavy metals, and nutritional supplements for fish. Despite this foundation, cyanobacterial polysaccharides remain an underutilized resource in biotechnology.

2.2 Chlorophyta

Green microalgae can be found in practically every habitat – freshwater, marine, and terrestrial ecosystems, including ice and snow – and they represent an amazing variety of diverse morphologies, from unicells to filaments and colonies. Green microalgae are common components of aquatic ecosystems ranging from plankton to surface scums, and many strains have been explored for their potential in nutraceutical, feedstocks, biofertilizer, and bioremediation applications. Cultures of *Chlorella*, *Dunaliella*, and *Tetraselmis* are commonly grown for feedstocks, food, and nutritional supplements. Species in these genera are known to produce abundant omega fatty acids and carotenoid pigments with anti-inflammatory and antioxidant properties.

Microalgae of the Chlorophyta have diverse cell wall polysaccharides as well as modified scales that may be composed of cellulose, mannans, xylans, SPS, glycoproteins, 2-keto sugars, as well as plant-like extensins and lignin. Rodrigues and Bon (2011) showed that species of *Chlorella* contained up to 80% carbohydrates, including cellulose. Although, Cheng et al. (2015) found that increasing concentrations of carbon dioxide increased the content of uronic acids and decreased the neutral sugars (e.g., glucose) in strains of *Chlorella*. In a strain of *C. protothecoides*, the presence of a nonhydrolysable sporopollenin layer was found outside the cellulosic cell wall (He et al. 2016). And in some species of *Chlorella*, there is another highly resistant outer layer of algaenan, a hydrophobic biopolymer, modified with amide and N-alkyl substituted pyrroles (Rodrigues and Bon 2011). The cell walls of the filamentous genus *Oedogonium* are highly complex, containing microfibrillar cellulose, homogalacturonan, rhamnogalacturonan, arabinogalactan, and extensin-like glycoprotein (Estevez et al. 2008). Of note, cells walls for organisms in the order Chlamydomonadales, e.g., *Chlamydomonas* spp., do not contain any cellulose; instead, the cell wall is made of crystalline aggregates of hydroxyproline-rich and glycine-rich glycoproteins (Imam et al. 1985).

The storage carbohydrate in chlorophytes is amylose starch composed of α-1,4-D-glucose, or amylopectin, a branched glucan consisting α1→4(α1→6) glucose residues. Starch granules can be seen dispersed throughout the chloroplasts and large granules can often be detected around the pyrenoid. The arrangement of starch in the pyrenoid creates a microcompartment that facilitates the concentration of carbon dioxide to generate a low-oxygen environment for carbon fixation by the enzyme RuBisCO.

Like their seaweed relatives, green microalgae produce numerous types of EPS and SPS with bioactive properties, including antiviral, antioxidant, and immunomodulatory effects. Fabregas et al. (1999) documented antiviral effects from intracellular and extracellular soluble polysaccharides extracted from cultures of *Chlorella autotrophica* and *Dunaliella tertiolecta* on viral hemorrhagic septicemia virus (VHSV) and African swine fever virus (ASFV). Both extracts were found to inhibit the replication of VHSV in salmonid fish in a dose-dependent manner. Extracts from *C. autotrophica* were also found to significantly inhibit in vitro replication of ASFV. While the extracts from *Chlorella* had the highest amount of SPS (15.9%) compared to extracts from other microalgal species, there was no apparent correlation between the inhibition of virus replication and sulfur content, suggesting that the sugar composition was responsible for the antiviral properties. Thus, such polysaccharide extracts could have potential as treatments for viral diseases in fish and mammals.

There are also numerous studies on the antioxidant potential of green microalgal polysaccharides. Chen et al. (2016) extracted *Chlorella pyrenidosa* polysaccharides (CPP) from whole biomass in water with ultrasonication, and ethanol was used to precipitate three discrete polysaccharide fractions. All three fractions were found to possess antioxidant properties as measured by various radical scavenging assays [e.g., hydroxyl radical, superoxide radical, and 2,2-diphenyl-1-picrylhydrazyl (DHHP) radical]. All three extracts responded in a dose-dependent manner. The hydroxyl radical scavenging activity of CPP85<CPP60<CPP70 had 52.71%, 81.46%, and 92.71% neutralizing capacity, respectively, at dosages of 1.2 mg/mL. CPP70 contained significantly more sugar (52.74%) and far less protein (0.74%) when compared to the composition of CPP60 (34.35% sugar and 7.75% protein) and CPP85 (23.85% sugar and 11.21% protein). Otherwise, the fractions were very similar by composition. CPP70 was comprised primarily of D-glucose (25.46%) and D-galactose (13.91%), with minor D-arabinose (3.26%), D-mannose (1.06%), D-xylose (1.0%), and L-rhamnose (1.47%) residues; no fructose was detected in any fraction. Of note, all three fractions contained a nominal amount of phenol (0.13–0.47%), but this chemical feature did not appear to have any direct correlation with radical scavenging activity.

In another study, structurally diverse mixed polysaccharides extracted from *Chlorella pyrenidosa* demonstrated immunostimulatory activity in THP-1 human monocytic leukemia cells (Pugh et al. 2001). This fraction was determined to contain high molecular weight (HMW) water-soluble polymeric substances, which was named Immurella. This glycan contained mostly arabinose (31.6%), galactose (26.8%), and rhamnose (12.4), followed by glucose (5.4%), xylose (2.4%), mannose

(2.3%), and ribose (1.9%), as well as more than a dozen methylated carbohydrates, deoxyhexoses, and amino sugars. The immune response was dose dependent and the bioactivity of this extract decreased by 50% with heat treatments and by 25% when treated with proteinase K, indicating there were protein constituents contributing to the immunostimulatory effects. Compared to other clinically used polysaccharides, it was estimated that Immurella had at least 1000× more macrophage stimulating activity.

Other polysaccharides have shown anticancer and antitumorigenic potential. Nomoto et al. (1983) examined the antitumor activity of PCM-4, a crude water-soluble polymeric extract from *C. regularis*, on murine transplanted tumors. The PCM-4 extract had 25.7% sugar, 51.5% protein, and 18.7% nucleic acids. Oral administration of PCM-4 in mice inhibited the growth of sarcoma 180 and Meth A masses; similarly, in rats, PCM-4 inhibited the growth of ascites hepatoma AH 44 and AH 41C. This extract also showed antitumor activity by intraperitoneal injection. However, PCM-4 had no direct inhibition on tumor cells *in vitro*, which suggested that the antitumor property was elicited from the host's immune system. Interestingly, crude polysaccharide extracts from yet a different species of *Chlorella*, *C. stigamotophora*, exhibited immunosuppressive effects in studies by Guzman et al. (2003).

In addition to EPS associated with sheath mucilage, unicellular algae like *Chlamydomonas*, *Botryococcus*, *Haematococcus*, and *Dunaliella* consistently produce and shed soluble polysaccharides, including SPS, into their surrounding environment. EPS can constitute up to 25% of the total organic matter in media as shown with cultures of *C. mexicana* (Lewin 1956). Galactose and arabinose were the primary constituents found in polysaccharides for many species of *Chlamydomonas*; although, glucose and xylose residues were more abundant in cultures of *C. ulvaenisis* (ibid.); fucose, rhamnose, mannose, and uronic acids were identified as minor components.

EPS from several green microalgae have been tested for their anti-inflammatory bioactivity. Park et al. (2011) isolated a water-soluble polysaccharide (~135 kDa) from the medium of *Haematococcus lacustris* cultures by ethanol precipitation. The purified polysaccharide was characterized as a galactomannan, which was found to induce the secretion of proinflammatory cytokine, TNF-α, in a dose-dependent manner. This compound was also found to increase the expression of COX-2 and iNOS genes, demonstrating its immunostimulatory properties. Of note, the addition of glyoxylate enhanced EPS production in cultures of *Ankistrodesmus angustus*, and it was postulated that glyoxylate is metabolized to serine via glycine as part of photorespiration, which corresponded to an increase in the production of intracellular polysaccharides and extracellular EPS (Bergman 1986). Therefore, the addition of glyoxylate to cultures could present a potential strategy to improve yields of bioactive EPS in microalgal cultures.

EPS from green microalgae also have been documented to chelate heavy metals in solution. EPS from *Chlorella stigmatophora* was examined for its ability to bind Zn^{2+}, Cd^{2+}, Pb^{2+}, and Cu^{2+} (Kaplan et al. 1987), and results showed that bound EPS increased with cell growth in cultures of *Chlorella*. However, dissolved EPS was not

detectable until after day 12 when cultures were entering stationary phase and dissolved EPS accounted for ~10% of the total polysaccharide. While standard trace metals were added to growth media, they could not be detected by voltammetry in cultures of *C. stigmatophora* and *C. salina* grown in EDTA-free media. After performing measurements by chemical addition, it was determined that EPS from *C. stigmatophora* had a Zn complexing capacity of 213 ng/mL, while the capacity for binding Zn^{2+} in cultures of *C. salina* was measured at 78 ng/mL. Dissolved EPS was also capable of binding Cu^{2+} and Cd^{2+}, but the binding capacity of Pb^{2+} was nominal. It was postulated that the binding capacity was related to negative surface charges, e.g., uronic acids and sulfate moieties. Correspondingly, there was nearly 3× more uronic acids in EPS from *C. stigmatophora* (30%) than *C. salina* (9%), although the sulfate content was roughly similar between the two samples. While not all algae produce EPS with the capacity to bind heavy metals, chelating properties depended on the polysaccharide concentration and composition, which can be highly variable among species and could have potential applications in therapeutics.

There are more than 5000 described species of green microalgae, and it is predicted there are likely >100,000 species, yet very few species/strains have been examined for their polysaccharides and potential bioactivity. Given the biochemical similarities of green microalgae, it is highly possible that related species also produce an array of mixed polysaccharides with antioxidant, antiviral, chelating, and immunomodulatory activities.

2.3 Rhodophyta

While the Rhodophyta are perhaps best known for the production of SPS, members of this phylum produce a wide range of polysaccharides with diverse functions and potential applications. These algae owe their distinct red pigmentation to the phycobiliprotein phycoerythrin. Microscopic genera of the Rhodophyta (*Porphyridium* and *Rhodella*) exist only as unicells and they are well known for the production of polysaccharides and SPS (Fig. 4d, e). Cultures of *Porphyridium* are often highly viscous as soluble SPS is continuously shed from cells into the surrounding medium (Fig. 3a). This sheath of SPS is thought to serve a wide range of biological functions, most of which centers on the protection of the microalgal cells from their environment. The continuous secretion of polysaccharide creates a loose mucilaginous capsule best described as a dynamic system with a concentration gradient of SPS that is highest at the cell wall (Ramus and Groves 1972). The production of cell wall and bound polysaccharide in these microalgae is driven by the Golgi apparatus, and inhibition of the Golgi apparatus has shown a decrease in the production of these polysaccharides (Keidan et al. 2009).

Rhodophytes produce a variety of low molecular weight (LMW) carbohydrates and starch for the purpose of short-term energy storage. Reserve carbohydrates are composed of unique monosaccharides and sugar alcohols, including floridoside, L-isofloridoside, D-isofloridoside, digeneaside, mannitol, sorbitol, and dulcitol. However, not every species in the Rhodophyta produces all, or even a fraction, of these sugar residues.

While all species investigated have been shown to contain L-isofloridoside, the genera *Porphyridium* and *Rhodella* were found to produce only floridoside and mannitol, respectively, in addition to L-isofloridoside (Karsten et al. 2003). The primary long-term storage product is an α-glucan known as floridean starch. This starch is thought to be made in the cytosol through UDP-glucose utilization and the structure is described as a semi-amylopectin, after amylopectin used for storage in land plants.

The cell wall polysaccharides of rhodophytes provide structural support and make up approximately 10% of the biomass in cultures of *Porphyridium* sp. (Bernaerts et al. 2018). The cell wall of these microalgae is an ever-changing, dynamic capsule that is continuously replenished as its soluble portion dissolves into the media. Thus, the soluble SPS and cell wall fractions share similar components. Both of these polysaccharides consist mainly of xylose, glucose, and galactose, and they exhibit anionic properties due to the incidence of glucuronic acid and sulfate moieties (Agustini 2017). The incorporation of D-glucose and D- and L-galactose isomers are characteristic of certain genera within this group. While the presence of both D-and L-isomers are often considered unusual, they are a hallmark for red microalgae. Despite similarities in the profile of the soluble SPS and cell wall components, the structural cell wall polysaccharides exhibit different rheological properties and are arranged into LMW polymer chains (Bernaerts et al. 2018), although they are poor thickening agents when compared to SPS from the same species. The composition of the cell wall polysaccharides can also change based on CO_2 concentration which may alter the partitioning of fixed carbon. Cultures of *Porphyridium* sp. provided with only air were found to have twice the ratio of galactose to xylose in the soluble fraction of the cell wall (Li et al. 2000).

SPS from *Porphyridium* and *Rhodella* differ in their composition, which varies between and within species. Examinations with *Porphyridium* have found SPS to consist of 36% hexose with the main sugars as xylose, glucose, and galactose; the SPS also contained about 8.5% uronic acid and 9% sulfate (Heaney-Kieras and Chapman 1976). In contrast, the characterization of SPS from *Rhodella grisea* and *R. maculata* contained xylose, ribose, and rhamnose as the dominant sugars, with 17.1% uronic acids (Capek et al. 2008) and 10% sulfate (Evans et al. 1974). Other studies with *Porphyridium* have shown that sulfate moieties are present in variable amounts (1–14%), which are often attached to glucose and galactose at the C3 and/or C6 of the hexose (Bernaerts et al. 2018).

SPS can have high viscosities and usually consist of HMW molecules. The viscous nature of the SPS remains unchanged over a wide range of pH and temperature values, although gels formed using SPS are weaker than agar gels (Geresh and Arad 1991). The ability of the microalgae to take up sulfate from its environment has been shown to be dependent on light. The uptake of sulfate by cells grown in the dark was 20% less when compared to cells grown in light as measured by the accumulation of radiolabeled sulfate within cells (Ramus and Groves 1972). The intensity of light was directly correlated to higher polysaccharide production while photosynthetic rates were inversely correlated. *Porphyridium* cultures grown under a high-photon flux produced 1.6–3× more SPS than cultures grown under low-photon flux (Friedman et al. 1991). Therefore, the production of SPS and cell

wall polysaccharides was controlled through metabolite partitioning instead of total photosynthetic load (Friedman et al. 1991; Li et al. 2000).

Polysaccharides from unicellular reds have demonstrated a wide range of bioactive properties, including anticancer, antimicrobial, antioxidant, biolubrication, and gastrointestinal effects. SPS from red microalgae have been shown to interfere with tumor and inflammation mediators as well as the growth of neoplastic mammalian cell lines and implanted tumors. The action of hyaluronidase, a known mediator of inflammation implicated in tumor metastasis, was inhibited by 50% at 210 μg/mL with treatments of an SPS isolated from *P. purpureum* (Mase et al. 2013). The growth of implanted tumors (S180 cells) was inhibited by LMW SPS (6.53 kDa) from *P. cruenteum* in a dose-dependent manner. The maximum inhibition was 53.3% with a dose of 200 mg/kg/day (Sun et al. 2012). Tumor growth was mainly inhibited through stimulation of the immune system, specifically, through the proliferation and activity of macrophage cells (ibid.). The degree of SPS sulfation has also been demonstrated to affect its potency as a cancer inhibitor. The growth of T cell lymphoma lines (i.e., 24-1, EL-4, and early myeloid line FD cells) were inhibited around 70% in the presence of oversulfated HMW SPS from *Porphyridium* sp. and *Rhodella reticulata* at concentrations as low as 7 μg/mL (Geresh et al. 2002). The oversulfated SPS were the product of sulfation reactions with native SPS samples whose inhibitory action against the cell lines was consistently below 50% (ibid.).

The antiviral effects of SPS from red microalgae are well documented to inhibit viruses from binding to cells and blocking viral glycoproteins. Alternatively, SPS may prevent viral entry into the cell, or it may act as an agent to prevent replication or release of virus particles upon lysis. SPS from *Porpyridium* sp. have been shown to inhibit the cytopathic effects of HSV-1 and HSV-2. Virus replication was inhibited by 50% when 1 μg/mL was applied to cells and the development of symptoms was prevented in rats and rabbits at 100 μg/mL (see Huleihel et al. 2002 and related investigations). Similar activity was found against Varicella zoster virus, the causative agent of shingles and chicken pox, at concentrations up to 250 μg/mL without cytotoxic effects (Huleihel et al. 2002). The production of the retrovirus Murine leukemia virus (MuLV) and cell transformation by retrovirus Murine sarcoma virus (MuSV-124) were inhibited by SPS from *Porphyridium* sp. (Talyshinsky et al. 2002). The formation of foci by MuSV-124 was reduced by 50% at a concentration of 10 μg/mL SPS and the reverse transcriptase activity of MuLV was reduced by half at a concentration of 5 μg/mL (ibid.). The most effective antiviral activity was observed when the polysaccharide was administered before, or at the time of, infection implying that some of the inhibition depended on the polysaccharide blocking viral receptors.

The bioactive properties of SPS can also vary widely between strains. SPS isolated from two strains of *P. cruentum*, one from Spain and another from Israel, were tested on a variety of pathogens, which included the viruses HSV 1 and 2, vaccinia virus, and vesicular stomatitis virus, and the bacteria *Escherichia coli*, *Salmonella enteritidis*, and *Staphylococcus aureus* (Raposo et al. 2014). These studies demonstrated that the Spanish strain exhibited higher antiviral activity than

the Israeli strain, on average. However, only the Israeli strain exhibited significant antibacterial activity. In addition to favorable properties, negative side effects such as anticoagulation can occur when SPS is introduced intravenously, which is characterized by a severe, but reversible, reduction in blood platelets (Radonic et al. 2010). Thus, any undesirable effects would need to be mitigated before such compounds could be used safely in a clinical environment.

SPS from various strains of red microalgae have significant antioxidant activity. A study investigating the relative antioxidant activity from *Rhodella reticulata* found that the crude, untreated SPS was the most potent antioxidant when analyzed by both ferrous oxidation (FOX) assay and autooxidation of linoleic acid compared to deproteinized and purified SPS (Chen et al. 2010). Crude SPS preparations scavenged free radicals against superoxide anions at a concentration of 1.6 mg/mL with a proficiency of 340.65 U/L, which was significantly higher than the positive control, α-tocopherol, at 174.03 U/L (ibid.).

There are also many reports of antioxidant activity for polysaccharides derived from strains of *Porphyridium*. Tannin-Spitz et al. (2005) measured antioxidant activity of polysaccharides from different strains of *Porphyridium* by autooxidation of linoleic acid, FOX assays, and oxidative damage to 3T3 cells using a dichlorofluorescein assay. The molar concentration of the whole SPS from *Porphyridium* sp. and *P. aerugineum* for effective oxidative inhibition was found to be approximately 10 μM with larger concentrations needed when using sonicated *Porphyridium* sp. samples. The antioxidant activity proved to be at least partially dependent on sulfate availability as SPS isolated from media containing 3% sulfate was found to be about 20% less effective than SPS from media with 4.5% sulfate content. SPS and assorted intracellular polysaccharides from *P. cruentum* exhibited antioxidant activity as measured by the reduction of free radicals (Agustini 2017). The extracellular SPS was determined to be more potent than the intracellular polysaccharides with a 50% inhibitory concentration (IC_{50}) of 150.586 mg/L compared to 145.998 mg/mL, respectively. The extracellular SPS was only slightly less toxic than the intracellular extracts as shown by the Brine Shrimp Lethality Test with lethal concentrations (50% mortality) of 513.175 and 521.823 mg/L, respectively (ibid.).

In addition to native bioactivity, the high viscosity of red microalgal SPS make them ideal biomaterials for use as viscosupplements in which aqueous solutions of purified SPS can be injected into joints to treat lubrication-related disorders, e.g., rheumatoid arthritis. The biolubrication properties of SPS from *Porphyridium* sp. have been shown to be superior to hyaluronic acid, resist degradation by hyaluronidase, and maintain high viscosity over a wide range of temperatures (Arad et al. 2006). Lastly, the algal biomass and SPS extracts from *Porphyridium* sp. have been shown to significantly lower cholesterol as well as induce a variety of gastrointestinal (GI) changes in rats (Dvir et al. 2000). While the biomass proved to be more effective at increasing fecal stool bulk and decreasing GI tract transit time (51–60%), the SPS extracts induced favorable metabolic and morphological changes, e.g., reduction in lipids in the blood and bile-inducing hormone cholecystokinin, earlier excretion of acidic bile and neutral sterols, and an increase in mucosal goblet cells (ibid.).

While more research is needed, the results of these studies indicate that *Porphyridium* sp. has potential as a nutritional prebiotic.

2.4 Select Chromistan Algae

This section will highlight polysaccharides derived from "golden algae" in the Chromista, a kingdom that contains >8 major photosynthetic phyla, >12 classes of microalgae, and >20,000 described species. This branch of the algae family tree is complicated and perpetually undergoing revisions (i.e., it would require a separate chapter to tease out the genetic relationships and discrete biochemical features!). Despite this vast diversity, reports of polysaccharides from chromistan algae and their bioactivity are limited. Nevertheless, examinations conducted thus far demonstrate there is immense biochemical and bioactive potential from golden algae.

The storage products of chromistan algae are highly variable across the different phyla. Inoflagellates and cryptophytes produce amylose, similar to chlorophyte algae. And like their phaeophyte relatives, microalgal ochrophytes produce laminarin and chrysolaminarin, linear polysaccharides that can be found in hydrated vacuoles. Laminarin is a linear polysaccharide of β1→3 linked glucose and β1→6 linked branches, with a 3:1 ratio of β1→3:β1→6. Chrysolaminarin, formerly known as leucosin, is also a linear β1→3(β1→6) glucose polymer, although the branching frequency is much lower with a ratio of 11:1. Diatoms and haptophyte algae are also known to synthesize chrysolaminarin that is stored in vacuoles.

Given the broad genetic diversity of the chromists, cell walls of golden microalgae vary widely in their composition and morphology. Haptophytes have a modified cell wall composed of ornate microcrystalline cellulosic scales that may be nonmineralized or mineralized with calcium carbonate; the morphology of these scales are species-specific traits. Dinoflagellates (Miozoa) possess a cell wall made up of armored cellulosic plates, known as a theca. And diatoms (Bacillariophyta) produce their own glass house, known as a frustule, through the biomineralization of silicon dioxide obtained from the environment. Whereas, ochrophytes may have no cell wall (e.g., Raphidophyceae) or cell walls comprised of cellulose (e.g., Eustigmatophyceae) and/or hemicellulose (e.g., Xanthophyceae).

Several studies have examined the antioxidant potential of polysaccharides from algae in the Chromista, including species of diatoms, haptophytes, and ochrophytes (see Raposo et al. 2014 for review). Bioactive polysaccharides extracted from *Pavlova viridis* (Haptophyta) and *Sarcinochrysis marina* (Ochrophyta) possessed antioxidant activity determined using DPPH, hydroxyl lipid peroxidase, and hydrogen peroxide assays (Sun et al. 2014a). Three fractions were isolated containing mixed polysaccharides from *P. viridis* (P_0, P_1, P_2), including HMW (3645 kDa), moderate MW (387 kDa), and LMW (55 kDa) samples. Similarly, four fractions were isolated from *S. marina*: HMW (2595 kDa), moderate MW (453 and 169 kDa), and LMW (8.7 kDa). The fractions were characterized as containing between 70% and 79% sugars, 15% and 17% sulfur, and 5% and 9% uronic acids. The composition of polysaccharides from *P. viridis* consisted mostly of glucose, D-fructose, and

mannose; rhamnose was also found in fractions P_0 (6.63%) and P_2 (35.9%). Whereas fractions from *S. marina* contained mostly L-arabinose, D-fructose, and glucose; there was no detectable rhamnose or mannose. All fractions were found to scavenge free radicals in a dose-dependent manner, wherein antioxidant activity increased with polysaccharide concentration. The highest scavenging activity was observed for the moderate MW fractions with DHHP assays where P_2 and S_3 both had >96% scavenging rate at 1.0 mg/mL; HMW fractions P_0 and S_0 had weaker DPPH scavenging. The hydroxyl scavenging rate for fractions ranged from 50% to 98% when applied at the same concentration. The highest inhibition in lipid peroxidase assays was observed for the LMW fractions P_2 (81.4%) and S_3 (88.9%) at dosages of 1.5 mg/mL, and overall lipid peroxidase inhibition corresponded to a decrease in MW. In peroxide-induced hemolysis assays, fractions P_2 (77.4%) and S_3 (78.4%) had the highest inhibition at concentrations of 2.0 mg/mL. These results suggested that free radical scavenging activity of the various polysaccharides was primarily related to the MW of the fraction and the degree of sulfation.

Antioxidant polysaccharides from another haptophyte alga, *Isochrysis galbana*, demonstrated radical scavenging activities with superoxide-, hydroxyl-, and hydrogen peroxide-based assays (Sun et al. 2014b). Three fractions were isolated (IPSI-A, IPSI-B, and IPSII) and characterized as containing uronic acids (15.6%, 6.6%, and 25.6%, respectively) and sulfate (2.2%, 1.1%, and 5.5%, respectively). All three fractions responded in a dose-dependent manner with antioxidant assays. The scavenging rates with hydrogen peroxide varied broadly from 10% to 48% among fractions; the ability to scavenge superoxide radicals had rates ~32–54% at a dosage of 3.2 mg/mL. Scavenging rates were somewhat higher with hydroxyl radicals, which ranged from ~34% to 64%. Overall, the scavenging activity of IPSII was significantly higher ($P < 0.05$) than IPSI-A and IPSI-B, which is likely due to the content of uronic acids and sulfur. However, the radical scavenging activity for IPSII was significantly lower than the scavenging activity rate of the positive control, ascorbic acid, at half of the concentration, indicating these fractions had low-to-moderate scavenging abilities. Another strain of *I. galbana* was also found to possess an intracellular polysaccharide with antiviral properties that inhibited the replication of VHSV in salmonid fishes (Fabregas et al. 1999), illustrating the potential of finding both antioxidant and antiviral polysaccharides in single species.

SPS extracted from the diatom *Phaeodactylum tricornutum* (Bacillariophyta) have demonstrated anti-inflammatory and immunomodulatory effects (Guzman et al. 2003). Crude polysaccharide extracts were ~7% of the aqueous extract and contained 32.82% reducing sugar, 7.5% sulfate, and 6.26% uronic acids. The crude extract was further separated by ion exchange chromatography, revealing four polysaccharide fractions with variable MW, ranging from 27 to 428 kDa. Discrete fractions were obtained by increasing the concentration of KCl from 0.15 to 1.0 M; the reducing sugar content decreased with increasing KCl concentration, and there was a significant increase in uronic acids and sulfate content across fractions. Both crude extracts and fractions exhibited anti-inflammatory and immunostimulatory effects in a dose-dependent manner in examinations using rat-paw edema, delayed hypersensitivity, and phagocytosis assays. Crude extracts reduced inflammation

caused by edema from ~50% in the control rats to less than 8% in treatments with a dosage of 17.1 mg/kg. This corresponded to an increase in inhibition, which was >83% at the same dosage. The anti-inflammatory effects were significant ($P < 0.05$) and the IC_{50} of 2.92 mg/kg was much lower than the positive control reagent, indomethacin, which had an IC_{50} of 8.58 mg/kg. Of note, crude extracts from *P. tricornutum* (5 and 10 mg/kg) caused a significant increase in paw thickness in assays measuring the delayed hypersensitivity response whether the extract was administered before or after sensitization, demonstrating stimulation of the immune response. Phagocytosis was also examined with in vitro and in vivo assays. In both, the crude extracts (5 mg/kg) had no significant effect on the phagocytic index until day 12, wherein the percentage of phagocytosis was significantly higher than in the control group.

Other EPS have shown potential as anticancer agents. A sulfated EPS with tumoricidal activity was extracted from the dinoflagellate *Gymnodinium impudicum* (Miozoa) (Bae et al. 2006). The cytotoxicity of B16 tumor cells by EPS-induced murine peritoneal macrophages was measured at concentrations of 0.1–10.0 μg/mL, and cytotoxicity was shown to be dose dependent. The roles of peroxide and superoxide were evaluated, although results suggested that reactive oxygen species and cytokines were not involved in EPS-induced macrophage/tumoricidal activity. Macrophages treated with EPS elicited NO synthase in the cytosol of the stimulated cells, suggesting that the EPS induced the production of NO in cells, and results support that NO from EPS-stimulated macrophages was the primary agent involved with tumoricidal activity. EPS treatments demonstrated a significant increase in NF-nB DNA binding to its cognate in a concentration-dependent manner. Although, cytokines, e.g., IL-1, IL-6, IFN-a/h, and TNF-a, were not affected by the EPS treatments and it was determined they did not play an obvious role in the death of tumor cells by macrophage activation. Results suggested that the tumoricidal activity induced by EPS was mainly due to NO production, and the activation of macrophages was mediated by the NF-nB and JNK pathways.

Antitumor activity was also observed for polysaccharides extracted from *I. galbana* (Sadovskaya et al. 2014). Extracts contained highly branched polysaccharides with a β1→6-linked glucan backbone wherein every monosaccharide residue could possess a single glucose residue or an oligosaccharide chain. The composition of extracts was approx. 15% glucose, 6% arabinose, 6% galactose, and 6% xylose as well as ~4% uronic acids. Immunomodulatory effects were tested by measuring the expression of IL-8 by inducing expression in U937 human leukemic monocyte lymphoma cells with lipopolysaccharide (LPS) and phorbol 12-myristate 13-acetate (PMA). Their findings found no significant effect on IL-8 expression in cultures treated with polysaccharide (1–200 μg/mL), and there was no apparent interference/interaction between the extract and LPS or PMA. The effects of the polysaccharide on apoptosis were measured after incubating cells with the extract and compared to camptothecin (positive control). Up to 30% cytotoxicity was documented for U937 cells with extract concentrations of 200 μg/mL ($P < 0.03$). Although, no measurable apoptosis was observed for extract treatments. Complementary cell lysis assays were performed using the lactate dehydrogenase assay, and

there were no detectable differences for untreated cells and cells treated with the extract at 100 μg/mL after 24 h. However, there was significant inhibition of cell replication after 72 h for 50 μg/mL treatments, and after 48 and 72 h for 100 μg/mL treatments. These results suggested the polysaccharide extract from *I. galbana* was responsible for inhibiting the proliferation of U937 cells rather than cell death or inducing an immune response, similar to other β1→3(β1→6) glucans enriched in β1→6 linkages.

The genetic and biochemical diversity of the Chromista is vast, yet only a few species and their polysaccharides have been investigated for their bioactive potential. There are many genera of diatoms, dinoflagellates, and haptophytes known to produce potent bioactive metabolites with cytotoxic and allelopathic effects. Thus, it is anticipated numerous bioactive polysaccharides will be found in other chromistan microalgae that possess anti-inflammatory, antioxidant, and anti-tumorigenic activities with potential applications in therapeutics.

3 Conclusions

Microalgae produce a complex array of mixed polysaccharides, including EPS and SPS that have demonstrated bioactive properties with potential applications in therapeutics and pharmaceuticals. Microalgal polysaccharides vary widely among strains, and the bioactivity often depends on the monosaccharide composition, degree of branching, as well as the content of uronic acids and sulfate concentration. While current studies have been limited to a few species, the advent of high-throughput methodologies should allow the rapid biochemical characterization and testing of polysaccharides derived from a wide variety of microalgae. There are innumerable species/strains of microalgae yet to be characterized, providing immense opportunities for metabolite discovery in the field of microalgal polysaccharides and natural products.

References

Agustini NWS. Potency of endo-exopolysaccharide from *Porphyridium cruentum* (S.F.Gray) Nägeli as antioxidant (DPPH) and biological toxicity (BSLT). KnE Life Sci. 2017;3(4):147–56. https://doi.org/10.18502/kls.v3i4.699.

Arad SM, Rapoport L, Moshkovich A, Moppes DV, Karpasas M, Golan R, Golan Y. Superior biolubricant from a species of red microalga. Langmuir. 2006;22(17):7313–7. https://doi.org/10.1021/la060600x.

Ascencio F, Gama NL, De Phillippis R, Ho B. Effectiveness of *Cyanothece* spp. and *Cyanospira capsulata* extracellular polysaccharides as antiadhesive agents for blocking attachment of *Heliobactor pylori* to human gastric cells. Folia Microbiol. 2004;41:64–70.

Bae SY, Yim JH, Lee HK, Pyo S. Activation of murine peritoneal macrophages by sulfated exopolysaccharide from marine microalga *Gyrodinium impudicum* (strain KG03): involvement of the NF-κB and JNK pathway. Int Immunopharmacol. 2006;6(3):473–84.

Bergman B. Glyoxylate induced changes in the carbon and nitrogen metabolism of the cyanobacterium *Anabaena cylindrica*. Plant Physiol. 1986;80(3):698–701.

Bernaerts TM, Kyomugasho C, Looveren NV, Gheysen L, Foubert I, Hendrickx ME, Loey AMV. Molecular and rheological characterization of different cell wall fractions of *Porphyridium cruentum*. Carbohydr Polym. 2018;195:542–50. https://doi.org/10.1016/j.carbpol.2018.05.001.

Bertocchi C, Navarini L, Cesàro A. Polysaccharides from cyanobacteria. Carbohydr Polym. 1990;12:127–53.

Brüll LP, Huang Z, Thomas-Oates JE, Paulsen BS, Cohen EH, Michaelsen TE. Studies of polysaccharides from three edible species of *Nostoc* (Cyanobacteria) with different colony morphologies: structural characterization and effect on the compliment system of polysaccharides from *Nostoc commune*. J Phycol. 2000;36:871–81.

Capek P, Matulová M, Combourieu B. The extracellular proteoglycan produced by *Rhodella grisea*. Int J Biol Macromol. 2008;43(4):390–3. https://doi.org/10.1016/j.ijbiomac.2008.07.015.

Cardemil L, Wolk CP. The polysaccharides from heterocysts and spore envelopes of a blue-green alga. J Biol Chem. 1979;254:736–41.

Chen B, You W, Huang J, et al. Isolation and antioxidant property of the extracellular polysaccharide from *Rhodella reticulata*. World J Microbiol Biotechnol. 2010;26:833–40. https://doi.org/10.1007/s11274-009-0240-y.

Chen YX, Liu XY, Xiao Z, Huang YF, Liu B. Antioxidant activities of polysaccharides obtained from *Chlorella pyrenoidosa* via different ethanol concentrations. Int J Biol Macromol. 2016;91:505–9.

Cheng YS, Labavitch JM, VanderGheynst JS. Elevated CO_2 concentration impacts cell wall polysaccharide composition of green microalgae of the genus *Chlorella*. Lett Appl Microbiol. 2015;60(1):1–7.

De Philippis R, Ena A, Paperi R, Sili C, Vincenzini M. Assessment of the potential of *Nostoc* strains from the Pasteur Culture Collection for the production of polysaccharides of applied interest. J Appl Phycol. 2000;12:401–7.

Dvir I, Chayoth R, Sod-Moriah U, Shany S, Nyska A, Stark AH, ... Arad SM. Soluble polysaccharide and biomass of red microalga *Porphyridium* sp. alter intestinal morphology and reduce serum cholesterol in rats. Br J Nutr. 2000;84(4):469–76. https://doi.org/10.1017/s000711450000177x.

Estevez JM, Leonardi PI, Alberghina JS. Cell wall carbohydrate epitopes in the green alga *Oedogonium bharuchae* F. minor (Oedogoniales, Chlorophyta) 1. J Phycol. 2008;44(5):1257–68.

Evans LV, Callow ME, Percival E, Fareed V. Studies on the synthesis and composition of extracellular mucilage in the unicellular red alga *Rhodella*. J Cell Sci. 1974;16:1–21.

Fabregas J, Garcıa D, Fernandez-Alonso M, Rocha AI, Gómez-Puertas P, Escribano JM, Otero A, Coll JM. In vitro inhibition of the replication of haemorrhagic septicaemia virus (VHSV) and African swine fever virus (ASFV) by extracts from marine microalgae. Antivir Res. 1999;44(1):67–73.

Friedman O, Dubinsky Z, Arad SM. Effect of light intensity on growth and polysaccharide production in red and blue-green rhodophyta unicells. Bioresour Technol. 1991;38(2–3):105–10. https://doi.org/10.1016/0960-8524(91)90139-b.

Garbacki N, Gloaguen V, Damas J, Hoffmann L, Tits M, Angenot L. Inhibition of croton oil-induced oedema in mice ear skin by capsular polysaccharides from Cyanobacteria. Naunyn Schmiedeberg's Arch Pharmacol. 2000;361:460–4.

Garozzo D, Impallomeni G, Spina E, Sturiale L. The structure of the exocellular polysaccharide from the cyanobacterium *Cyanospria capsulata*. Carbohydr Res. 1998;307:113–24.

Ge H, Zhang J, Zhou X, Xia L, Hu C. Effects of light intensity on components and topographical structures of extracellular polymeric substances from *Microcoleus vaginatus* (Cyanophyceae). Phycologia. 2014;53:167–73.

Geresh S, Arad S. The extracellular polysaccharides of the red microalgae: chemistry and rheology. Bioresour Technol. 1991;38(2–3):195–201. https://doi.org/10.1016/0960-8524(91)90154-c.

Geresh S, Mamontov A, Weinstein J. Sulfation of extracellular polysaccharides of red microalgae: preparation, characterization and properties. J Biochem Biophys Methods. 2002;50(2–3):179–87. https://doi.org/10.1016/s0165-022x(01)00185-3.

Guzman S, Gato A, Lamela M, Freire-Garabal M, Calleja JM. Anti-inflammatory and immunomodulatory activities of polysaccharide from *Chlorella stigmatophora* and *Phaeodactylum tricornutum*. Phytother Res. 2003;17(6):665–70.

Hayashi K, Hayashi T, Morita N, Kojima I. An extract from *Spirulina platensis* is a selective inhibitor of herpes simplex virus type 1 penetration into HeLa cells. Phytother Res. 1993;7:76–80.

Hayashi K, Hayashi T, Jojima I. A natural sulfated polysaccharide, calcium spirulan, isolated from *Spirulina platensis*: *in vitro* and *ex vivo* evaluation of anti-herpes simplex virus and anti-human immunodeficiency virus activities. AIDS Res Hum Retrovir. 1996;12:1463–71.

He X, Dai J, Wu Q. Identification of sporopollenin as the outer layer of cell wall in microalga *Chlorella protothecoides*. Front Microbiol. 2016;7:1047. https://doi.org/10.3389/fmicb.2016.01047.

Heaney-Kieras J, Chapman DJ. Structural studies on the extracellular polysaccharide of the red alga, *Porphyridium cruentum*. Carbohydr Res. 1976;52(1):169–77. https://doi.org/10.1016/s0008-6215(00)85957-1.

Hernández-Corona A, Nieves I, Meckes M, Chamorro G, Barron BL. Antiviral activity of *Spirulina maxima* against herpes simplex virus type 2. Antivir Res. 2002;56:279–85.

Huleihel M, Ishanu V, Tal J, Arad SM. Activity of *Porphyridium* sp. polysaccharide against herpes simplex viruses in vitro and in vivo. J Biochem Biophys Methods. 2002;50(2–3):189–200. https://doi.org/10.1016/s0165-022x(01)00186-5.

Imam SH, Buchanan MJ, Shin HC, Snell WJ. The *Chlamydomonas* cell wall: characterization of the wall framework. J Cell Biol. 1985;101(4):1599–607.

Kanekiyo K, Lee J-B, Hayashi K, Takenaka H, Hayakawa Y, Endo S, Hayashi T. Isolation of an antiviral polysaccharide, nostoflan, from a terrestrial cyanobacterium, *Nostoc flagelliforme*. J Nat Prod. 2005;68:1037–41.

Kaplan D, Christiaen D, Arad SM. Chelating properties of extracellular polysaccharides from *Chlorella* spp. Appl Environ Microbiol. 1987;53(12):2953–6.

Karsten U, West JA, Zuccarello GC, Engbrodt R, Yokoyama A, Hara Y, Brodie J. Low molecular weight carbohydrates of the bangiophycidae (Rhodophyta)1. J Phycol. 2003;39(3):584–9. https://doi.org/10.1046/j.1529-8817.2003.02192.x.

Keidan M, Friedlander M, (Malis) Arad S. Effect of Brefeldin A on cell-wall polysaccharide production in the red microalga *Porphyridium* sp. (Rhodophyta) through its effect on the Golgi apparatus. J Appl Phycol. 2009;21:707. https://doi.org/10.1007/s10811-009-9406-0.

Lewin RA. Extracellular polysaccharides of green algae. Can J Microbiol. 1956;2(7):665–72. https://doi.org/10.1139/m56-079.

Li S, Shabtai Y, Arad S. Production and composition of the sulphated cell wall polysaccharide of *Porphyridium* (Rhodophyta) as affected by CO_2 concentration. Phycologia. 2000;39(4):332–6. https://doi.org/10.2216/i0031-8884-39-4-332.1.

Liao H-F, Wu T-J, Tai J-L, Chi M-C, Lin L-L. Immunomodulatory potential of the polysaccharide-rich extract from the edible cyanobacterium *Nostoc commune*. Med Sci. 2015;3:112–23.

Mader J, Gallo A, Schommartz T, Handke W, Nagel C-H, Günther P, Brune W, Reich K. Calcium spirulan derived from *Spirulina platensis* inhibits herpes simplex virus 1 attachment to human keratinocytes and protects against herpes labialis. J Allergy Clin Immunol. 2016;137:197–203.

Mase T, Yamauchi M, Kato Y, Esaki H, Isshiki S. Hyaluronidase-inhibiting acidic polysaccharide isolated from *Porphyridium purpureum*. J Sugiyama Jogakuen Univ Nat Sci. 2013;44:105–13.

Mishima T, Murata J, Toyoshima M, Fujii H, Nakajima M, Hayashi T, Kato T, Saiki I. Inhibition of tumor invasion and metastasis by calcium spirulan (Ca-SP), a novel sulfated polysaccharide derived from blue-green alga, *Spirulina platensis*. Clin Exp Metastasis. 1998;16:541–50.

Nicolaus B, Panico A, Lama L, Romano I, Manca MC, De Giulio A, Gambacorta A. Chemical composition and production of exopolysaccharides from representative members of heterocystous and non-heterocystous cyanobacteria. Phytochemistry. 1999;52:639–47.

Nomoto K, Yokokura T, Satoh H, Mutai M. Antitumor activity of *Chlorella* extract, PCM-4, by oral administration. Gan To Kagaku Ryoho Cancer Chemother. 1983;10(3):781.

Panoff J-M, Priem B, Morvan H, Joset F. Sulphated exopolysaccharides produced by two unicellular strains of cyanobacteria, *Synechocystis* PCC 6803 and 6714. Arch Microbiol. 1988;150:558–63.

Park JK, Kim ZH, Lee CG, Synytsya A, Jo HS, Kim SO, Park JW, Park YI. Characterization and immunostimulating activity of a water-soluble polysaccharide isolated from *Haematococcus lacustris*. Biotechnol Bioprocess Eng. 2011;16(6):1090–8.

Pugh N, Ross SA, El Sohly HN, El Sohly MA, Pasco DS. Isolation of three high molecular weight polysaccharide preparations with potent immunostimulatory activity from *Spirulina platensis*, *Aphanizomenon flos-aquae* and *Chlorella pyrenoidosa*. Planta Med. 2001;67(08):737–42.

Radonic A, Thulke S, Achenbach J, Kurth A, Vreemann A. Anionic polysaccharides from phototrophic microorganisms exhibit antiviral activities to vaccinia virus. J Antivir Antiretrovir. 2010;02(04). https://doi.org/10.4172/jaa.1000023.

Rajasekar P, Palanisamy S, Anjali R, Vinosha M, Elakkiya M, Marudhupandi T, Tabarsa M, You S, Prabhu NM. Isolation and structural characterization of sulfated polysaccharide from *Spirulina platensis* and its bioactive potential: *In vitro* antioxidant, antibacterial activity and Zebrafish growth and reproductive performance. Int J Biol Macromol. 2019;141:809–21.

Ramus J, Groves ST. Incorporation of sulfate into the capsular polysaccharide of the red alga *Porphyridium*. J Cell Biol. 1972;54(2):399–407. https://doi.org/10.1083/jcb.54.2.399.

Raposo MFDJ, de Morais AMMB, de Morais RMSC. Bioactivity and applications of sulphated polysaccharides from marine microalgae. Mar Drugs. 2014;93:479–86. https://doi.org/10.1016/j.lfs.2013.08.002.

Reshef V, Mizrachi E, Martezki T, Silberstein C, Loya S, Hizi A, Carmeli S. New acylated sulfoglycolipids and digalactolipids and related known glycolipids from cyanobacteria with a potential to inhibit the reverse transcriptase of HIV-1. J Nat Prod. 1997;60:1251–60.

Rodrigues MA, Bon EP. Evaluation of *Chlorella* (Chlorophyta) as source of fermentable sugars via cell wall enzymatic hydrolysis. Enzyme Res. 2011;2011. Article ID 405603, 5 pages.

Sadovskaya I, Souissi A, Souissi S, Grard T, Lencel P, Greene CM, Duin S, Dmitrenok PS, Chizhov AO, Shashkov AS, et al. Chemical structure and biological activity of a highly branched (1→3,1→6)-β-D-glucan from *Isochrysis galbana*. Carbohydr Polym. 2014;111:139–48.

Singh S, Das S. Screening, production, optimization and characterization of cyanobacterial polysaccharide. World J Microbiol Biotechnol. 2011;27:1971–80.

Sun L, Wang L, Zhou Y. Immunomodulation and antitumor activities of different-molecular-weight polysaccharides from *Porphyridium cruentum*. Carbohydr Polym. 2012;87(2):1206–10. https://doi.org/10.1016/j.carbpol.2011.08.097.

Sun L, Wang L, Li J, Liu H. Characterization and antioxidant activities of degraded polysaccharides from two marine Chrysophyta. Food Chem. 2014a;160:1–7.

Sun Y, Wang H, Guo G, Pu Y, Yan B. The isolation and antioxidant activity of polysaccharides from the marine microalgae *Isochrysis galbana*. Carbohydr Polym. 2014b;113:22–31.

Talyshinsky MM, Souprun YY, Huleihel MM. Anti-viral activity of red microalgal polysaccharides against retroviruses. Cancer Cell Int. 2002;2:8. https://doi.org/10.1186/1475-2867-2-8.

Tannin-Spitz T, Bergman M, van-Moppes D, et al. Antioxidant activity of the polysaccharide of the red microalga *Porphyridium* sp. J Appl Phycol. 2005;17:215–22. https://doi.org/10.1007/s10811-005-0679-.

Wang L, Wang X, Wu H, Liu R. Overview on biological activities and molecular characteristics of sulfated polysaccharides from marine green algae in recent years. Mar Drugs. 2014;12(9):4984–5020.

Polysaccharides of Biomedical Importance from Genetically Modified Microorganisms

29

Regina Sharmila Dass, K. R. Anand, Damini Saha, Joy Elvin Dhinakar, and Pooja Thorat

Contents

R. S. Dass (✉) · K. R. Anand · D. Saha · P. Thorat
Fungal Genetics and Mycotoxicology Laboratory, Department of Microbiology, School of Life Sciences, Pondicherry University, Pondicherry, India

J. E. Dhinakar
Department of Studies in Biochemistry, University of Mysore, Mysore, India

J. M. Oliveira et al. (eds.), *Polysaccharides of Microbial Origin*,
https://doi.org/10.1007/978-3-030-42215-8_38

Abstract

Polysaccharides are a group of biopolymers which play a pivotal role in all living organisms ranging from macro- to microorganisms. The synthesis of polysaccharides using natural methods is a sluggish and slow process. Hence, an amalgamation of several methods for the production of polysaccharides to innovatively increase yields is the need of the hour. This is also to meet the growing population and industrial demands. Genetically modified organisms are continually being explored and scrutinized. The impeccable adoption and integration of the methods lies in producing polysaccharides through genetically modified microorganisms. Naturally synthesized polysaccharides consume a huge amount of time, with smaller yields compared to the actual requirement. The role of microbes in such situations is not just demanding, but also crucial in the large-scale production of polysaccharides. The microbial polysaccharides are a renewable source in the synthesis process, especially in industries and pharmaceuticals. Genetic engineering technologies offer a tremendous hike in the synthesis of polysaccharides/biopolymers in shorter time spans. However, very few microbial polysaccharides have been commercialized. Progression toward diversification of microbial polysaccharides can be achieved to enhance production via physical and biochemical reactions. *Agrobacterium tumefaciens*, *Azotobacter*, *Gluconoacetobacter xylanicans*, *Leuconostoc dextranicum*, *L. mesenteroide*s, *Pseudomonas aeruginosa*, *P. putida*, *Rhizobium meliloti*, *Sphingomonas elodea*, *Xanthomonas* species, etc. are some examples of microorganisms which have been used in production of polysaccharides. However, there is a soaring load of work to be done. One of the ways to achieve this is to adopt genetic engineering techniques, which undoubtedly offer promising results as well as pose scientific and technical challenges.

Keywords

Biomedical · Microbial · Natural biopolymers · Polysaccharides · Genetically modified microorganisms

1 Introduction

In science, biomolecules are biological molecules which include either small metabolites or large molecules. These biomolecules are categorized into four major classes: carbohydrates, proteins, nucleic acids, and lipids. These classes of molecules either singly or in complex combinations with themselves or each other perform several functions within the living system. Polysaccharides are one such branch of biomolecules, synthesized by the living system in supporting life forms.

Polysaccharides make up a major part of the class of natural biopolymers. They have been expounded as high molecular weight carbohydrate polymers with simple monosaccharide units as their building block elements. The monosaccharide units are linked to one another by *O*-glycosidic bonds either in straight linear chains or as branches attached to each other at specific regions, forming linear or branched chain polysaccharides, thus these polysaccharides can be hydrolyzed into more than one monosaccharide units. Polysaccharides form structural units as well as energy storage units in all forms of life, in case of humans and animals they also have nutritional value. Because of its nontoxicity toward mammalian tissues, they have found major industrial utility, mostly in cosmetics and pharmaceuticals.

Polysaccharides can be classified based on several characteristics like their composition, function, origin, etc. One such important characteristic is its composition of monosaccharide units. A polysaccharide with a single type of monosaccharide unit is called a homopolysaccharide, whereas a polysaccharide with two or more types of repetitive monosaccharide units is referred to as heteropolysaccharide. An example of a heteropolysaccharide is hyaluronic acid which is composed of two types of monosaccharide units, namely D-glucuronic acid and *N*-acetyl-D-glucosamine linked with alternating β (1→4) and β (1→3) glycosidic bonds. On the contrary, starch which is simply composed of glucose subunits is an example of homopolysaccharides (BeMiller and Whistler 2012). Polysaccharides also entitled as glycans are polymers of glycose residues linked by glycosidic bonds. It should be noted that a polysaccharide is not a synonym for polyglycose, because in polyglycose multiple glycose residues are not linked via glycosidic linkages. Polysaccharides with a major amount of amino sugar residues are also termed as glycosaminoglycan.

2 Types of Polysaccharides

Polysaccharides are broad and huge macromolecules with high molecular weight, having monomeric units linked by glycosidic bonds. They play a very important role in rigidity as well as integrity of biological systems. Polysaccharides are of different types, they are classified on the basis of biological, biochemical, and physical properties. Biologically they can be classified as the following:

2.1 Food Storage Polysaccharides

They are called so because of their unique ability to act as a store house of preserved food. They are naturally available in the form of fruits, vegetables, leaves, pulses, grains, nuts, milk, meat, etc. These can be for human consumption as they are much simpler polysaccharides and can be easily metabolized by the human digestive system. Food storage polysaccharides are classified into three groups based on their origin. These origins are plant, animals, and microbes (Kato 2002) (Table 1).

Table 1 Various polysaccharides used for consumption

Sl. No.	Polysaccharide	Origin	Sources		Subunits	
1.	Starch	Plant	Cereals, grains, legumes, fruits		Amylose amylopectin	and
2.	Inulin	Plant	Tubers of *Dahlia* and plants	related	Glucose fructose	and
3.	Glycogen	Animal	Meat, animal cell extract		Glucose	
4.	Agar agar	Algae	Sea weeds		Agarose agaropectin	and

Starch is formed as the end product of photosynthesis, and is stored as a reserve for food in the chloroplasts and leucoplasts of plant cells. They are stored as microscopic granules known as starch grains (Li et al. 2018). On the other hand, polysaccharides like glycogen is a form of reserved food found in bacteria, fungi, and animals, stored as small granules in the liver and muscles and hence is also known as animal starch. Inulin, a furan storage product in tubers of *Dahlia* and related plants, is not metabolized in human body and therefore it is released readily in the urine.

2.2 Structural Polysaccharides

These types of polysaccharides are associated with the cell wall of animals, plants, and microbes. The main function of these polysaccharides is to maintain the shape and rigidity of the cells and also give mechanical support to the cell. They are very complex molecules and are difficult to rupture. The most abundant structural polysaccharide in this universe is cellulose. Structurally they are tough and complex. A single cellulose chain has more than 6000 glucose units and is associated with all kind of plants. The various forms of cellulose include lignin, hemicellulose, pectin, and wax. Fifty percent of cotton fibers are made up of cellulose. Wood rich in cellulose is used in making furniture. In recent days, cellulose is also used as a source of biofuel by enzymatic saccharification. Normally, these polysaccharides are not digested by human beings and other animals. However, some bacteria, fungi, protozoa, and ruminant animals can utilize cellulose because of their ability to produce an enzyme called cellulase. It also used for biomedical applications to make bandages and other medical strappings. A principal portion of cellulose is used for manufacturing of paper. The second most abundant structural polysaccharide is chitin, commonly present in fungi and invertebrate animals. It is made up of monomers of *N*-acetyl glucosamine. In comparison to cellulose, chitin is lathery, soft, and has good elasticity. It is also referred to as fungal cellulose (Brown and Gordon 2003). Peptidoglycan, common in microbes like bacteria and archaea, is another major type of structural polysaccharide. Peptidoglycan makes up a thick cell wall around the cell. It is a heteropolysaccharide made up of monomeric units *N*-acetyl

Table 2 Structural polysaccharides and their biological roles

Sl. No.	Polysaccharide	Natural sources	Special properties
1.	Keratin sulphate	Skin and cornea of animal cells	Provide strength and flexibility to eye muscles
2.	Chondroitin sulphate	Matrix of cartilage and connective tissue	Mechanical support and elasticity
3.	Pectin	Fruits and middle lamellae of plants	Used for making jelly and jams
4.	Peptidoglycan	Cell wall of bacteria	Mechanical support
5.	Lipopolysaccharides	Associated with cell walls of gram-negative bacteria	Responsible for endotoxic activity
6.	Mucoproteins	Nasal secretion, stomach, intestine, and vagina	Antibacterial; protective in function

muramic acid and *N*-acetyl glucosamine. The thickness of the layer is different in different bacteria. It is thicker in gram-positive bacteria as compared to gram-negative bacteria. It is also responsible for the virulence of the bacteria and therefore can be used for the production of polysaccharide vaccines (Grage et al. 2009).

2.3 Mucoid Polysaccharides

These are a heterogeneous group of polysaccharides having unique ability to associate with protein, lipids, and other organic molecules. These also called as membrane-associated polysaccharides present in animals, plants, and microorganisms. Generally, they are considered as associative polysaccharides as it binds to the proteins in cell wall and connective tissues and are quite common in the mucus membranes of the animal tissue. Following are the most common examples of mucoid polysaccharides (Roca et al. 2015) (Table 2).

3 Naturally Occurring Polysaccharides

Nature is a source of different types of polysaccharides. This is one of the reason for sustainability of life on earth. Such polysaccharides are known as biological polysaccharides or natural polysaccharides. The spectrum of biological polysaccharides ranges from unicellular bacteria to plants; more than 80% of biological polysaccharides are derived from plants. The most abundant and naturally occurring polysaccharide is glycan sugars. Their molecular structure is either branched or linear, as in gum Arabic and cellulose, respectively. Complex heteropolysaccharides occur in plant gum, such as gum Arabic from *Acacia* and gum tragacanth from *Astragalus*, with several of them containing glucuronic acid and various sugars. These gums are mainly formed in the bark of the tree, due to attack by certain bacteria, insects, or fungi. Plant gums are used in the preparation of cosmetics as an adhesive agent

Table 3 Polysaccharides of plant origin (Li et al. 2018)

Sl. No.	Polysaccharide	Occurrence
1.	Glycogen and starch	Storage carbohydrate for plants and animals
2.	Dextran	Glucose contains homopolysaccharide found in plants; synthesized extracellularly by certain bacteria
3.	Pentosans	Woods, nuts, and other plant products
4.	Fructans (Levans)	Present as inulin forms in roots and tubers
5.	Mannose	Ivory nuts (*Phytelephas* species), orchid, bark of pine trees, fungi, and bacteria
6.	Pectins	Found in fruits like berries commercially used as a gelling agent

(Kögel-Knabner 2002). Polysaccharides from animals and plants were widely used in ancient times. The major disadvantage associated with this is growing plants. It requires large area for cultivation and also the conditions like temperature, water, and hydrostatic pressure should be suitable for the plant growth. The long duration (6–12 months) associated with the harvest of the product is another drawback (Klok 2005). Most of the food storage polysaccharides can be industrially produced by using plants. Previously food industries used gelatin and collagen from animals like pigs, buffalos, cow, and goats. This gave rise to ethical and religious problems in various countries like India, UAE, USA, etc. Vegetarians also shunned the use of animal products as food additives. These problems led to the search for better alternatives for the production of industrially important polysaccharides (Table 3).

4 Microbial Polysaccharides

Polysaccharides derived using natural methodologies from animals and plants are time consuming and energy intensive. Microbial polysaccharides are found to be a better alternative. Many researchers have reported the efficiency of microbial polysaccharides as a source of antioxidants, viscosifying, and gelling agents in food industries and in medicine (Gonçalves et al. 2009; Falconer et al. 2011). Microbes like bacteria, yeast, fungi, protozoa, etc. are the source of various biomaterials. Most of the microbial-derived polysaccharides play an important role in their physiology. Fungal cell walls are rigid and tuff biomaterials. It provides strength and maintains the architecture of fungal cell wall. It is dynamic in nature and is fundamentally based on the type of cell wall polysaccharides present in them. Cell walls are composed of repeating units of various polysaccharides cemented with proteins. Gastebois et al. (2009) reviewed the cell wall polysaccharides of the pathogenic fungus *Aspergillus fumigatus* and its biosynthesis. It consisted α-(1, 3)-glucan (35–46%), galactosaminoglycan, β-(1, 3)-glucan (20–35%), galactofuran (20–25%), mannan, and chitin (7–15%). The organization of these polysaccharides is a complex process. It can be extracted by enzymatic digestion with endo-β-(1, 3)-glucanase and endo-β-(1, 6)-glucanase (Gastebois et al.

2009). Microbial polysaccharides are of three classes based on their morphological location and are intracellular, span the cell wall, and extracellular of which bacterial exopolysaccharides are most common. Bacteria are the most widely exploited organisms for the production of various polysaccharides.

4.1 Bacterial Exopolysaccharides

Bacterial exopolysaccharides are important intracellular polysaccharides and are associated with the cytoplasmic membrane through covalent bonding or through weak binding to cell surface to form slime. Exopolysaccharides get wide attention due to its applicability as a food additive. For example, Xanthan and gellan gum. Under conditions of stress, certain bacteria can release large number of exopolysaccharides like xanthan gum (40 g/L). The use of such bacteria for the production of exopolysaccharides is advantageous, because it takes a long duration for synthesis. The costs associated with production can be reduced by replacing conventional methods with biotechnological methods. A large number of bacteria are known to produce extracellular polysaccharides. These are biopolymers which possess high molecular weight, displaying enormous diversity with respect to their chemical structures and composition. This makes it possible for them to be used in a wide range of applications, depending upon the presence of rare sugars and physical and chemical properties. Sugars such as D-galactose, D-fructose, D-xylose, D-ribose, and L-arabinose are most abundant in the extracellular polysaccharides and some rare sugars like L-fructose, L-rhamnose, or uronic acids are also associated with extracellular polysaccharides (Czaja et al. 2007b).

The exopolysaccharides from bacteria are an interesting source in predicting their identity, because certain sugars are specifically present in certain strains of bacteria. Polysaccharides containing rare sugars are also found in plants, seaweeds, and animals. Microbial production of such polymers is an advantage over the others because of several reasons; one reason is the production not being affected by environmental factors. Some of these exopolysaccharides are used for the making of artificial cartilage. Widely studied bacterial exopolysaccharides include colonic acid, fucogel, clavan, gellan, and welan gum. Many of these polymers are being studied for new applications in pharmaceuticals, cosmetics, food products, etc. Exopolysaccharides containing rhamnose belonging to the class of spingans are produced by strains of *Sphingomonas*. Other important polysaccharides which come under this group are gellan, diutan, welan gum, etc. There is a variation in the structure of spingans, mainly due to the difference in number of repeating units of rhamnose or mannose sugars in its side chain. *Klebsiella* I-174 produces exopolysaccharides with a high rhamnose content, consisting of hexasaccharide units of L-rhamnose, D-galactose, and D-glucuronic acid in a molar ratio of 3:2:1 (Roca et al. 2015).

Clavan is a fucose-containing exopolysaccharide formulated of tetrasaccharide repeating units of glucose, galactose, fucose, and pyruvic acid, in a molar ratio of 1:1:2:1. These kinds of polysaccharides are produced by *Clavibacter michiganensis*. Another class of fucose exopolysaccharide is solabia-A. It is a linear anionic

polymer produced by *Klebsiella pneumoniae*, having trisaccharide repeating units of galactouronic acid of L-fucose and D-galactose. *Enterobacter cloacae* produce polymers composed of fucose, galactose, glucose, and glucuronic acid (Roca et al. 2015). Strains of *Butyrivibrio fibrisolvens* and *Streptococcus zooepidermidis* produce illustrious amounts of exopolysaccharide containing L-allose and L-iduronic acid. *Pseudomonas viscogena* produces exopolysaccharide containing allose, a C2 epimer of altrose, but present only in negligible proportions (Nasi et al. 2011).

4.1.1 Bacterial Polysaccharides Used for Industrial Applications

Up until now not many polysaccharides have been commercialized. The limited availability of polysaccharides and also applicability of various microbial polysaccharides is not well known and understood in industries. Till now the most commonly accepted polysaccharides commercialized for industrial purpose are xanthan gum (Simsek 2009), succinoglycan, gellan gum (Gonçalves et al. 2009), and dextran (Falconer et al. 2011).

Xanthan Gums

It is an extracellular polysaccharide produced by the bacterium *Xanthomonas campestris*. It is an anionic polysaccharide. Its activity depends up on the ionic strength of the solution. It is composed of β-(1, 4)-linked glucan with repeating units of mannose. USA Food and Drug Administration (FDA) has approved xanthan gum as a food grade additive. In the food industry, xanthan gum is used as an emulsifier, thickener, and stabilizing agents, because of its high viscosity at lower concentration, stability in higher temperature, freezing conditions, and highly acidic and basic conditions. A team of workers (Zhao et al. 2009) examined the consistency of whipping cream, in which they found that consistency increases with 0.1% increase in xanthan gum. Simsek (2009) reported freeze dough formation at increased xanthan concentration. It was also reported that xanthan is an edible starch and used for making PGX (poly glycopleX), a tertiary mixture of glucomannan and sodium alginate. More recently, large-scale production of xanthan gum is possible by using *X. campestris* (Garcia et al. 2000) (Table 4).

Gellan Gum

"Gellan gum" is the trivial name given for the exopolysaccharides produced by *Pseudomonas elodea*. It is composed of β-D-glucose, L-rhamanose, and D-glucouronic acid in molar ratio of 2:1:1 with a high molecular weight. Gellan gum is used as an additive in jams, jellies, juices, and in dairy products like ice creams, milkshake, and yogurt (Banik and Santhiagu 2006). Gellan exhibits gelation property and is dependent upon acetylating process. Under acetylated conditions gellan forms soft, elastic, and thermo-reversible gels and in deacetylated conditions it forms hard, brittle, and thermo-irreversible gels.

Dextran

It is an exopolysaccharide produced by several bacteria and consists of repeating units of α-(1, 6)-glucopyranase units. Bacteria like *Streptococcus* and *Acetobacter*

Table 4 Showing various polysaccharides of bacterial origin

Sl. No.	Polysaccharide	Microbial source
1.	Levan	*Halomonas eurihalina* (Béjar et al. 1998) *Zygomonas mobils* (Bekers et al. 2005)
2.	Glucan	*Leuconostoc deaticum* (Majumder and Goyal 2009)
3.	Gellan gum	*Sphingomonas paucimobilis* (Banik and Santhiagu 2006) *Sphingomonas elodea* (Gonçalves et al. 2009)
4.	Xanthan gum	*Gluconobacter xylinum*
5.	Dextran	*Leuconostoc mesenteroides* (Falconer et al. 2011)
6.	Alginate	*Pseudomonas aeruginosa* (Schürks et al. 2002) *Pseudomonas putida*
7.	Succinoglycan	*Agrobacterium radiobacter* *Rhizobium melioti*

have been reported to produce dextran. Some bacteria produce enzymes like dextrasucrase to convert sucrose to produce dextran. Dextran is used as gelling agent, thickener, and emulsifier in jams, jellies, and ice creams. It is highly soluble in water and biodegradable polymer. A wide range of gram-positive and gram-negative bacteria produce dextrans, namely *Leuconostoc mesenteroides* (Üretimi 2005) and *Streptococcus mutans*, respectively. These have been used as blood plasma expanders in biomedical sciences. Colloidal blood plasma expanders, namely dextrans, are used to restore blood plasma, lost due to excessive bleeding. The commercial production of dextrans is carried out by adopting batch fermentation, using the lactic acid bacterium (LAB), namely *Leuconostoc mesenteroides*, in culture broths containing sucrose, an organic nitrogen source, and an inorganic phosphate. Cell-free extracts are also used to produce dextrans, with the addition of dextrasucrase, which transforms sucrose to dextrans in a cell-free nutrient solution, with pH and temperature optimizations (pH 5.0–5.5; 25–30 °C).

Cellulose

Microbial cellulose is a versatile polymer used in electronic and biomedical devices. It possesses special properties like wound healing properties (Czaja et al. 2006). *Acetobacter xylinum* is a widely used strain for the industrial production of cellulose (Czaja et al. 2007a). Synthesis of microbial cellulose is a complex process, in which glucose present in medium polymerizes into β-1, 4-glucan chains. This is followed by the extracellular release of these chains, which further crystallize to form microfibrils of cellulose and finally produce a matrix in the medium. Bacterial celluloses possess very high water holding capacity.

The nanoporous consistency of these molecules prevents the entry of bacteria and other microbes. It differs from plant cellulose owing to its nanoporosity and high elasticity. Medically, it is used for healing wounds temporarily along with dressings. In tissue engineering, microbial cellulose is used as a material for creating temporary artificial tissues and cartilages. There are a multitude of polymers which need to be commercialized for industrial applications. Clinical trials are under process for validating the use of such polysaccharides.

5 Synthetic Polysaccharides

Synthetic polysaccharides are otherwise called as artificial polysaccharides, which generally are formulated by a chemical process. Further, their property can be improved by a chemical or physical manufacturing process. Most of these polysaccharides are semisynthetic. Naturally occurring polysaccharides like cellulose possess chemical inertness that can be overcome by chemical processes like esterification, acidification, condensation, proteolysation, etc. Most of the synthetic process aims to improve the property of naturally occurring polymers. Polyglucose is a high molecular weight polycondensation product of α-D-glucose formed under temperature range of 140–170 °C. It requires an acidic catalyst like phosphoric acid, and would result in polymerization of glucose to polyglucose under reaction conditions of reduced pH. Biomedical industries use polyglucose for making capsule shells.

Alginates are anionic polymers typically obtained from brown algae namely *Laminaria hyperborea* and *L. japonica,* which are its natural producers. Some common bacterial producers of alginates are *Azotobacter* and *Pseudomonas* species. Bacterial synthesis of alginates provides these polymers with defined chemical structures and properties. Alginates are now understood to be an entire family of linear copolymers containing blocks of (1, 4)-linked β-D-mannuronic (M) and α-L-glucouronic (G) acid residues.

Alginate extracts from different sources have different proportion of mannurate and gluconate residues. Mainly glucouronic (G) acid residues or G blocks as they are referred to are believed to be involved in the intermolecular cross-linking with divalent cations. M alginates are more immunogenic and result in more cytokine production. The main derivatives of alginates are amphiphilic alginates. These are synthetic alginates produced by introducing alkyl chain and hydrophobic polymers to its main chain. There is another class of alginate containing cell-adhesive peptides. These derivatives are typically prepared by introducing peptide as side chain radical linked via carbodiimide to coupling with adhesivity. Alginate basically lack properties such as adhesivity to mammalian cells. Appropriate ligands are crucial to promote and regulate this cellular interaction (Young et al. 2006).

Another class of polysaccharides which biologically interacts with divalent cations such as calcium and magnesium ions are egg-box polysaccharides. They possess a perfect coordination and cooperativity in noncovalent interactions like ionic bonds and electrostatic force of attractions with divalent cations. Most of the egg-box polysaccharides are "Gels" with threefold skew symmetric arrangement. Helical chains of polysaccharides interact with divalent cations, and the subsequent aggregation results in dimerization and conversion to gel structure. Naturally occurring polysaccharides are used for making egg-box polysaccharides. In three-dimensional view it appears as "eggs-arranged in an egg box" and hence the name. Some of the examples are following.

1. **Pectin** – present in plant cell walls, linear polymer of polygalactouronic acid (PGA) with varying methyl esterification. Low methoxy-pectin gels in presence of calcium.

2. **Algin** – it is a seaweed extract, consisting of mannopyranosyl uronic acid and L-glucopyronic acid. It gels in the presence of calcium ions.
3. **Carageenan** – seaweed gum, linear polymer of D-galactopyranosyl with sulfate ester residues. It exists as a gel.

Egg-box polysaccharides find wide application in preparation of jams, candies, and processed fruit products in food industry. Gums in chemical industry (for example, xanthan gums) can also be applied as a carrier particle in pharmaceutical industry as low-viscosity emulsifiers and shear-thinning thickeners in dairy industries (Ahmad et al. 2015).

6 Biological Macromolecules

The word macro defines something that is large in size or occupies a large amount of space. A molecule is the smallest functional unit of a pure substance that can take part in a chemical reaction. The Nobel laureate Hermann Staudinger first coined the term "*macromolecule*" in the 1920s, although his first relevant publication on this field only mentioned about molecules with large number of atoms and not about their size. Thereafter, the term macromolecule was used to define large molecules produced by the polymerization of small subunits called monomers into large polymeric forms. Typically, macromolecules are polymers comprising of thousands of atoms or more specifically smaller molecules that chemically combine to form a larger one. Also, in the field of chemistry, macromolecules were defined as aggregates of two or more molecules held together by intermolecular forces rather than covalent bonds but which do not readily dissociate. Macromolecules can be both naturally occurring/biological as well as artificially synthesized synthetic polymers.

Biological macromolecules are large molecules, necessary for life, that are built from smaller organic molecules – commonly these include biopolymers like nucleic acids, proteins, and carbohydrates and large nonpolymeric molecules such as lipids and macrocycles. On the other hand, synthetic macromolecules include synthetic polymers (plastics and synthetic fibers), graphenes, and carbon nanotubes. Synthetic polymers are either inorganic polymers or geopolymers.

The biological macromolecules are synthesized by the living systems either intracellularly or extracellularly. These molecules make up for majority of the cell's dry mass within the living systems and hence is mandatory for the survival and adequate functioning of the living systems in general.

7 Biomedicine

The term biomedicine first arose in the early twentieth century. Since the very beginning, biomedicine and biomedical research has been designated as medicine that is closely associated with experimentation and the laboratory instead of completely relying on the experience of physicians and the clinic. The pursuit of therapeutic ambitions in the laboratory received further support in the late nineteenth

century through the works of Louis Pasteur and Robert Koch in innovating techniques in bacterial culture and the microbiological theories of disease etiology. These developments have made it possible for scientists to investigate the biological processes within an organism responsible for causing disease and allowing them to utilize these in developing a more potential and effective form of medicine.

The science of medicine ever since its initial discovery has undergone drastic, new developments and reformation, with the sole aim to provide better efficacy in treatment and reduced side effects. In modern day, medicine aims primarily in prevention and a more focused treatment for a particular disease. Detailed studies into the disease-causing mechanism of a particular organism and the knowledge of the exact molecules that are involved in disease development have improved scientist and researchers in developing medicine which is more effective in controlling the development of the disease and curing it specifically.

In the present-day context, medicine aims at preventing disease first and cure afterward. Prevention is indispensable for all kinds of diseases and not just for infectious ones. One way to achieve this is the development of vaccines for diseases – both infectious and noninfectious. In the present world, research is being conducted for the development of vaccines for lifestyle disorders like diabetes as outlined in studies conducted by Richardson et al. (2009), hypertension (Phisitkul 2009), cancers, especially cervical (Jenkins 2008), schizophrenia, and other mental disorders (Tomlinson et al. 2009). Not only vaccination but a healthy lifestyle change is also required in the prevention and control of a disease, including checks on sanitation, food habits, diet, etc.

Macromolecules like carbohydrates, proteins, lipids, and nucleic acid play a major role in the formulation of these medicines and vaccines. This is because of the wide range of properties they exhibit. Each of these macromolecules and their properties are described briefly in this section.

7.1 Proteins

Proteins are macromolecules consisting of one or more long chains of amino acid residues linked via amide linkages and folded into a three-dimensional structure that is essential for its proper functioning. Proteins are an essential part of every living organism and participate in every biological process occurring within the cell. Some of the properties of proteins include enzymatic activity, cell signaling, immune response, cell adhesion, dietary supplement for essential amino acids, etc. All these properties have been well exploited by researchers in the development of medicine. For example, the production of immunoglobulins for the treatment of infectious diseases, development of recombinant proteins like hormones – insulin, somatotropin, etc. for treatment of noninfectious diseases, development of vaccines and antitoxins, replacement for deficient enzymes or proteins like **lactaid** to digest lactose, augmentation of an existing pathway like **epogen** for erythropoesis stimulation for treating anemia, proteins providing a novel function – enzymatic

degradation of small metabolites like use of L-asparginase to treat acute lymphocytic leucopoenia, etc. (Leader et al. 2008).

7.2 Carbohydrates

Carbohydrates are biomolecules composed of carbon (C), hydrogen (H), and oxygen (O) atoms, usually with hydrogen–oxygen atoms in the ratio 2:1 (as in water). They have an empirical formula $C_m(H_2O)_n$ where m and n can have different values that hold true for almost all monosaccharide units except a few, for example, deoxyribose sugar present in DNA has an empirical formula $C_5H_{10}O_4$. According to the American Diabetes Association (ADA), carbohydrates are the body's main source of energy. Carbohydrates are macronutrients and are required for the proper functioning of the body tissues and organs like the brain, heart, liver, and so on. Carbohydrates are simple or complex based on their chemical structure and ease with which they can be digested. Monosaccharides are simple carbohydrates, whereas polysaccharides are complex (for example, starches and fibers) mostly present in vegetables and grains, especially legumes. Because of the hydrophilic nature, carbohydrates are mostly present on the outer surface and are therefore widely involved in cell–cell recognition, cell differentiation processes, and cell–external agent interactions. These interactions can initiate beneficial biological events, such as fertilization, cell growth and differentiation (e.g., during embryogenesis), and immune responses. A comprehensive understanding of these surface glycans and their interaction with lectins is required for the development of a rational drug design of carbohydrate-based therapeutic agents. An example of carbohydrate-based drugs includes acemannan, a complex carbohydrate that is extracted from the *aloe vera* plant is used for treating burns and skin inflammation (Pelley and Strickland 2000). Some of the major strategies used in the development of these drugs involve the disruption of the carbohydrate–lectin interactions that are involved in the initiation or development of specific diseases; identification of carbohydrate antigens that are unique to a disease for the development of vaccines; inhibition of enzymes that are responsible for the biosynthesis of the disease- associated carbohydrates; replacement of carbohydrate-processing enzymes that are absent in diseased cells; and application of carbohydrate and lectin interactions to deliver drugs specifically to diseased cells (Osborn et al. 2004).

7.3 Lipids

Lipids are organic esters of fatty acids or substances associated with them and are mostly insoluble in water but soluble in nonpolar solvents like benzene, etc. Functional lipids are considered to be the ones that promotes good health and reduces diseases (Roberfroid 2000). Some major functional lipids include the omega-3 fatty acids including alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), omega-6 fatty acids, etc. Since some of these are

polyunsaturated fatty acids, they are not synthesized by the human body and thus need to be taken as an external source in the diet. Therefore, they are called essential fatty acids. Of these, omega-3 and omega-6 fatty acids are essential components of the cell membrane phospholipids and they have several other functional roles (Hardman 2004). Some common fatty acids and their importance includes: DHA status is important to ensure optimum neural and visual functions (Innis 2003; Birch et al. 2007; Eilander et al. 2007); medium-chain triglycerides are required for fat source in people with abnormalities in fat digestion, metabolism, and storage (Marten et al. 2006); phytosterols inhibit cholesterol absorption and so help in controlling the total blood cholesterol, LDL, and HDL levels which further helps modify the risk of cardiovascular disease (Lichtenstein and Deckelbaum 2001); epidemiological studies suggest a protective role of x-3 PUFA against Alzheimer's disease (Barberger et al. 2007); and in some humans, fish consumption or administration of DHA has been associated with cognitive improvement (Barberger et al. 2002). The healthy ratio of x-6 to x-3 fatty acids in the human diet is said to range from 1:1 to 4:1 (Simopoulos and Bhat 2003).

7.4 Nucleic Acids

Nucleic acids are biological macromolecules that form an essential component of all life forms. It is commonly the DNA and RNA present in living cells and contains all the genetic information of an organism. Nucleic acids are linear polymers (chains) of nucleotides and differ from one another primarily in the sequence of nucleotides. Nucleotide sequences are of great importance in biology since they carry the ultimate instructions that encode all biological molecules, molecular assemblies, subcellular and cellular structures, organs and organisms, and directly enable cognition, memory, and behavior. The advances in acquisition of genetic sequence information and the ability to manipulate small quantities of nucleic acid have led to the emergence of multiple technologies, enabling scientist to exploit nucleic acids for research, diagnostic, and therapeutic purposes. The sequencing of the genomes of various organisms have benefited the various fields of biology, mostly biotechnology and genetic engineering (Adrio and Demain 2010), which in turn has had a greater impact in the diagnosis and detection of diseases, and newer more precise ways of therapy like gene therapy and production of recombinant proteins like insulin for the treatment of diabetes. Antisense RNA are small oligonucleotides with sequences that are complementary to RNA transcribed from the target gene and therefore are able to inhibit translation of sequences with mutations, i.e., inhibition of expression of genes responsible for causing diseases. This was first instituted in the 1970s, when isolated antisense RNA complementary to region of the 35S RNA of the Rous sarcoma virus was to inhibit its replication and prevent infection. Another therapeutic purpose is gene therapy where genes are transferred into s cell to compensate for a defective gene. Initial trials were for single-gene disorders like severe combined immunodeficiency wherein a defect in the ADA gene (adenosine deaminase) is responsible for the condition. The success in this trial caused researchers to invest more resources in

the development of this therapy. Even though all these possibilities make it a rapidly evolving field of research in modern day, various challenges in the development of these oligonucleotides and their delivery to target cells, immune system, and reactions against the vectors carrying the nucleic acids and the nucleic acid itself have reduced progress in using these as a commercial and an efficient therapeutic method. Nonetheless, with interdisciplinary approaches to overcoming these challenges, the future stands bright in using nucleic acids in biomedicine.

7.5 Synthetic Macromolecules in Biomedicine

Macromolecules are high molecular weight polymers having same or different types of monomeric units. These polymers are generally prepared from natural inorganic matter, hence it is also known as geopolymers. The association of macromolecules with inorganic elements enables its properties and stability as like other biopolymers. There are different types of macromolecules used in biomedical applications, important ones among them are following.

7.6 Synthetic Plastics

Plastics are one of the most widely used synthetic polymers. Modern system of health care and treatment is highly dependent on plastic materials. Plastics have made health care quite simple, easy, less expensive, and affordable. Previously, things which were made up of glass were beings used for medical purposes. Later on, plastics replaced glass. Things which are used for health care purpose such as disposable syringes, intravenous blood bags, and heart valves are now available in plastic. The weight of eyeglass frames and lenses have been considerably reduced due to their manufacture in plastics.

They are the principal components of modern health care devices such as an open MRI machine to small injection vials where it confers greater flexibility, comfort, and mobility. Plastics are also used for making artificial hip and knees to provide smooth working, trouble-free joints. Other exceptional properties of plastic like light weight, low cost, durability, and transparency make it as a material for packages. In general, most of the innovative medical research is dependent on plastics. The basic starting material for synthetic plastic is ethylene ($CH_2{=}CH_2$). Ethylene itself is a monomer which on heating under 100–400 °C under high pressure gives rise to polyethylene. The routinely used plastics in medical industry are polyvinyl chloride (PVC), polypropylene (PP), polyethylene (PE), polystyrene (PS) as well as nylon, polyethylene terephthalate (PET), polyimide (PA), polycarbonate (PC), acrylonitrile butadiene (ABS), polyetheretherketone (PEEK), and polyurethane (PU). The most extensively used plastic material in the medical applications is PVC followed by PE, PP, PS, and PET. PVC is the most widely used in presterilized single-use medical applications.

There are number of synthetic plastics which can be safely applicable in human tissues. One such best polymer is methyl methacrylate ("lucite," "plexiglas," and "perspex") and nylon. Results of experimental studies on animals showed that these polymers are well tolerated by cells and tissues and it can be used for clinical applications. Methyl methacrylate is a rigid, brittle, hard, and transparent synthetic plastic suitable for cranioplasty dentures and soft tissue prostheses in plastic surgery (Raja and Berg 2007). Many other plastics have been used in clinical surgical practice but discarded for various reasons. The main reason is all these synthetic plastics are from nonbiological origin and also nonbiodegradable. Some of the synthetic plastics were causing toxicity in peoples. On the flip side, in the current scenario, plastic is considered as one of the most common environmental contaminants. Nations are trying to reduce the use of plastics and looking for better alternatives. Most of the medical vials like syringe, catheters, tubes, etc. are for only one-time use. So, the reusability of these plastics is rare.

Bioplastics are eco-friendly, biodegradable synthetic plastics made from the polymerization of biological raw materials. The production of bioplastic has advantages; it would reduce the use of fossil fuel resources for plastic production. Further, it would scale down carbon footprint by undergoing faster decomposition. Bioplastic is also less toxic and does not contain bisphenol A (BPA), a hormone disrupter that is often found in traditional plastics. PLA (polylactic acid) is a bioplastic made from biomaterials like corn starch, cassava, or sugarcane. It is an edible starch and biodegradable. The conversion of corn starch into plastic is a multiple-step process. The starch consists of long-chains monomers which are made up of carbon; it resembles the long chain of plastics which are made from fossil fuel resources. Sometimes citric acids are used as stabilizing agents to form a long-chain polymer that is the building block for plastic. PLA can look and behave like polyethylene (used in plastic films, packing. and bottles), polystyrene (Styrofoam and plastic cutlery), or polypropylene (packaging, auto parts, and textiles). PHA (polyhydroxyalkanoate) are inclusion bodies produced by certain bacteria especially *Bacillus* sp. These are organic materials having plastic-like properties, biodegradability, and cause no toxicity or harm to living tissue. Microorganisms produce PHA as granules under nutrient-deprived conditions. PHA acts as a carbon resource. It has a chemical structure similar to that of traditional plastics. PHA is often used for medical applications such as sutures, slings, bone plates, and skin substitutes; it is also used for single-use food packaging.

7.7 Polyurethane

An important class of synthetic polymers, namely polyurethanes, are used widely in the field of automobile, medical, and food industries. It is a unique type of polymer formed by the polycondensation of polyisocyanates and polyalcohols. The monomers of polyurethane are of nonbiological origin, but it was found to be susceptible for biodegradation by microorganisms present in soil. The chemical properties and structure of this polymer is quite similar to biomolecules like proteins. The microbial

degradation of it mainly focuses on properties like molecular orientation, crystallinity, cross-linking, and chemical groups present in the polymer chain.

Nowadays polyurethanes have been used for wide range of applications, because it is provided with good abrasion resistance, flexural endurance, and biocompatibility. It is possible to use polyurethanes for making artificial hearts, catheter tubing, devices used for dialysis, feeding tubes, surgical drains, intra-aortic balloon pumps, nonallergenic gloves, medical garments, hospital bedding, and wound dressings (Alvarez et al. 2004). Polyurethane elastomers are another group of polymers having molecular structures of protein of human origin. It possesses high antithrombotic property. It will slow down the cascade of blood coagulation due to high protein absorption. This makes them as an ideal candidate for a variety of medical applications which require adhesive strength and unique biometric properties. For example, polyurethane elastomers are currently being used as a special sealant to bind bundles of hollow fibers in artificial dialysis cylinders.

7.8 Silicon Gels

Another type of polymers used in medical industries is silicon gels. It does not contain repeated units of silica or any other metal as per the name. It is made up of smooth, cross-linked silicon elastomer and the polymer chains are swollen due to silicon fluids. Silicon gels are light with a rubbery consistency with a dual-part fluid system supported via a platinum-catalyzed addition reaction. It has been used in medical industries as soft tissue implants for tissue replacement or to back up the appearance of breasts after cancer surgery. Silicon gels are also used for making garments, such as brassieres and other external breast prostheses. There are numerous other synthetic macromolecules which are used in biomedical applications like synthetic proteins, ribonucleic acids, lipids, etc. Each and every material has its own utility and significance. Most of the synthetic macromolecules have a biological background. So, the preliminary understanding about biomolecules of biomedical applicability is very helpful in making synthetic macromolecules.

8 Fungi as a Source of Macromolecules

Fungi are mostly regarded as the organisms responsible for causing diseases in humans or animals, and also responsible for infections in plants and food crops like rotting. Regardless of these, fungi are responsible for a large amount of health benefits in humans, plants, and animals. Fungi have been used in several industrial fermentation processes, such as the production of enzymes, vitamins, polysaccharides, polyhydric alcohols, pigments, lipids, and glycolipids. A large number of secondary metabolites and primary metabolites produced by these organisms has medicinal purposes. Some of the fungi are sources of nutrition; for example, *Agaricus campestris*, a common mushroom, forms a part of human diet. Another method is the involvement of fungi in the production of fermented foods like alcohol

and bread. *Saccharomyces cerevisiae* or baker's yeast is an important ingredient in bread, a staple food in human diet.

As *Saccharomyces cerevisiae* is an organism utilized in food industry, it is used as a safe host for the production of pharmaceutical proteins. Mammalian genes have been cloned and expressed in *S. cerevisiae*, including human interferon, human epidermal growth factor, and human hemoglobin. It has also been used in the production of vaccines. Hepatitis B vaccine was the first successful vaccine produced using *Saccharomyces cerevisiae.*

Most fungi are made up of a cell wall, which is a complex compartment consisting of polysaccharides (Rodrigues et al. 2011) like chitin, glucans, and mannans. Most of these play a major function in virulence of the organism or activation of the innate immune response. The α- and β-glucans and complex mannans are mostly associated with immune activation (Brown and Gordon 2003; Van de veerdonk et al. 2009). Some fungal species with polysaccharides having immune function include *Candida albicans, Aspergillus fumigatus, Histoplasma capsulatum,* and *Cryptococcus neoformans* (Chai et al. 2011; Monari et al. 2006). Chitin, a water-soluble polymer with β-1→4 linkage between *N*-acetylglucoseamine units, showed in a study that high molecular dimension was immunologically inert whereas fractions with low molecular dimension showed effective stimulation of the innate immune system (Da Silva et al. 2009).

Polysaccharide krestin (PSK) and polysaccharopeptide (PSP) are two of the best-known commercial fungal polysaccharide–protein complexes derived from *Coriolus versicolor.* They have been studied to have various therapeutic values and also have been used to treat cancer and infectious diseases. Both PSK and PSP have been showing high immunological activity and therefore have been used in cancer therapy as an adjuvant. Both are brown powders with a dark coloration, with a light delicate odor with a pH ranging from neutral to slightly acidic. The PSP/PSK polymers are only soluble in hot water and insoluble in methanol, pyridine, chloroform, benzene, and hexane. PSK and PSP are protein- bound polysaccharides, chemically similar except one difference: PSK contains fructose whereas PSP contains rhamnose and arabinose. The common polysaccharides contain 74% glucose, 4% xylose, 2.7% galactose, 2.4% fructose, and 1.5% mannose.

There is evidence to show that PSK can inhibit HIV infection by the modification of the viral receptor or by preventing HIV from binding with lymphocytes. PSP shows analgesic action in mice subjected to hot-plate-induced pain and stress (Chan and Yeung 2006). Both have a hydroxyl and superoxide scavenging activity. The antimicrobial activity of PSK has been shown in *Escherichia coli*, *Listeria monocytogenes*, and *Candida*. Phagocyte activation and reactive nitric oxide production of macrophages are suggested mechanisms contributing to the antimicrobial activity of PSK. In commercial markets around the world, they are sold as capsules, food additives, teas, tablets, and syrups (Wan 2013).

In the previous years, a significant increase in international literature dealing with the lipid production from microbial sources, i.e., the "single cell oils or SCOs" produced by the "oleaginous" microorganisms, has been recorded. Oleaginous (oil-bearing) microbes are microbial species that have the ability to accumulate

amounts of lipid higher than 20% w/w of their dry cell weight (DCW) during growth, on glucose metabolism, inside their cells, or mycelia (Papanikolaou and Aggelis 2011; Patel et al. 2017). SCOs can be employed as substitutes of expensive lipids rarely found in the plant or animal kingdom. For example, oils contain high quantities of the medically important γ-linolenic acids, other nutritionally important polyunsaturated fatty acids (PUFAs), or finally substitutes of several important exotic fats like the cocoa butter, the shea butter, etc. (Ratledge and Wynn 2002; Papanikolaou et al. 2007; Papanikolaou and Aggelis 2011; Wei et al. 2017). Production of SCOs is an expensive procedure because of the maintenance of aseptic conditions, cost of fermentation, and capability of microorganisms to produce lipids efficiently. These challenges have caused the development of better methods for large-scale and effective production.

The neutral lipids form the large part of the storage lipids of most oil-bearing single- celled (yeasts) and mycelial fungi (Fakas et al. 2008) under appropriate conditions. The portion of these compounds varies drastically during the life cycles of fungi belonging to different classes, from fungal species to species at corresponding stages of development, and is influenced by ecological and biological factors such as the incubation temperature, cultural pH, and the source of carbon used as a nutritional base. Triacylglycerols are the most common form of neutral lipids produced in fungi and yeast, and have energy storage functions.

They also produce sterol esters in marginal quantities (Mličková et al. 2004). Diglucosyldiacylglycerol (DGDG) and monogalactosyldiacylglycerol (MGDG) have been documented in the black mold *Aspergillus niger*.

Several growth conditions have been suggested to affect lipid content and composition of fungi. Under high carbon-to-nitrogen ratio circumstances, *A. niger* can convert approximately 90% of the carbohydrate present into fats. *Candida utilis*, when grown on a defined media, showed an increase in the lipid content as the culture ages, till the stationary phase is reached, but with the reduction of glucose content, a rapid decrease in cellular lipid content was observed. Lipid content in yeasts is also observed to vary with the concentration of sodium chloride in the medium. Although high salt levels inhibit growth, an increase from 0% to 10% w/v increases the lipid content of *C. albicans* from 0.32% to ~6.29%. A theory suggested that sodium ions speed up the fermentation of glucose in yeast, and in *C. albicans* this causes the accumulation of lipid at the expense of other cellular components. This phenomenon has also been observed in *S. cerevisiae*. Anaerobic conditions reasonably influence the lipid production.

In recent years yeasts, yeast-like fungi, and *Ustilago maydis* were the only ones in which glycolipids were reported and identified. *C. bogoriensis* produced two members of this family of lipids when cultured on glucose yeast extract medium. The structure of one of these was established as 13-[(2'-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy] docosanoic acid 6',6"-diacetate and was reported to be attached to the yeast cells as crystals.

These fatty acids have a large number of applications in industries as well as in human life. Fatty acids like PUFAs are precursors for the production of prostaglandins and leucotrienes that are important hormones for the proper functioning of

human body. Polyunsaturated fatty acids are known to have important roles when present in the diet rather than administered as pseudodrugs. The low incidence of heart disease in Iceland, in Eskimo populations, and some Japanese populations has been attributed to the high proportions of marine fish in their diet, which has various polyunsaturated fatty acids (Sancholle et al. 2004). The first was SCO rich in γ-linolenic acid (GLNA) produced using *Mucor circinelloides*, and the others were SCO rich in arachidonic acid (ARA) and docosahexaenoic acid (DHA) produced using *Mortierella spp*. GLNA has long been regarded as a "cure-all," which is best found in evening primrose seed oil and used for the treatment of eczema. GLNA is effective in diseases such as Alzheimer's, cancer, depression, peroxisomal disorders, 6-polyunsaturated fatty acids, hyperactivity, etc. The dihomo-γ-linolenic and arachidonic acid have very important roles in mammalian biochemistry as precursors of prostaglandins (Akpinar 2014).

9 Genetically Modified Organisms

Microorganisms are prolific producers of a large number of polysaccharides. Such polysaccharide production can be compared to the processes that occur during the biosynthetic pathways of especially their cell walls. An array of nearly more than one hundred enzymatic reactions is involved, in the synthesis of these polysaccharides, directly or indirectly. Under cultural conditions, the production of polysaccharides invariably increases or alters the viscosity of the culture broths, thereby enabling their recovery through the use of precipitation by salts, acids, or organic solvents.

Genetic engineering is a method of incorporating novel genetic sequences into target cells in order to alter a recipient microbial cell (bacterial or fungal origin mostly) for the purpose of changing its characteristics. Insertion of a new gene form possesses a new desirable trait or feature that poses a huge impact and can be extensively used for large-scale production. Among the various uses of genetic engineering, one of them is to produce proteins in organisms that cannot synthesize these proteins as such. One of the classical examples of techniques of genetic engineering has been used for the synthesis of proteins; bacteria, which synthesize human insulin, were developed in 1979 and were first used commercially for treatment purposes about 3 years later (1982). The first human antibodies were produced in plants in 1988 (Alexander 2003). Selection of specific host is crucial and very necessary to carry out such genetic engineering processes in order to aim at a potential/higher yield as well as easy downstreaming process with cost-efficient methods (Nielsen 2013).

10 Polysaccharides Produced by Genetically Modified Microorganisms

Genetic modifications can fundamentally boost the productivity and quality of products. Genetic engineering, hand in hand with recombinant DNA (rDNA) technology, is progressing rapidly with reference to organisms. Genetically modified

organisms like bacteria, fungi, and yeast are widely used in food industries. Basic understanding of various microorganisms and the science of microbiology will stimulate the manipulation of genetically modified microorganisms of industrial relevance.

The synthetic potential of microorganisms is well known and has gained attention worldwide. Exploration of various metabolic pathways of microorganisms has made possible metabolic engineering in microbes. Today, genetically modified microorganisms (GMMs) have found applications in various sectors of human health, agriculture, bioremediation, and in industries such as food, paper, and textiles. Genetic engineering renders maximum genetic diversity and chemical selectivity. Also, it confers sufficient production of desired product and reduces the cost associated with product formation. Microorganisms serve as important tools in molecular biology and genetic engineering because they are easy to culture, maintain, limited genetic complexities, and easy to get ethical clearance. Manipulations of genes within a species involve direct transfer of genes between organisms. This characteristic process can be done under in vitro conditions.

Most of the polysaccharides are hydrocolloids. They are hydrophilic and are highly soluble in water. Commonly these polymers can be used as thickeners, emulsifiers, and gelling agent in food industries for making candies, bread, and cake. It was also used as tablet capsule, matrix, and agent for drug delivery in biomedical industries. Most of the microbial polysaccharides have not been commercialized because of its poor quality and physical properties. By genetic engineering one can rewrite the genetic code and make possible the establishment of highbred strains. Metabolic engineering in microbes effectively improves the desired product formation.

Xanthan gum is an extracellular polysaccharide produced by *Xanthomonas campestris*. It is an efficient viscosifier in aqueous solutions. In a particular study, *Xanthomonas* cells were generated by genetic engineering and these mutants produced variants of xanthan gums. The rheological properties of gums produced in this manner are similar to that of xanthan gums. It was then concluded that by mutation variants of xanthan gum were produced with different viscosity properties. Mutation in the genome of bacteria will result in changes of the chemical properties of the polysaccharides formed. So, the micromutations in gene cause broad changes in chemical as well as physical properties of the final product.

Yeasts are unicellular eukaryotes. They are the sources of various polysaccharides like laminarin, mannose protein, glycan, and chitin. The widely used yeast for industrial purpose is *Saccharomyces cerevisiae*.

It is also a model organism for various genetic studies. It consists of total 20–30% of cell dry mass in which beta-glucans was about 85–90%. In general, the protocol for the extraction of cell wall polysaccharides is by controlled disruption of cell wall and precipitated to get the product followed by downstream process like chromatography for further purification. Other studies reported an optimized and simple protocol for the extraction of cell wall polysaccharides like beta-glucan, mannose, and chitin from a cell wall–defective mutant of *S. cerevisiae*. On comparison with wild-type strains, it was found that the quality and quantity of polysaccharides were

better in mutants (Dallies et al. 1998). Genetic engineering modifies strains for the industrial production. The major disadvantage in this process is that it is very challenging to produce strains which ensure high productivity. This is because most of the polysaccharides production is controlled by metabolism of the concerned organism. It creates problems in controlling their metabolism. Also, the production of polysaccharides by genetic engineering is complicated as compared to proteins, amino acids, enzymes, and nucleic acids.

11 Bacteria as the Most Preferred Models

Earlier, bacteria were the first microorganisms to be manipulated in scientific research, due to their simplicity of their genetic material and the considerable ease with which they could be handled compared to other microbes. These organisms are now been used elaborately in the scientific world for various reasons, and have lent themselves extremely well as candidates in the production of large amounts of pure human proteins for biomedical purposes. A large part of our preliminary understanding of the molecular biology of bacteria stems from the study of the model colon bacterium *Escherichia coli*. Other examples include yeast forms like *Saccharomyces cerevisiae* and *C. ammoniagenes* (Ruffing and Chen 2006).

12 Polymers Produced Through Genetic Engineering Technologies

Some benefits of genetic engineering in the synthesis of polymers are increased polymer yields in short time span, reduced costs for production/cost-effective methods of production of polymers, need for natural production of polymers is lowered, enhanced nutrient composition and quality, supply is enhanced if produced in large quantities, and medical benefits to the world's growing population. Large-scale production of these polymers is important as they are mainly required for the production of drugs in the pharmaceutical companies of the medical field. To curb the dearth for polymers, the production has to be looked into keenly (Gomes et al. 2012).

13 Risks Associated with Genetic Engineered Polymers

Specific substrates required and the same methodologies for the production may not work in all cases and with all target organisms. Cost-effective methods have to be considered and chosen. Basically, trial and error methods have to be implemented until the right, altered combination is accomplished. Moreover, particular and specific strains of microbes are required for the production of polymers. Espousing strains of organisms is the most decisive and crucial part of the production (Koller et al. 2010).

14 Why Genetic Engineering?

The development of new biopolymers becomes possible through genetic engineering, wherein the newly synthesized polymers resemble the natural polymers formed in nature, in their complexity and functionality. The usage of synthetic DNA makes it possible to combine the various functional domains for a specific fusion protein, for example, properties like the adhesion of cells, its migration, mechanical features like its response during the alteration of a specific gene, the manifestation of antimicrobial factors, etc., especially when multifunctional biomaterial systems are being involved. The exploration of genetic engineering techniques for the purpose of tissue engineering and regenerative medicine/biomedical applications has been used as a tool and has witnessed a significant progress for the generation of tailor-made biomaterials (Gomes et al. 2012).

15 The Downstreaming Process, Easy or Hard?

The downstream process poses a paramount challenge to the scientists for the production of biopolymers. Synthetic production of these polymers is a task by itself, unless the downstream process is feasible enough to produce a higher amount of yield rather than its diminishment. Thereby, this mainly depends on the suitability of specific substrates to be used for the production of the biopolymers in question, which portrays extensive use in the medical industry. So, the downstream process turns out to be convenient, if the substrate and the methodologies chosen to work compatibly with their respective enzymes. If not, the downstreaming process turns out to be a challenging one to produce polymers alternatively in large amounts (Koller et al. 2010).

16 Conclusion

The revolution in the recombinant deoxyribonucleic acid (DNA) technology for the production of desired products spanning different sectors like agriculture and industry has harnessed the metabolic potentials of microorganisms in several ways. As a result of such exploitation, there are nearly thousands of products which have found application in agriculture, bioremediation, and human health/pharmaceuticals and industrially in food, paper, textiles, etc. The increase in the global population has driven man to embrace the use of genetically modified microorganisms (GMMs), particularly bacteria and fungi, for the welfare of humankind. Production of polysaccharides by natural methods has been in practice since time immemorial. The introduction and use of GMMs could improve the existing processes and lead to the design of desired or novel polysaccharides with increased performance and safety. The successful application of GMMs in the production of genetically engineered polysaccharides may be affected by the lack of information about the role of specific genes in the process itself. There is tremendous potential for the use of microbes in

the enhanced production of polysaccharides like dextrans, etc., for biomedical purposes. Thus, in future we can expect more microbial polysaccharides in industries. Productivity can be scaled by using strains which are genetically manipulated. In this way, economic feasibility can be achieved.

References

Adrio JL, Demain AL. Recombinant organisms for production of industrial products. Bioeng Bugs. 2010;1(2):116–31.

Ahmad NH, Mustafa S, Che Man YB. Microbial polysaccharides and their modification approaches: a review. Int J Food Prop. 2015;18(2):332–47.

Akpinar BA. Fungal lipids: the biochemistry of lipid accumulation. Int J Chem Eng Appl. 2014;5(5):409.

Alexander DR. Uses and abuses of genetic engineering. Post Med J. 2003;79:249–51.

Alvarez OM, Patel M, Booker J, Markowitz L. Effectiveness of a biocellulose wound dressing for the treatment of chronic venous leg ulcers: results of a single center randomized study involving 24 patients. Clin Res Pract. 2004;16(7):224–33.

Banik RM, Santhiagu A. Improvement in production and quality of gellan gum by *Sphingomonas paucimobilis* under high dissolved oxygen tension levels. Biotechnol Lett. 2006;28(17):1347.

Barberger GP, Letenneur L, Deschamps V, Pérès K, Dartigues JF, Renaud S. Fish, meat, and risk of dementia: cohort study. BMJ. 2002;325(7370):932–3.

Barberger GP, Raffaitin C, Letenneur L, Berr C, Tzourio C, Dartigues JF, Alpérovitch A. Dietary patterns and risk of dementia: the Three-City cohort study. Neurology. 2007;69(20):1921–30.

Béjar V, Llamas I, Calvo C, Quesada E. Characterization of exopolysaccharides produced by 19 halophilic strains of the species *Halomonas eurihalina*. J Biotechnol. 1998;61(2):135–41.

Bekers M, Upite D, Kaminska E, Laukevics J, Grube M, Vigants A, Linde R. Stability of levan produced by *Zymomonas mobilis*. Process Biochem. 2005;40(5):1535–9.

BeMiller JN, Whistler RL. Industrial gums: polysaccharides and their derivatives. Academic; 2012.

Birch EE, Garfield S, Castañeda Y, Hughbanks-Wheaton D, Uauy R, Hoffman D. Visual acuity and cognitive outcomes at 4 years of age in a double-blind, randomized trial of long-chain polyunsaturated fatty acid-supplemented infant formula. Early Hum Dev. 2007;83(5):279–84.

Brown GD, Gordon S. Fungal β-glucans and mammalian immunity. Immunity. 2003;19(3):311–5.

Chai LY, Vonk AG, Kullberg BJ, Verweij PE, Verschueren I, Van Der Meer JW, … Netea MG. *Aspergillus fumigatus* cell wall components differentially modulate host TLR2 and TLR4 responses. Microb Infect. 2011;13(2):151–9.

Chan SL, Yeung JH. Polysaccharide peptides from COV-1 strain of *Coriolus versicolor* induce hyperalgesia via inflammatory mediator release in the mouse. Life Sci. 2006;78(21):2463–70.

Czaja W, Krystynowicz A, Bielecki S, Brown RM Jr. Microbial cellulose – the natural power to heal wounds. Biomaterials. 2006;27(2):145–15.

Czaja WK, Young DJ, Kawecki M, Brown RM. The future prospects of microbial cellulose in biomedical applications. Biomacromolecules. 2007a;8(1):1–12.

Czaja W, Krystynowicz A, Kawecki M, Wysota K, Sakiel S, Wróblewski P, Bielecki S. Biomedical applications of microbial cellulose in burn wound recovery. In: Cellulose: molecular and structural biology. Springer; 2007b. p. 307–21.

Da Silva CA, Chalouni C, Williams A, Hartl D, Lee CG, Elias JA. Chitin is a size-dependent regulator of macrophage TNF and IL-10 production. J Immunol. 2009;182(6):3573–82.

Dallies N, François J, Paquet V. A new method for quantitative determination of polysaccharides in the yeast cell wall. Application to the cell wall defective mutants of *Saccharomyces cerevisiae*. Yeast. 1998;14(14):1297–306.

Eilander A, Hundscheid DC, Osendarp SJ, Transler C, Zock PL. Effects of n-3 long chain polyunsaturated fatty acid supplementation on visual and cognitive development throughout

childhood: a review of human studies. Prostaglandins Leukot Essent Fat Acids. 2007;76(4): 189–203.
Fakas S, Čertik M, Papanikolaou S, Aggelis G, Komaitis M, Galiotou PM. γ-Linolenic acid production by *Cunninghamella echinulata* growing on complex organic nitrogen sources. Bioresour Technol. 2008;99(13):5986–90.
Falconer DJ, Mukerjea R, Robyt JF. Biosynthesis of dextrans with different molecular weights by selecting the concentration of Leuconostoc mesenteroides B-512FMC dextransucrase, the sucrose concentration, and the temperature. Carbohydr Res. 2011;346(2):280–4.
Garcia OF, Santos VE, Casas JA, Gómez E. Xanthan gum: production, recovery, and properties. Biotechnol Adv. 2000;18(7):549–79.
Gastebois A, Clavaud C, Aimanianda V, Latge JP. *Aspergillus fumigatus*: cell wall polysaccharides, their biosynthesis and organization. Future Microbiol. 2009;4(5):583–95.
Gomes S, Leonor IB, Mano JF, Reis RL, Kaplan DL. Natural and genetically engineered proteins for tissue engineering. Progress Polym Sci. 2012;37(1):1–17.
Gonçalves VM, Reis A, Domingues MRM, Lopes-da-Silva JA, Fialho AM, Moreira LM, Coimbra MA. Structural analysis of gellans produced by *Sphingomonas elodea* strains by electrospray tandem mass spectrometry. Carbohydr Polym. 2009;77(1):10–9.
Grage K, Jahns AC, Parlane N, Palanisamy R, Rasiah IA, Atwood JA, Rehm BH. Bacterial polyhydroxyalkanoate granules: biogenesis, structure, and potential use as nano-/micro-beads in biotechnological and biomedical applications. Biomacromolecules. 2009;10(4):660–9.
Hardman WE. (n-3) fatty acids and cancer therapy. J Nutr. 2004;134(12):3427S–30S.
Innis SM. Perinatal biochemistry and physiology of long-chain polyunsaturated fatty acids. J Pediatr. 2003;143(4):1–8.
Jenkins D. A review of cross-protection against oncogenic HPV by an HPV-16/18 AS04-adjuvanted cervical cancer vaccine: importance of virological and clinical endpoints and implications for mass vaccination in cervical cancer prevention. Gynecol Oncol. 2008;110(3):S18–25.
Kato AKIO. Industrial applications of Maillard-type protein-polysaccharide conjugates. Food Sci Technol Res. 2002;8(3):193–9.
Klok HA. Biological–synthetic hybrid block copolymers: combining the best from two worlds. J Polym Sci Part A: Polym Chem. 2005;43(1):1–17.
Kögel-Knabner I. The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter. Soil Biol Biochem. 2002;34(2):139–62.
Koller M, Salerno A, Dias M, Reiterer A, Braunegg G. Modern biotechnological polymer synthesis: a review. Food Technol Biotechnol. 2010;48(3):255–69.
Leader B, Baca QJ, Golan DE. Protein therapeutics: a summary and pharmacological classification. Nat Rev Drug Discov. 2008;7(1):21–39.
Li Q, Niu Y, Xing P, Wang C. Bioactive polysaccharides from natural resources including Chinese medicinal herbs on tissue repair. Chin Med. 2018;13(1):7.
Lichtenstein AH, Deckelbaum RJ. Stanol/sterol ester–containing foods and blood cholesterol levels. Am Heart Assoc Inc. Circulation. 2001;103:1177–9.
Majumder A, Goyal A. Rheological and gelling properties of a novel glucan from *Leuconostoc dextranicum* NRRL B-1146. Food Res Int. 2009;42(4):525–8.
Marten B, Pfeuffer M, Schrezenmeir J. Medium-chain triglycerides. Int Dairy J. 2006;16:1374–82.
Mlíckova K, Roux E, Athenstaedt K, d'Andrea S, Daum G, Chardot T, Nicaud JM. Lipid accumulation, lipid body formation, and acyl coenzyme A oxidases of the yeast *Yarrowia lipolytica*. Appl Environ Microbiol. 2004;70(7):3918–24.
Monari C, Bistoni F, Vecchiarelli A. Glucuronoxylomannan exhibits potent immunosuppressive properties. FEMS Yeast Res. 2006;6(4):537–42.
Nasi R, Taber A, Van Vliet N. Empty forests, empty stomachs? Bushmeat and livelihoods in the Congo and Amazon Basins. Int Forest Rev. 2011;13(3):355–68.
Nielsen J. Production of biopharmaceutical proteins by yeast: advances through metabolic engineering. Bioengineering. 2013;4(4):207–11.
Osborn HM, Evans PG, Gemmell N, Osborne SD. Carbohydrate-based therapeutics. J Pharm Pharmacol. 2004;56(6):691–702.

Papanikolaou S, Aggelis G. Lipids of oleaginous yeasts. Part 1: biochemistry of single cell oil production. Part 2: technology and potential applications. Eur J Lipid Sci Technol. 2011;113: 1030–51, 1052–73.

Papanikolaou S, Galiotou PM, Fakas S, Komaitis M, Aggelis G. Lipid production by oleaginous Mucorales cultivated on renewable carbon sources. Eur J Lipid Sci Technol. 2007;109(11): 1060–70.

Patel A, Arora N, Mehtani J, Pruthi V, Pruthi PA. Assessment of fuel properties on the basis of fatty acid profiles of oleaginous yeast for potential biodiesel production. Renew Sustain Energy Rev. 2017;77:604–16.

Pelley RP, Strickland FM. Plants, polysaccharides, and the treatment and prevention of neoplasia. Crit Rev Oncol. 2000;11:3–4.

Phisitkul S. CYT-006-AngQb, a vaccine against angiotensin II for the potential treatment of hypertension. Curr Opin Investig Drugs (London, Engl: 2000). 2009;10(3):269–75.

Raja SG, Berg GA. Impact of off-pump coronary artery bypass surgery on systemic inflammation: current best available evidence. J Card Sur. 2007;22(5):445–55.

Ratledge C, Wynn JP. The biochemistry and molecular biology of lipid accumulation in oleaginous microorganisms. Adv Appl Microbiol. 2002;51:1–52.

Richardson SJ, Willcox A, Bone AJ, Foulis AK, Morgan NG. The prevalence of enteroviral capsid protein vp1 immunostaining in pancreatic islets in human type 1 diabetes. Diabetologia. 2009;52(6):1143–51.

Roberfroid MB. Prebiotics and probiotics: are they functional foods? Am J Clin Nutr. 2000;71(6): 1682S–7S.

Roca C, Alves VD, Freitas F, Reis MA. Exopolysaccharides enriched in rare sugars: bacterial sources, production, and applications. Front Microbiol. 2015;6:288.

Rodrigues M, Nimrichter L, Cordero R, Casadevall A. Fungal polysaccharides: biological activity beyond the usual structural properties. Front Microbiol. 2011;2:171.

Ruffing A, Chen RR. Metabolic engineering of microbes for oligosaccharide and polysaccharide synthesis. Microb Cell Factories. 2006;5:25.

Sancholle M, Lösel DM, Laruelle E. Lipids in fungal biotechnology. In: Genetics and biotechnology. Springer; 2004. p. 391–423.

Schürks N, Wingender J, Flemming HC, Mayer C. Monomer composition and sequence of alginates from *Pseudomonas aeruginosa*. Int J Biol Macromol. 2002;30(2):105–11.

Simopoulos AP, Bhat RV. Street foods. Am J Clin Nutr. 2003;75(2):337–8.

Simsek S. Application of xanthan gum for reducing syruping in refrigerated doughs. Food Hydrocol. 2009;23(8):2354–8.

Tomlinson M, Rudan I, Saxena S, Swartz L, Tsai AC, Patel V. Setting priorities for global mental health research. Bull WHO. 2009;87(6):438–46.

Üretimi D. Production of dextran by newly isolated strains of Leuconostoc mesenteroides PCSIR-4 and PCSIR-9. Tur J Biochem. 2005;31(1):21–6.

Van de Veerdonk FL, Marijnissen RJ, Kullberg BJ, Koenen HJ, Cheng SC, Joosten I, Netea MG. The macrophage mannose receptor induces IL-17 in response to *Candida albicans*. Cell Host Microbe. 2009;5(4):329–40.

Wan JMF. Polysaccharide krestin (PSK) and polysaccharopeptide (PSP). In: Abba Kastin (Ed.), Handbook of Biologically Active Peptides: Fungal Peptides, Elsevier Inc., USA, 2013, pp. 180–4.

Wei L, Xin Y, Wang Q, Yang J, Hu H, Xu J. RNA i-based targeted gene knockdown in the model oleaginous microalgae *Nannochloropsis oceanica*. Plant J. 2017;89(6):1236–50.

Young CC, Rekha PD, Lai WA, Arun AB. Encapsulation of plant growth-promoting bacteria in alginate beads enriched with humic acid. Biotechnol Bioeng. 2006;95(1):76–83.

Zhao Q, Zhao M, Yang B, Cui C. Effect of xanthan gum on the physical properties and textural characteristics of whipped cream. Food Chem. 2009;116(3):624–8.

Sulfation of Microbial Polysaccharides

30

Cristiana Gonçalves, Hajer Radhouani, Joaquim Miguel Oliveira, and Rui L. Reis

Contents

Abstract

Microbial polysaccharides are valuable sources of structurally distinct bioactive compounds. The understanding of the composition, structure, and physical properties of microbial polysaccharides can usefully be employed to define their roles in several industries. In this chapter, the potential of these polysaccharides, for diverse applications, has been exposed. This discussion focuses on its modifications, specifically the sulfation of these molecules. The sulfation could bring to these polysaccharides the well-known advantages of their naturally sulfated antagonists. Those benefits are related to a broad range of biological activities, such as antioxidant, antitumor, immunomodulatory, inflammation, anticoagulant, antiviral, antiprotozoan, antibacterial, and antilipemic; as well as an excellent physicochemical performance with resulting potential health benefits. The most common sulfation reagents mentioned in literature are concentrated sulfuric, complexes of SO_3 with pyridine, N,N-dimethylformamide, liquid SO_2, trialkylamine, dimethyl sulfoxide, and also chlorosulfonic acid in formamide or pyridine. The sulfation conditions not only should consider the chemistry involved but also make use of tools for the

C. Gonçalves (✉) · H. Radhouani · J. M. Oliveira · R. L. Reis
3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Zona Industrial da Gandra, Braga, Guimarães, Portugal

ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães, Portugal

J. M. Oliveira et al. (eds.), *Polysaccharides of Microbial Origin*,
https://doi.org/10.1007/978-3-030-42215-8_39

optimization of processes since a high complexity is associated with the diverse characteristics that these natural compounds could have. Considering the advantages in the use of sulfation of microbial polysaccharides, research related to this field should be encouraged.

Keywords

Microbial · Polysaccharides · Sulfation · Optimization

1 Introduction

Polysaccharides represent one of the first polymeric materials used by humans in manufacturing (Christian 2011). Polysaccharides are polymers (or copolymers) of 20–60,000 monosaccharide repeating units linked by glycosidic bonds with a specific stereochemistry (Maji 2019). Monosaccharides can be subcategorized as (i) aldoses, if it contains an aldehyde functional group; and (ii) ketoses, if it contains a ketone functional group (Sparkman et al. 2011). These may be linked together in numerous (over 134 million) ways to form disaccharides, oligosaccharides, and polysaccharides (Stick and Williams 2010). Polysaccharides have high molecular weight and widely occur in nature (Partain III 2000; Udayan et al. 2017). Its structure is highly branched and can be composed of equal (homopolysaccharide) or distinct (heteropolysaccharide) monosaccharide units (Udayan et al. 2017). There is considerable commercial interest in using these polymeric carbohydrates in cosmetic and pharmaceutical applications since many polysaccharides are nontoxic and nonthreatening to mammalian tissues (Partain III 2000).

Polymers with repeated units built on sugars are classified as natural polysaccharides (Christian 2011). They are abundantly produced from numerous natural resources. In fact, natural polysaccharides can be found in all living organisms, representing the most abundant and essential group of compounds in the biosphere (Beserra et al. 2016). The importance of natural-based biomaterials for tissue engineering applications is exponentially rising in recent years. Polysaccharides and proteins are being intensely studied and even used to produce new functional and structural biomaterials (Lahaye et al. 1998; Robic et al. 2008; Amin and Panhuis 2011; Bhardwaj and Kundu 2011; Zhao et al. 2011; Shang et al. 2013; Lai et al. 2014). The advantages of these materials are mainly due to its plant and animal origin, (a) being more eco-friendly and cost-effective than synthetic ones, (b) having superior compatibility with human hosts, and (c) better bioactivity and biodegradation (Shang et al. 2013; Ivanova et al. 2014).

Natural polysaccharides have two significant roles in living organisms: (i) structural component of the plant cell wall, and (ii) food storage (starch in plants and glycogen in mammalians) (Maji 2019). The sugar repeating units, on the backbone of natural polysaccharides, are composed of several chiral (a carbon atom with nonidentical substituents) centers. These centers establish the exact saccharide. For instance, they can have the same nominal formula ($C_6H_{12}O_6$),

being at the same time markedly distinct due to their chiral nature (Christian 2011). The repeating units of these organic compounds are connected via the oxygen on carbon 1, which forms a glycosidic bond to carbon four on another molecule with the subsequent elimination of water. Natural polysaccharides usually hold a variety of biological properties, such as antioxidant, anti-inflammatory, anti-HIV, antitumor, and anticoagulant, owing to their structure (Maji 2019). Slight structural differences confer different physical and chemical properties (Udayan et al. 2017). Natural polysaccharides or their modified structures have extensive applications, both in pharmaceutical and biomedical fields (Maji 2019; Yadav and Karthikeyan 2019). Their commercial value is associated not only with their functional properties but also with their environmental-friendly characteristics. In fact, natural polysaccharides are renewable products, from natural resources, and commonly are nontoxic and also biodegradable (Freitas et al. 2009).

Natural polysaccharides could have plant/algae origin (e.g., cellulose or alginate), animal/crustacean origin (e.g., chitin or hyaluronic acid), and microbial origin (e.g., gellan) (Freitas et al. 2009). Microbial polysaccharides include a significant amount of versatile biopolymers with a growing interest in chemical, food, and pharmaceutical industries, being their market currently expanding and scientific interest in this field growing (Giavasis 2013).

Polysaccharides excreted by microorganisms are produced by numerous bacteria, yeast, and fungi (Giavasis 2013). However, these processes are still costly, compared to traditional ones (mostly involving extraction and purification processes), mainly due to the high costs of reactor operation and especially the costs of the carbon sources used (Huang and Tang 2007). Consequently, microbial polysaccharides only occupy a small fraction of the biopolymers market (Freitas et al. 2009). Nevertheless, most recently, the interest in these polysaccharides is growing since most of them possess extraordinary characteristics, such as antitumor, anti-inflammatory, antimicrobial, or even hypocholesterolemic and hypoglycemic properties (Giavasis 2013). Moreover, using microorganisms to produce polysaccharides might be the best choice, after all. Their production processes allow higher growth rates and are more accessible to the manipulation of production conditions. It is possible to work at large scale, achieve a product with a stable chemical structure and characteristics, and it is possible to have an unconstrained availability in the market. For that to happen, it is critical to perform a preliminary analysis for process optimization and a proper scale-up. The critical point is to search for less expensive carbon sources, to decrease the production costs (Freitas et al. 2009; Giavasis 2013). The microorganisms usually employed for microbial polysaccharides production are (i) fungi of the Basidiomycetes family, (ii) numerous Gram-negative (e.g., Xanthomonas, Pseudomonas, Alcaligenes) and Gram-positive bacteria as well as, and (iii) a few yeasts, mostly belonging to the Saccharomyces genus (Giavasis 2013). The fermentation process for polysaccharide synthesis generally does not have any uncommon conditions (Fig. 1). It requires the adequate microorganism or consortium, an excess of carbon substrate, limiting nitrogen quantities, optimal temperature, carbon/nitrogen ratio and pH, agitation, aeration (good oxygen flow and distribution), and usually batch fermentation system, but fed-batch and continuous is also found (Mironescu

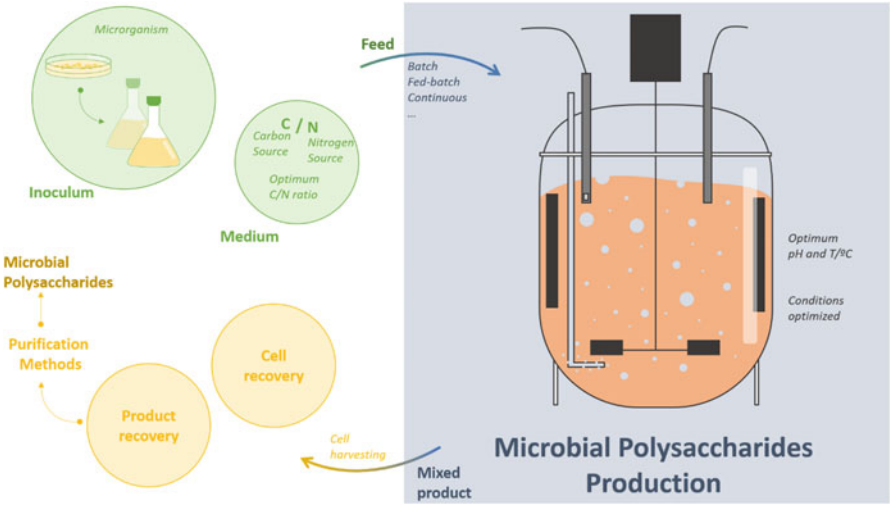


Fig. 1 Schematic representation of microbial production of polysaccharides

MJAUCSEFT 2003). The exopolysaccharides are the ones excreted by the cells, found in association with the cells or the medium, and have significant commercial importance. The produced exopolysaccharides just need to be extracted and purified at the end of the fermentation time needed, i.e., they are firstly and roughly extracted by precipitation (using salts, acids, or organic solvents), and then recovered by employing several adequate purification methods (Freitas et al. 2009).

The most commonly studied microbial polysaccharides are Cellulose, Xanthan, Kefiran, Dextran, Alginate, Sclerroglucan, Gellan, Pullulan, Curdlan, Emulsan, Baker's yeast glycan. Moreover, there are certain microbes able to produce linear polysaccharides known as glycosaminoglycans (GAGs). The enzymes of those microorganisms can catalyze the polymerization of microbial GAGs, such as chondroitin, hyaluronan (Hyaluronic acid, HA), keratan, and heparan sulfate/heparin (or the precursor heparosan). Interestingly, the microbial version of these gags is not sulfated, while the animal one usually is O-sulfated or N-sulfated (De Angelis 2002). In addition to these microbial polysaccharides, there is another category attributed to the marine microbial polysaccharides mainly produced by marine bacteria and also fungi and microalgae (such as α-D-1,6-homoglucan, Yancheng polysaccharide (YCP) and spirulan). Those are having a significant interest in the biomedical field due to their promising biological activities, mostly immunomodulatory but also anticancer effects (Li et al. 2018). Bacterial glucans could be divided into two types, the water (i) soluble and (ii) insoluble glucans. The first group (i) includes curdlan, bacterial cellulose, and the second (ii) selecan, β-(1,3)(1,6)-glucan, cyclosophorans, dextran, alternan, and reuteran. Glucans can also be divided into α-glucans (reuteran, dextran, mutan, and alternan) and β-glucans (cellulose and curdlan).

Microbial polysaccharides typically have both mechanical and biochemical functions. These carbohydrates also affect the rheology of the microenvironment

surrounding the cell (Sletmoen et al. 2010), changing the solution's flow behavior. For this reason, these polysaccharides have high commercial value as a gelling and thickening agents, binders, coagulants, emulsifiers, film formers, lubricants, stabilizers, and suspension agents (Huang and Tang 2007). Nevertheless, their diverse and interesting properties make them attractive in the most diverse areas, from chemical and cosmetics to food and medical industries (Freitas et al. 2014). In fact, these polysaccharides play critical functions in protein sorting, cell–cell interaction, cell adhesion, and molecular recognition in the immune response (Sletmoen et al. 2010). Microbial polysaccharides can also show antiviral and anticancer properties owing to their natural biological activity and skill to form polymeric matrices (Gupta et al. 2020).

Sulfated polysaccharides (SPs) have attracted considerable attention in biomedicine, being one of the most valuable groups in the field of polysaccharide research. Their relevance is mainly due to their broad range of biological activities, such as antioxidant, antitumor, immunomodulatory, inflammation, anticoagulant, antiviral, anti-protozoan, antibacterial, and antilipemic as well as excellent physicochemical performance (Raveendran et al. 2013; Patel 2012), with resulting potential health benefits.

SPs have astounding immunological activities, mainly based on macrophages modulation (Patel 2012; Huang et al. 2019) being also known by their antioxidant, immunomodulatory, inflammation, anticoagulant, antitumor, antiviral, antibacterial, antilipemic effects, among others. The therapeutic mechanisms of these molecules are not well defined yet. Marine algae are the most important source of nonanimal SPs (Patel 2012), followed by mushrooms, vegetables, and others.

Regarding the immunological effects, it was found that macrophages can be programmed and activated differently and profoundly influence immune responses (Liu and Yang 2013). Macrophages are crucial immune cells of different types that regulate the immune system, disease progression, and wound healing (Kang et al. 2019). They produce and release substances that stimulate the production of cells involved in inflammatory and immune processes, being essential for the functioning of the immune response. These phagocytic hematopoietic cells have a crucial function in the maintenance of homeostasis by changing their function according to the tissue (Patel 2012). Those different types of macrophages are present in connective tissues, being distributed by several organs (such as: lungs, liver, kidneys, blood, or bone tissue) with functions that diverge according to the location where it is found, but all types perform phagocytosis. SPs may have potential biomedical applications in stimulating the immune system or in controlling macrophage activity to reduce associated adverse effects (Patel 2012). The immunological activity of those polysaccharides will be different depending on the source of the sulfated polysaccharide and its structural characteristics, for instance, degree of sulfation (DS) and molecular weight (Patel 2012; Huang et al. 2019). In fact, immunological activity is a vital biological activity, allowing the management of the immune system function through several pathways by macrophage function regulation, natural killer cells, and T/B lymphocytes and the immune responses of lymphocytes complement system (Huang et al. 2019).

The anticoagulant activity is mostly ascribed to the inhibition of thrombin, mediated by heparin cofactor II (Ciancia et al. 2010). The number of sulfate groups and the glycosidic linkage per monosaccharide unit significantly impacts the potential for the anticoagulant activity (Ciancia et al. 2010).

Another reported biological feature of the SPs is their anti-inflammatory effect. The inflammation is, by default, a useful rapid response of the immune system to combat injuries and infections. Nevertheless, the regulation of such a response could fail, becoming chronic and causing the spread and progress of the disease (Sousa et al. 2018). The causes leading to anti-inflammation are attributed to selectin blockade, inhibition of the enzyme, and complement cascade (Patel 2012).

Sulfated polysaccharides also show antiviral activity against crucial viruses, such as respiratory syncytial (RSV), herpes simplex (HSV), dengue (DENV), Japanese encephalitis virus (JEV), and human cytomegalovirus virus (HCMV) (Chen and Huang 2018). The degree of sulfation and the distribution of sulfate groups on the constituent polysaccharides play an essential role in the antiviral activity (Patel 2012). The antiviral activity is based on the development of similar complexes to prevent the interaction between the viruses and the cells (Damonte et al. 2004) by adsorption and internalization steps (Patel 2012).

The main properties of microbial polysaccharides, and polysaccharides in general, hang on their primary chemical composition, structure, and molecule configuration, as well as their interactions on a molecular level (Sletmoen et al. 2010; Patel 2012). SPs can be naturally or chemically sulfated. The molecular modification via chemical, physical, and biological techniques is highly desirable to achieve properties for the envisaged biomedical application (Maji 2019). It is possible, for instance, to change the molecule solubility, hydrophobicity, physicochemical, and biological characteristics by using chemical functionalization. The methods typically used to modify polysaccharides comprise grafting, cross-linking, polymer-polymer blending, and derivative formation. The derivative formation involves the addition of functional groups to the molecule. For this, several reactions are well-known and used, such as (a) carboxymethylation, (b) carbamoylethylation, (c) cyanoethylation, (d) acetylation, (e) deacetylation, (f) phosphorylation, (g) sulfation, (h) esterification, and (i) selenylation (Ngwuluka 2018; Li et al. 2016).

2 Sulfation Reactions

2.1 Modification Reactions

The structural modification of molecules allows the preparation of several structural types of derivatives by chemical, physical, and biological methods. Throughout those methods, polysaccharides could be changed at distinct levels: molecular weight, dimensional structure, and types, number, and positions of substituent groups. Those changes foresee structural changes in favor of desirable physicochemical properties and bioactivities (Li et al. 2016).

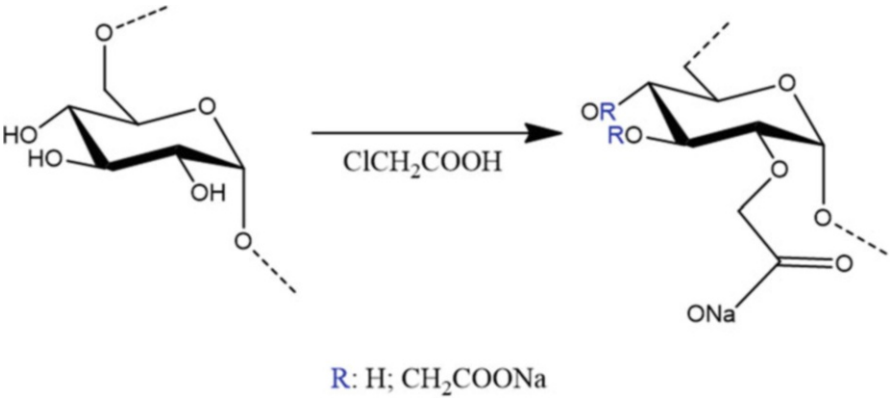


Fig. 2 Example of a carboxymethylation reaction of dextran

A straightforward chemical modification of polysaccharides, achieved through the acid-catalyzed reaction of the polymer, is the carboxymethylation (Silcock 2010), Fig. 2. This modification generally occurs by the reaction established by Alexander Williamson (1850), known as "Williamson ether synthesis" or "Williamson-type etherification." In this reaction, the polysaccharide is firstly protonated with sodium hydroxide (NaOH) and then reacts with chloroacetic acid ($ClCH_2COOH$), generating a derivative of carboxymethyl through an S_N2 reaction. As a reminder, the S_N2 reaction mechanism is a kind of nucleophilic substitution reaction mechanism that happens in one synchronous step where one bond is broken while another is formed. Several examples could be found in the literature about the use of this reaction, even for microbial polysaccharides, such as carboxymethyl cellulose (CMC), carboxymethyl scleroglucan (CMSG), carboxymethylated Gellan gum (CMG), Carboxymethylated Pullulan (CMP), carboxymethylated curdlan (CMc), Carboxymethyl glucan (CMg), and carboxymethyl hyaluronan (CMHA). For instance, carboxymethyl dextran (or Carboxymethyldextran) can be obtained to present heparin-like biological properties and several other applications. The reaction of dextran with chloroacetic acid occurs in alkaline conditions (Fig. 2). Besides the hydroxyl functional groups, carboxymethyldextran also has carboxyl functional groups, a benefit for straightforward chemical modification. Moreover, this dextran derivative also has advantages related to water solubility, biocompatibility, and biodegradability (Vasić et al. 2020).

Carbamoylethylation is the chemical reaction that comprises a covalent bond of carboxylic groups ($–CH_2CH_2COOH$) at the sixth position of the aldohexose units (such as galactose, glucose, and mannose), by replacing the -OH group (Fig. 3). This reaction usually occurs under alkaline conditions, sodium hydroxide, and the polysaccharide then reacts with acrylamide. It is widespread for guar gum, a galactomannan polysaccharide widely used in the food industry as a thickener, stabilizer, emulsifier. Carbamoylethylated guar gum has cold-water solubility, solution stability, and clarity as well as improved rheological properties (Sharma et al. 2004). The optimization of the reaction conditions is essential to achieve the

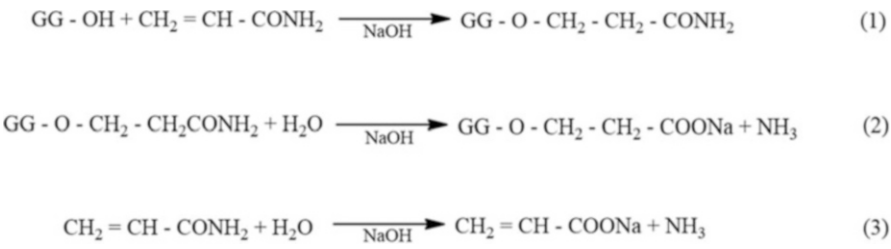


Fig. 3 Main reactions occurring during carbamoylethylation of guar gum. (Adapted reprint from Sharma et al. (2004), copyright (2004), with permission from Elsevier)

productivity and final characteristics of the product. The guar gum carbamoylethylation is achieved in the following optimum conditions: 1.0 mol of acrylamide, 0.75 mol sodium hydroxide, and 0.061 mol of guar gum, at 30 °C for 2 h. Nevertheless, for cassia tora gum the values are quite different: 1.12 mol of acrylamide, 1.25 mol sodium hydroxide, and 0.197 mol of cassia tora gum, at 30 °C for 1 h (Sharma et al. 2003).

Cyanoethylation is a reaction that introduces a cyanoethyl group (C_3H_4N), by a Michael-addition reaction type. Usually, acrylonitrile (AN), or substituted acrylonitrile, is used in this versatile reaction that typically occurs under alkali conditions (with sodium hydroxide) (Fig. 4). This reaction could also occur under acid conditions by using, for instance, acetic acid. In contrast to Williamson-type etherifications, the base is not consumed in this nucleophilic addition of acrylonitrile. This is a reversible and thermodynamically controlled reaction. Cyanoethylation is a lot common for wood or bamboo. Nevertheless, it is also used for polysaccharides such as cellulose, inulin, and starch, as well as dextran and pullulan (Fiege et al. 2012). Once again, this reaction requires an optimization stage since different conditions yield different degrees of substitution (DS) and properties (thermal behavior, solubility) of the resulting cyanoethylated polysaccharide. For instance, cyanoethylated pullulan (CEP) or cyanoethylated dextran (CED) could be obtained with DS ranging from 0.7 to 2.4 and 0.8 to 2.4 (Fiege et al. 2012).

Acetylation and deacetylation are also extensively used to modify molecules, and these reactions involve the addition or removal of an acetyl group. These versatile and straightforward reactions are applied to several polysaccharides, but the reaction method complexity depends on the molecule to be acetylated. One of the most common and relatively simple to obtain is acetylated cellulose. It is easily acetylated by acetic anhydride (acetylation reagent), sulfuric or citric acid (catalyst), and acetic acid (dispersal agent), Fig. 5. In fact, esters of cellulose are valuable derivatives in several fields (such as plastics, films, fibers, membranes, coatings, textiles, and cigarette industries). The well-known dextran has also been subjected to this modification (Ac-DEX) since 2008, foreseeing several applications. Acetylated dextran is obtained with acetic anhydride in the presence of pyridine (Dueramae et al. 2017).

Regarding alginate, interestingly, those of bacterial origin are naturally acetylated, while alginates from algae origin do not have acetyl groups. The non-acetylated alginates are acetylated with a reaction also involving acetic anhydride in the

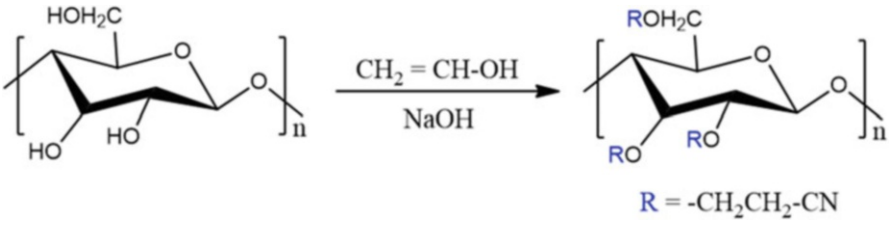


Fig. 4 Example of a cyanoethylation reaction of cellulose

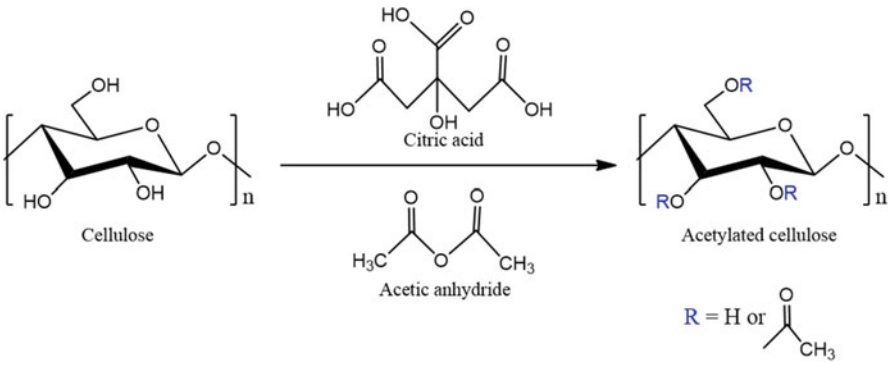


Fig. 5 Example of acetylation reaction of bacterial cellulose

presence of pyridine (Straatmann et al. 2004). More precisely, the alginate solution is transferred to a calcium chloride solution and left overnight; then, the pyridine is added to the precipitate, that is left to decant, and later a solution containing pyridine and acetic anhydride is added; finally, the purification steps are performed (Straatmann et al. 2004). Just as bacterial alginate, several other microbial polysaccharides are naturally acetylated, while others need to be subjected to acetylation methods, resulting in pullulan acetate (PA, AcPL), 9-O-acetylated glycan, acetylated hyaluronic acid (HA-Acet), N-acetyl-heparosan, among others.

The acetylation/deacetylation degree plays a vital role in achieving specific properties for the final application of the polysaccharides (e.g., rheology, solubility, antimicrobial activity). For instance, the chitosan degree of deacetylation (DDA) influences the olfactory ensheathing cells (OECs) expansion and condition (Foster et al. 2015). The degree of deacetylation (DDA) establishes the number of free amino groups in the structure. Several polysaccharides are subjected to deacetylation, such as cellulose acetate, gellan gum, and fungal chitin. For instance, xanthan deacetylation can be done by mild alkaline treatment (sodium and potassium hydroxide) with no additional structure modification (Fig. 6).

Regarding the phosphorylation, various approaches are explored for the phosphorus introduction on organic backbones (Illy et al. 2015). Examples of phosphorylated polysaccharides are bacterial cellulose, chitin, chitosan, dextran, and curdlan. For instance, curdlan or dextran could be reacted with phosphorous acid in molten

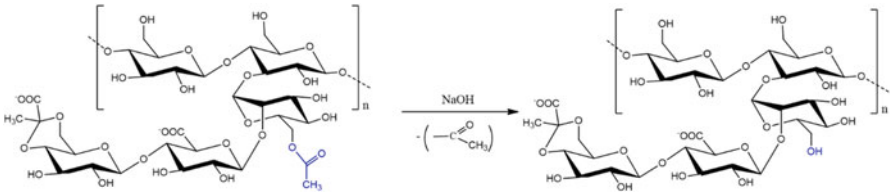


Fig. 6 Example of a reaction of xanthan deacetylation

urea curdlan, at hight temperature and inert atmosphere, to generate phosphorylated products (Fig. 6). The benefits of subjecting curdlan to this reaction are related to enhancements on its solubility in water, polyelectrolyte performance, or even biocompatibility and structure strength during the drug release progression (Zhang 2015). Fully water-soluble and molecule higher flexibility was achieved with phosphorylated dextran (Suflet et al. 2010) (Fig. 7).

As sulfated polysaccharides play an essential role in the biomedical field due to their therapeutic properties, the sulfation procedures emerge as one of the most important modification methods among the available methods for modifying the structures of polysaccharides. Note that sulfonation is different from sulfation, and the main difference is related to the bond formed. The sulfonation implies a sulfur-carbon bond, though the sulfation indicates the existence of a carbon-oxygen-sulfur bond. The first one is more stable and, thus, more advantageous.

2.2 Reaction of Sulfation

Significant interest has been centered on the sulfated polysaccharides' biological effects. Consequently, aiming for new pharmacological agents, a great effort is being made to produce sulfated polysaccharides (Moura Neto et al. 2011) or even to oversulfate the naturally sulfated structures.

The sulfation reaction has been reported, for instance, on cellulose, dextran, pullulan, starch, and chitosan (Mihai et al. 2001). This reaction could be performed in pyridine: formamide mixture utilizing chlorosulfonic acid as the sulfation reagent (Moura Neto et al. 2011), but a variety of other sulfation reagents could be employed with the necessary study of the optimal conditions. Sulfuric acid has the drawback of producing highly degradative sulfated products. On the other hand, sulfation reagents such as chlorosulfonic acid (HSO_3Cl) and sulfur trioxide (SO_3) used with Lewis bases produce more resistant sulfated products. Those can produce a variety of sulfation degrees depending on concentration, temperature, and the bound of the acid with its Lewis base (Whistler et al. 1967). The most common sulfation reagents mentioned in literature are concentrated sulfuric acid (alone or combined with organic solvents), complexes of SO_3 with pyridine, N,N-dimethylformamide (DMF), liquid SO_2, trialkylamine, dimethyl sulfoxide (DMSO), and also chlorosulfonic acid in formamide or pyridine (Mihai et al. 2001). Takano et al. (1996) have sulfated polysaccharides such as starch, agarose, κ-carrageenan, and porphyrin using sulfuric acid and

dicyclohexylcarbodiimide (DCC) as a condensation reagent (Takano et al. 1996). These authors found that the sulfation at 0–6 is predominant and also that the selective protection and deprotection of the hydroxyl groups (-OH) is usually challenging but entirely avoidable if the appropriate sulfation method with a suitable selectivity is chosen. For instance, Takano et al. (1996) found that applying a method using the sulfuric acid-DCC system, applicable to the regioselective sulfation of polysaccharides to neutral polysaccharides and into sulfated polysaccharide (over-sulfation) is effective (Takano et al. 1996). Remember that regioselectivity is the preference for one orientation of chemical bond over another, in the arrangement of a reaction product. Consequently, a regiospecific reaction will provide a more specific product.

Wood cellulose pulp can be sulfated with sulfuric acid as the sulfation reagent while using deep eutectic solvent (DES). Cellulose nanofibers were sulfated using this easy-to-handle and environment-friendly method, and the authors (Sirviö et al. 2019) agreed that this material could be used to adjust rheology or to reinforce additives. In another study (Whistler et al. 1967), the authors started by producing a stable solid complex composed of sulfur trioxide and dimethyl sulfoxide, and then using this complex cellulose was sulfated to substitution degrees 1.3–2.0 in 15–30 min. Song et al. (2018) sulfated bacterial cellulose membranes by firstly immersing them into pyridine for 1 h; then, SO3/Py (6:1) was added and reacted for 3 h at room temperature and magnetic stirring. In the end, pyridine and unreacted SO3/Py were removed, and the membranes neutralized (Song et al. 2018). Moreover, these membranes showed potent anticoagulant properties. Carboxymethyl cellulose sulfates can be prepared by firstly reacting cellulose with sodium monochloroacetate in the presence of NaOH, and then this carboxymethyl cellulose reacts with trisulfonated sodium amine ($N(SO_3Na)_3$), Fig. 8, previously produced by sodium bisulfite and sodium nitrite in aqueous solution (Fan et al. 2014). The authors of this study concluded that the insertion of sulfate groups into the carboxymethyl cellulose enhanced the molecule anticoagulant activity as well as wound healing.

Dextran sulfate is produced by sulfation of dextran. This polyanionic derivative of dextran can be obtained by dextran esterification with chlorosulfonic acid. Dextran sulfate has been studied for a long time, being found several exciting properties and applications such as heparin-like properties (Walton 1952), stabilization of basic fibroblast growth (Kajio et al. 1992), detection of procollagen

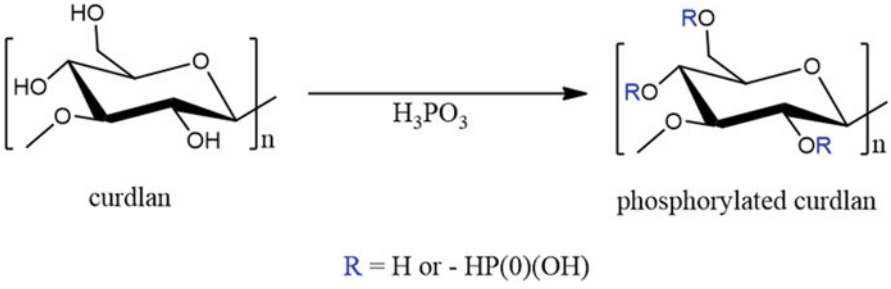


Fig. 7 Example of a reaction of curdlan phosphorylation

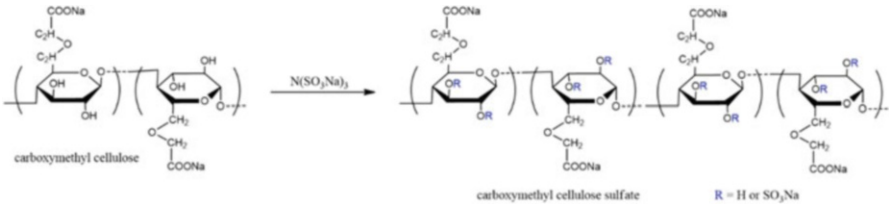


Fig. 8 Example of a reaction of sulfation of carboxymethyl cellulose. (Adapted reprint from Fan et al. (2014), copyright (2014), with permission from Elsevier)

processing defects, and electrophoretic abnormalities (Bateman and Golub 1990). Currently, it is widespread and can be acquired ready-to-use. It is commercially prepared using chlorosulfonic acid. The sulfation of dextran could also occur by dimethyl sulfoxide, which is one of the most successful catalysts for alcoholic sulfation with sulfamic acid compounds. However, better results could be achieved when using piperidine-N-sulfonic acid than those of sulfamic acid and other sulfamic acid derivatives (Nagasawa et al. 1972).

The alginate sulfation has also been described in several other studies using multiple strategies (Fig. 9) (Arlov and Skjåk-Bræk 2017). More recently, the sulfated modification of alginates has been made envisaging, for instance, the development of delivery systems based on electrostatic interactions for small molecule drugs. These authors (Nazemi et al. 2020) found that the sulfated modification was responsible for a remarkable effect on drug-polymer complexation, which may be useful for the preparation of various drug carriers. Daemi and coworkers (2018) developed a facile fabrication method of sulfated alginate electrospun nanofibers and found that decreasing the intramolecular hydrogen bonding of alginate helps its electrospinnability. Moreover, these authors had sulfated the alginate through a reaction of sodium alginate and chlorosulfonic acid in formamide.

Mihai et al. (2001) found that the sulfation of pullulan is influenced not only by the complex used but also by the temperature of the reaction. Moreover, these authors discovered that dextran and pullulan have different behavior on sulfation depending on the function of the SO3·organic base complex or the solvent used. The sulfation of pullulan was performed by Alban and coworkers (Alban et al. 2002), envisaging the development of new anticoagulants as heparin alternatives. The sulfation reaction was carried out by using the optimized conditions with SO_3–pyridine complex in DMF. The obtained sulfated pullulan reached the heparin anticoagulant efficiency, increasing with a growing degree of sulfation (DS) and molecular weight (MW), and with more sulfate groups (in 2, 3, and 4 positions) of the molecule (Alban et al. 2002).

Matsugasaki and his team workers have successfully sulfated curdlan, dextran, and starch at high temperatures (60–80 °C) in DMSO with sodium methyl sulfate or with pyridinium methyl sulfate (Takano et al. 2000). Curdlan sulfated with high DS, using piperidine N-sulfonic acid or SO3-dimethylformamide complex, have shown high anti-HIV (AIDS virus) activity (Yamamoto et al. 1990). Another sulfation process on curdlan with the assistance of ultrasonication yields even better

Fig. 9 Schematic representation of developed methods for chemical sulfation of alginate using different reagents. (Adapted from Arlov and Skjåk-Bræk (2017), an open-access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium)

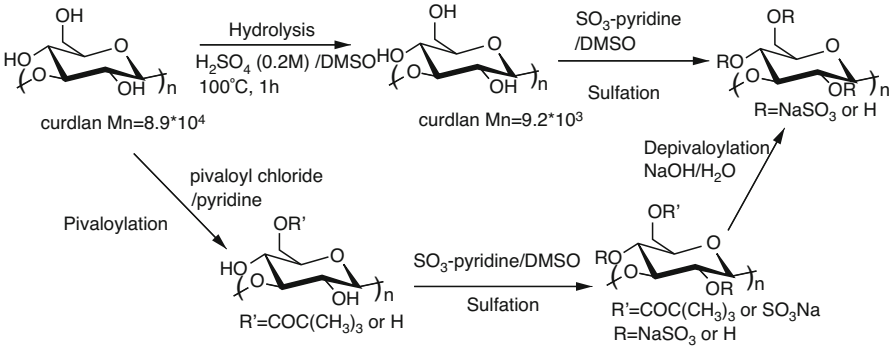


Fig. 10 Synthetic routes of regioselectively substituted curdlan sulfates. (Reprinted from Cai and Zhang (2017) copyright (2017), with permission from Elsevier)

physicochemical properties with potential for biomedical application (Wong et al. 2010). Moreover, regioselectively substituted curdlan sulfates with medium molecular weight were produced to examine the effect of position of sulfate groups on anti-HIV activity. Thus, the curdlan was modified by pivaloyl chloride to substitute the C6 hydroxyl groups, then followed by sulfation and depivaloylation (Fig. 10) (Cai and Zhang 2017). However, the authors found that the anti-HIV activity varied with the degree of sulfation but not with the sulfate groups' position (Gao et al. 1997).

2.3 Tools to Support the Study of New and Modified Polysaccharides

Research on new microbial polysaccharides and their sulfation is a field with not only many opportunities but also many challenges. Occasionally, it is not enough to have vast knowledge in some fields of chemistry to have success. For this reason, the experimental design emerges as an essential tool, which together with several characterization methods, will help the researcher to follow the best direction to success. The design of experiments (DOE) is not only a statistical but also a mathematical tool to organize the experiments systematically and efficiently

examine the obtained data, conducting the researchers/engineers/scientists throughout a process optimization (Das and Dewanjee 2018). In addition to this, DOE will do it at a reduced cost and time. The DOE's vulnerability is related to the range of the study that frankly influences the prediction.

DOE could be classified into four main groups: (i) Taguchi DOE, (ii) Factorial DOE, (iii) Response surface methodology (RSM)-based DOE, and (iv) Latin square DOE (Vikram et al. 2014). The choice between those DOE will depend on the researcher's experience together with other factors (objective, time, resources, team experience). For instance, while Taguchi DOE is based on the prior choice of the most probable interactions, in standard fractional factorial designs, the interactions are selected afterward with the evaluation of the initial results. Ali and coworkers concluded that the full factorial design performs better than the Taguchi method (Rafidah et al. 2014). Regarding the aim of using DOE, if it is optimization, the range of DOE alternatives reduces. For example, the most suitable designs for obtaining a response surface are central composite or Box-Behnken.

Several characterization techniques are helping the researcher to pursue the structural characteristics of their product. Usually, to identify the molecule or if a modification was successfully performed, Fourier Transform Infrared (FTIR) and Nuclear magnetic resonance (NMR) techniques are used. Other chromatographic techniques can also be used as High-performance liquid chromatography (HPLC) or Gel permeation chromatography (GPC). However, to insert a response/consequence of the experiment carried out into DOE software, relevant and quantitative answers are needed so that the program can calculate the effects, draw 3D curves, and draw the Pareto charts. It is, therefore, crucial that researchers select the methods accurately to be used to characterize the product. Those methods should not only be quantitative, but also have an appropriate relevance to the objectives of that product. For example, to optimize a product that should primarily have anticoagulant effects, its anticoagulant activity value must be monitored and used as a response in the DOE.

3 Final Remarks

The purpose of this chapter was to illustrate the vast diversity of modifications that can be applied to microbial polysaccharides, highlighting the sulfation of microbial polysaccharides and their potential application. The use, study, and commercialization of microbial polysaccharides have a long story. Nevertheless, there is still plenty of room for research on this topic nowadays, since technological difficulties stay high. Therefore, the pursuit of new and improved materials remains a crucial pillar for the development and marketing authorization of many products.

These molecules are heterogeneous and structurally distinct, which makes their research very challenging. Nevertheless, it is as challenging as it is advantageous. In fact, microbial polysaccharides have clear advantages regarding low cost, high efficiency, reproducibility, and ease of production. When those advantages are combined with the benefits obtained in their sulfation (for instance, to produce

therapeutic materials or bio-lubricants), it is possible to assert that this is a field with great potential and should be addressed for the benefit of the population.

Acknowledgments C. Gonçalves and H. Radhouani were supported by the Foundation for Science and Technology (FCT) from Portugal, with references SFRH/BPD/94277/2013 and CEECIND/00111/2017, respectively. This work was funded by the R&D Project KOAT – Kefiran exopolysaccharide: Promising biopolymer for use in regenerative medicine and tissue engineering, with reference PTDC/BTMMAT/29760/2017 (POCI-01-0145-FEDER-029760), financed by FCT and co-financed by FEDER and POCI.

References

Alban S, Schauerte A, Franz G. Anticoagulant sulfated polysaccharides: part I. Synthesis and structure–activity relationships of new pullulan sulfates. Carbohydr Polym. 2002;47(3):267–76.

Amin KAM, Panhuis Mih. Polyelectrolyte complex materials from chitosan and gellan gum. Carbohydr Polym. 2011;86(1):352–8.

Arlov Ø, Skjåk-Bræk G. Sulfated alginates as heparin analogues: a review of chemical and functional properties. Molecules. 2017;22(5):778.

Bateman JF, Golub SB. Assessment of procollagen processing defects by fibroblasts cultured in the presence of dextran sulphate. Biochem J. 1990;267(3):573–7.

Beserra FP, Rozza AL, Vieira AJ, Gushiken LFS, Pellizzon CH. Antiulcerogenic compounds isolated from medicinal plants. Stud Nat Prod Chem. 2016;47:215–34.

Bhardwaj N, Kundu SC. Silk fibroin protein and chitosan polyelectrolyte complex porous scaffolds for tissue engineering applications. Carbohydr Polym. 2011;85(2):325–33.

Cai Z, Zhang H. Recent progress on curdlan provided by functionalization strategies. Food Hydrocoll. 2017;68:128–35.

Chen L, Huang G. The antiviral activity of polysaccharides and their derivatives. Int J Biol Macromol. 2018;115:77–82.

Christian P. Polymer chemistry. In: Electrospinning for tissue regeneration. Elsevier; 2011. p. 34–50.

Ciancia M, Quintana I, Cerezo AJ. Overview of anticoagulant activity of sulfated polysaccharides from seaweeds in relation to their structures, focusing on those of green seaweeds. Curr Med Chem. 2010;17(23):2503–29.

Daemi H, Mashayekhi M, Modaress MP. Facile fabrication of sulfated alginate electrospun nanofibers. Carbohydr Polym. 2018;198:481–5.

Damonte EB, Matulewicz MC, Cerezo AS. Sulfated seaweed polysaccharides as antiviral agents. Curr Med Chem. 2004;11(18):2399–419.

Das AK, Dewanjee S. Optimization of extraction using mathematical models and computation. In: Computational phytochemistry. Elsevier; 2018. p. 75–106.

De Angelis PLJG. Microbial glycosaminoglycan glycosyltransferases. Glycobiology. 2002;12(1):9R–16R.

Dueramae I, Yoneyama M, Shinyashiki N, Yagihara S, Kita R. Self-assembly of acetylated dextran with various acetylation degrees in aqueous solutions: studied by light scattering. Carbohydr Polym. 2017;159:171–7.

Fan L, Zhou X, Wu P, Xie W, Zheng H, Tan W, et al. Preparation of carboxymethyl cellulose sulfates and its application as anticoagulant and wound dressing. Int J Biol Macromol. 2014;66:245–53.

Fiege K, Lünsdorf H, Atarijabarzadeh S, Mischnick PJ. Cyanoethylation of the glucans dextran and pullulan: substitution pattern and formation of nanostructures and entrapment of magnetic nanoparticles. Beilstein J Org Chem. 2012;8(1):551–66.

Foster LJR, Ho S, Hook J, Basuki M, Marçal H. Chitosan as a biomaterial: influence of degree of deacetylation on its physiochemical, material and biological properties. PLoS One. 2015;10(8): e0135153.

Freitas F, Alves VD, Pais J, Costa N, Oliveira C, Mafra L, et al. Characterization of an extracellular polysaccharide produced by a Pseudomonas strain grown on glycerol. Bioresour Technol. 2009;100(2):859–65.

Freitas F, Alves VD, Reis MA, Crespo JG, Coelhoso IM. Microbial polysaccharide-based membranes: current and future applications. J Appl Polym Sci. 2014;131(6).

Gao Y, Fukuda A, Katsuraya K, Kaneko Y, Mimura T, Nakashima H, et al. Synthesis of regioselective substituted curdlan sulfates with medium molecular weights and their specific anti-HIV-1 activities. Macromolecules. 1997;30(11):3224–8.

Giavasis I. Production of microbial polysaccharides for use in food. In: Microbial production of food ingredients, enzymes and nutraceuticals. Elsevier; 2013. p. 413–68.

Gupta J, Rathour R, Medhi K, Tyagi B, Thakur IS. Microbial-derived natural bioproducts for a sustainable environment: a bioprospective for waste to wealth. In: Refining biomass residues for sustainable energy and bioproducts. Elsevier; 2020. p. 51–85.

Huang W-C, Tang I-C. Bacterial and yeast cultures–process characteristics, products, and applications. In: Bioprocessing for value-added products from renewable resources. Elsevier, Burlington; 2007. p. 185–223.

Huang L, Shen M, Morris GA, Xie J. Sulfated polysaccharides: immunomodulation and signaling mechanisms. Trends Food Sci Technol. 2019;92:1–11.

Illy N, Fache M, Menard R, Negrell C, Caillol S, David G. Phosphorylation of bio-based compounds: the state of the art. Polym Chem. 2015;6(35):6257–91.

Ivanova EP, Bazaka K, Crawford RJ. Natural polymer biomaterials: advanced applications. In: Science E, editor. New functional biomaterials for medicine and healthcare, vol. 2. Oxford: Woodhead; 2014. p. 32–70.

Kajio T, Kawahara K, Kato K. Stabilization of basic fibroblast growth factor with dextran sulfate. FEBS Lett. 1992;306(2–3):243–6.

Kang H, Yang B, Zhang K, Pan Q, Yuan W, Li G, et al. Immunoregulation of macrophages by dynamic ligand presentation via ligand–cation coordination. Nat Commun. 2019;10(1):1–14.

Lahaye M, Inizan F, Vigoureux J. NMR analysis of the chemical structure of ulvan and of ulvan-boron complex formation. Carbohydr Polym. 1998;36(2–3):239–49.

Lai GJ, Shalumon KT, Chen SH, Chen JP. Composite chitosan/silk fibroin nanofibers for modulation of osteogenic differentiation and proliferation of human mesenchymal stem cells. Carbohydr Polym. 2014;111:288–97.

Li S, Xiong Q, Lai X, Li X, Wan M, Zhang J, et al. Molecular modification of polysaccharides and resulting bioactivities. Compr Rev Food Sci Food Saf. 2016;15(2):237–50.

Li J, Shen B, Nie S, Chen K. Marine microbial polysaccharides: promising immunomodulatory and anticancer potential. In: Marine polysaccharides. Milton: Jenny Stanford; 2018. p. 13–28.

Liu G, Yang HJ. Modulation of macrophage activation and programming in immunity. J Cell Physiol. 2013;228(3):502–12.

Maji B. Introduction to natural polysaccharides. In: Functional polysaccharides for biomedical applications. Amsterdam: Elsevier; 2019. p. 1–31.

Mihai D, Mocanu G, Carpov A. Chemical reactions on polysaccharides: I. Pullulan sulfation. Eur Polym J. 2001;37(3):541–6.

Mironescu M. Microbial polysaccharides production, characterisation and properties. Acta Universitatis Cibiniensis Series E. Food Technol. 2003;7(2):26–38.

Moura Neto ÉD, Maciel JDS, Cunha PL, de Paula RCM, Feitosa J. Preparation and characterization of a chemically sulfated cashew gum polysaccharide. J Braz Chem Soc. 2011;22(10):1953–60.

Nagasawa K, Harada H, Hayashi S, Misawa T. Sulfation of dextran with piperidine-N-sulfonic acid. Carbohydr Res. 1972;21(3):420–6.

Nazemi Z, Nourbakhsh MS, Kiani S, Daemi H, Ashtiani MK, Baharvand H. Effect of chemical composition and sulfated modification of alginate in the development of delivery systems based on electrostatic interactions for small molecule drugs. Mater Lett. 2020;263:127235.

Ngwuluka NC. Responsive polysaccharides and polysaccharides-based nanoparticles for drug delivery. In: Stimuli responsive polymeric Nanocarriers for drug delivery applications, vol. 1. San Diego: Elsevier; 2018. p. 531–54.
Partain III EM. Industrially important polysaccharides. In: Applied polymer science: 21st century. Amsterdam: Elsevier; 2000. p. 303–23.
Patel S. Therapeutic importance of sulfated polysaccharides from seaweeds: updating the recent findings. 3 Biotech. 2012;2(3):171–85.
Rafidah A, Nurulhuda A, Azrina A, Suhaila Y, Anwar I, Syafiq R. Comparison design of experiment (doe): Taguchi method and full factorial design in surface roughness. Appl Mech Mater. 2014;660:275–9.
Raveendran S, Yoshida Y, Maekawa T, Kumar DS. Pharmaceutically versatile sulfated polysaccharide based bionano platforms. Nanomedicine. 2013;9(5):605–26.
Robic A, Sassi JF, Lahaye M. Impact of stabilization treatments of the green seaweed Ulva rotundata (Chlorophyta) on the extraction yield, the physico-chemical and rheological properties of ulvan. Carbohydr Polym. 2008;74(3):344–52.
Shang S, Zhu L, Fan J. Intermolecular interactions between natural polysaccharides and silk fibroin protein. Carbohydr Polym. 2013;93(2):561–73.
Sharma BR, Kumar V, Soni P. Carbamoylethylation of Cassia tora gum. Carbohydr Polym. 2003;54 (2):143–7.
Sharma BR, Kumar V, Soni P. Carbamoylethylation of guar gum. Carbohydr Polym. 2004;58(4): 449–53.
Silcock D. Collagen-based dressings as therapeutic agents for wound healing. In: Drug-device combination products. Elsevier; 2010. p. 280–310.
Sirviö JA, Ukkola J, Liimatainen H. Direct sulfation of cellulose fibers using a reactive deep eutectic solvent to produce highly charged cellulose nanofibers. Cellulose. 2019;26(4):2303–16.
Sletmoen M, Klein DC, Stokke BT. Single-molecule characterization of microbial polysaccharides. In: Microbial glycobiology. Elsevier; 2010. p. 253–68.
Song W, Zeng Q, Yin X, Zhu L, Gong T, Pan C. Preparation and anticoagulant properties of heparin-like electrospun membranes from carboxymethyl chitosan and bacterial cellulose sulfate. Int J Biol Macromol. 2018;120:1396–405.
Sousa SG, Oliveira LA, de Aguiar MD, de Brito TV, Batista JA, Pereira CMC, et al. Chemical structure and anti-inflammatory effect of polysaccharide extracted from Morinda citrifolia Linn (Noni). Carbohydr Polym. 2018;197:515–23.
Sparkman OD, Penton Z, Kitson FG. Gas chromatography and mass spectrometry: a practical guide. Amsterdam: Academic; 2011.
Stick RV, Williams S. Carbohydrates: the essential molecules of life. Amsterdam: Elsevier; 2010.
Straatmann A, Windhues T, Borchard W. Effects of acetylation on thermodynamic properties of seaweed alginate in sodium chloride solutions. Progr Colloid Polym Sci. 2004;127:26–30.
Suflet DM, Chitanu GC, Desbrières J. Phosphorylated polysaccharides. 2. Synthesis and properties of phosphorylated dextran. Carbohydr Polym. 2010;82(4):1271–7.
Takano R, Yoshikawa S, Ueda T, Hayashi K, Hirase S, Hara S. Sulfation of polysaccharides with sulfuric acid mediated by dicyclohexylcarbodiimide. J Carbohydr Chem. 1996;15(4):449–57.
Takano R, Nagai T, Wu X, Xu X-Y, Huy NT, Kamei K, et al. Sulfation of polysaccharides using monomethyl sulfate. J Carbohydr Chem. 2000;19:1185–90.
Udayan A, Arumugam M, Pandey A. Nutraceuticals from algae and cyanobacteria. In: Algal green chemistry. Amsterdam: Elsevier; 2017. p. 65–89.
Vasić K, Knez Ž, Konstantinova EA, Kokorin AI, Gyergyek S, MJR L, et al. Structural and magnetic characteristics of carboxymethyl dextran coated magnetic nanoparticles: from characterization to immobilization application. React Funct Polym. 2020;148:104481.
Vikram KA, Narayana KS, Kumar GP, Skandha C. Prediction of optimality and effect of machining parameters on surface roughness based on taguchi design of experiments. Int J Theoret Appl Res Mech Eng. 2014;3:2319–3182.
Walton K. The biological behaviour of a new synthetic anticoagulant (dextran sulphate) possessing heparin-like properties. Br J Pharmacol Chemother. 1952;7(3):370.

Whistler RL, King AH, Ruffini G, Lucas F. Sulfation of cellulose with sulfur-trioxide-dimethyl sulfoxide. Arch Biochem Biophys. 1967;121(2):358–63.
Wong S-S, Ngiam ZRJ, Kasapis S, Huang D. Novel sulfation of curdlan assisted by ultrasonication. Int J Biol Macromol. 2010;46(3):385–8.
Yadav H, Karthikeyan C. Natural polysaccharides: structural features and properties. In: Polysaccharide carriers for drug delivery. Amsterdam: Elsevier; 2019. p. 1–17.
Yamamoto I, Takayama K, Honma K, Gonda T, Matsuzaki K, Hatanaka K, et al. Synthesis, structure and antiviral activity of sulfates of cellulose and its branched derivatives. Carbohydr Polym. 1990;14(1):53–63.
Zhang R. Regioselective synthesis of curdlan derivatives. Virginia Tech; 2015.
Zhao D, Huang J, Hu S, Mao J, Mei L. Biochemical activities of N,O-carboxymethyl chitosan from squid cartilage. Carbohydr Polym. 2011;85(4):832–7.

Part V

Pharmaceutical Applications

Dextran Pharmaceutical Applications

31

Suneela Dhaneshwar, Neha Bhilare, and Supriya Roy

Contents

S. Dhaneshwar (✉)
Department of Pharmaceutical Chemistry, Amity Institute of Pharmacy, Lucknow, Amity University Uttar Pradesh, Noida, India
e-mail: sdhaneshwar1@lko.amity.edu

N. Bhilare
Department of Pharmaceutical Chemistry, Arvind Gavali College of Pharmacy, Satara, India

S. Roy
Department of Pharmacology, Amity Institute of Pharmacy, Lucknow, Amity University Uttar Pradesh, Noida, India
e-mail: sroy@lko.amity.edu

J. M. Oliveira et al. (eds.), *Polysaccharides of Microbial Origin*,
https://doi.org/10.1007/978-3-030-42215-8_40

Abstract

Among the different polymers derived from natural origin, dextrans are one of the widely explored classes in the field of drug delivery. This chapter describes the chemistry, physicochemical properties, and reactivity of dextrans. An elaborative account of various drug-dextran conjugates, dextran hydrogels, dextran-micellar nanocarrier systems, dextran-magnetic layered composites, and bioadhesive oral delivery systems of dextran along with various methods of synthesis of these conjugates has been presented. Dextran is a nontoxic and biocompatible polysaccharide that has wide applications in pharmaceutical, biomedical, and industrial fields. The chapter provides an overview of these aspects apart from most significant developments in this field.

Keywords

Dextran · Polymer · Polysaccharide · Conjugates · Prodrugs · Drug delivery systems

1 Introduction

The α-glucans whose monosaccharides present exclusive configuration are extensively utilized as an energy source by living organisms such as glycogen (energy source for animals) and starch (energy source used by plants). However, α-glucans also possess further interesting anti-inflammatory, antimicrobial, immunomodulatory, and antioxidant properties. However, not all α–glucans have been widely assessed for their biological potential, for example, dextrans. Nowadays dextran

derivatives, dextran conjugates, dextran micelles, and hydrogels are broadly used as nanocarriers and nanomedicine on account of its simple and nonimmunogenic biopolymeric nature. Various properties of dextran like temperature sensitivity, hydrophobicity, hydrophilicity, ionic strength sensitivity, and pH sensitivity multiply their applications in drug delivery systems. They are also quite popular for their stabilizing, texturizing, emulsifying, and viscosifying characteristics in food industry and own the capability to be employed as novel ingredient replacing the commercial hydrocolloids in bakery and other related food industries (Bhavani and Nisha 2010).

2 Origin of Dextran

The progressively growing demand of many natural polymers for various industrial applications has led to the exploration of microbial exopolysaccharide (EPS) in recent years. Among several EPS, dextran has gained worldwide recognition due to its biodegradability and biocompatible properties (Patel et al. 2010). The EPS dextran was first discovered by Louis Pasteur in 1861 as a microbial product in wine. Scheilber in 1874 confirmed that this microbial polysaccharide has a positive (dextrorotatory) optical rotation with the empirical formula $(C_6H_{10}O_6)_n$ and therefore named as "dextran." The physiological roles of EPS in the microbial host are not yet completely understood, however their role in protection against dehydration, pathogenicity, biofilm formation, and quorum sensing is well documented in the literature. The presence of a dextran layer around the bacterial cell may have paramount effects on the cellular diffusion properties. Dextran has gained importance owing to its applications in the food, pharmaceutical, biomaterial, photofilm manufacturing, and fine chemical industries. A huge number of lactic acid bacteria are known to produce dextran (Varshosaz 2012; Kothari et al. 2014).

3 Structure and Physicochemical Properties

Dextrans are a family of high-molecular-weight polymers composed of D-glucose units interconnected with α-1,6 linkages, with a variable degree of side branches via α-1,2, α-1,3, or α-1,4 linkages. In most cases, the length of the side chains is short, and branched residues vary between 5% and 33%. Commercial dextrans are about 95% α-1,6-linked and 5% α-1,3-linked.

The structure of dextran is quite complex with a branched polysaccharide composed of several glucose molecules joined into chains of varying lengths (Fig. 1). The straight chain consists of α (1→6) glycosidic linkages between glucose molecules, while branches begin from α (1→3) linkages (and in some cases, α1→2 and α1→4 linkages as well). Dextran is biosynthesized by a wide variety of bacterial strains including *Leuconostoc mesenteroides, Gluconobacter oxydans,* and *Streptococcus mutans* through the use of specific enzymes like glucansucrases. Their physicochemical properties are mainly associated with molecular weight (Mw) and degree of branching, which in turn depend on the source of production (Heinze et al. 2006).

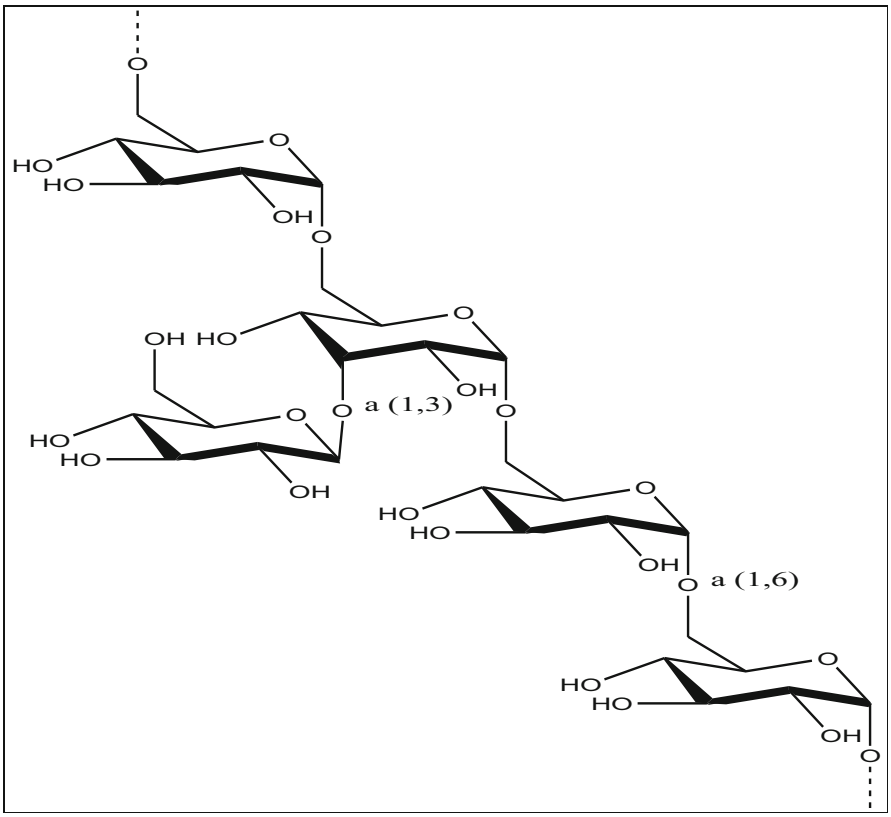


Fig. 1 Chemical structure of dextran

Dextran B-512(F) is freely soluble in water, methyl sulphoxide, formamide, ethylene glycol, glycerol, 4-methylmorpholine-4-oxide, and hexamethylphosphoramide (a carcinogenic). Some dextran fractions may adopt a certain degree of crystallinity and may only be brought into solution by strong heating. Dextran polymers have a noteworthy multiplicity in chain length and in physical and chemical properties as a result of difference in degree of branching that exists in their glucose backbone. Generally, dextran is found to be readily soluble in water, formamide, ethylene glycol, dimethyl sulfoxide, and glycerol; however it is insoluble in monohydric alcohols, e.g., methanol, ethanol, and isopropanol, and also most of the ketones, e.g., acetone and 2-propanone. However, the water solubility of dextrans is subject to the pattern of branched linkage. Linear dextrans have high water solubility, and the aqueous solutions behave as Newtonian fluids (Tsuchiya 1960). Dextran solution's viscosity depends on its concentration, temperature, and molecular weight. Variations in salt concentration or pH do not considerably influence the viscosity as dextran is reported to be a neutral polysaccharide. Dextrans with>43% branching through α (1-3) linkages are water insoluble. Dextrans have molecular weight in the range of 3–500,000 kDa. Dextrans with a molecular weight of 2000–10,000 kDa exhibit the

properties of an expandable coil, and at lower molecular weights (<2000 kDa) dextran is more rod-like. Low-molecular-weight dextrans (40, 60, and 70 kDa) are generally preferred in clinical applications (Virnik et al. 1975). The surface morphological studies of dextran revealed a porous structure. Dextran has excellent thermal stability with degradation temperature of 300 °C. Dextran does not undergo hydrolysis easily in the presence of α-amylase or simulated gastric juice. Dextran supports growth of probiotic bacteria but does not promote the growth of unwanted *Escherichia coli* (Walker 1978).

4 Reactivity

The reactivity of dextran involves principally an exploration of the comparative reactivities of the secondary, equatorially orientated hydroxyl groups, 2-OH, 3-OH, and 4-OH. A lesser percentage of the hydroxyl groups in dextran are primary (approx. 1.5%), although this figure rises marginally at lower molecular weights owing to the nonreducing end groups. Attempts to tritylate the primary hydroxyls selectively in methyl sulphoxide/pyridine have been reported. Good agreement between the experimental and theoretical distribution of methyl ethers was only obtained when allowance was made for the enhanced reactivity at 3-OH as a result of substitution at 2-OH or 4-OH. The relative reactivities of the hydroxyl groups toward ethylene oxide closely follow those for methylation (De Belder and Norrman 1969). At higher degrees of substitution, however, the pattern of substitution becomes complex owing to the introduction of primary hydroxyl sites. Acylations may differ from alkylations in that they may be subject to thermodynamic control owing to migration of the substituents.

5 Pharmacokinetic Fate

Various physical and chemical properties like hydrophilic-lipophilic balance, molecular size and shape, flexibility, and charge influence the pharmacokinetics of dextran. Metabolism of dextrans by dextranases and extrarenal excretion account for only 2–10% of the total drug expelled from the body. Persistence of dextrans in the systemic circulation and elimination by the renal route are dependent on the size of dextrans and their molecular weight distribution. Dextran species with a molecular weight below 15,000 Da are filtered unrestricted, and consequently the elimination half-life of dextran 1 is relatively short (2 h) and that of dextran 40 (10 h) or dextran 60 (42 h) much longer. In patients with renal insufficiency, elimination is impaired, parallel to the reduction in glomerular filtration rate, and smaller doses are advisable in these patients.

Dextrans with molecular weight in the range 70,000–250,000 kDa show prolonged survival in circulatory system. The high polarity of dextrans excludes their transcellular passage and their size prevents their passage through gastrointestinal tract (Klotz and Kroemer 1987).

6 Dextran Conjugates

Linking of dextran conjugates can either be irreversible or reversible type. Methods to link dextran conjugates irreversibly include various immobilization techniques. Metal dextran enzyme conjugates, dextran metal complexes, and dextran hormone complexes are some examples of irreversible linking. Reversible dextran conjugates include dextran esters, dextran ethers, and dextran amides.

6.1 Irreversible Dextran Conjugates

Dextran derivatives which consist of ligand irreversibly connected to the carrier have been employed extensively in experimental medicine and find wide applications in the field of biotechnology and related areas. Some examples are summarized below:

6.1.1 Dextran Enzyme Conjugates

Enzymes are widely used in medical diagnosis and their clinical applications are vast. Enzymes are also used as therapeutic agents since they catalyze complex chemical reactions under appropriate physiological conditions. For coupling enzymes with dextran, several mild synthetic procedures have been employed, many of which were previously used for immobilization of enzymes on insoluble polysaccharide support. These conjugates have demonstrated several benefits like increase in the biological half-life of enzyme, better thermal stability, improvement of enzyme stability at physiological pH, and lowered risk of emergence of allergic reactions on repeated administration (Varshosaz 2012).

Examples of enzymes linked by this approach include α-amylase, arginase, asparaginase, carboxypeptidase, glucose oxidase, catalase, α-galactosidase, hyaluronidase, NAD+, streptokinase, papain, α-chymotrypsin, and trypsin (Wileman 1991; Altikatoglu et al. 2010). Some of the examples have been described below.

Asparaginases isolated from *Escherichia coli* and *Erwinia carotovora* are used in the treatment of acute lymphoblastic leukemia. Unfortunately, their use has been limited by their relatively short circulatory half-lives and by immune reactions that develop in response to repeated injections of the enzymes. Dextran, a biocompatible polymer of D-glucose, is used to improve therapeutic potential by binding it covalently to the surface of asparaginase. The resulting soluble dextran-asparaginase conjugates show increased circulatory persistence and markedly reduced antigen reactivity. Studies have shown that it is possible to use dextran to create a steric barrier around asparaginase that not only protects the enzyme from degradation in vivo but also slows its inactivation by the immune system. Soluble dextran conjugates provide a means of avoiding the biological limitations to the use of microbial enzymes in therapy (Wileman 1991).

Multipoint covalent bonding of glucose oxidase (EC 1.1.3.4) to dextran and optimization of procedures to obtain, with enhanced temperature and pH stabilities, were studied by Altikatoglu et al. Purified enzyme was conjugated with dextrans of different molecular weights in different ratios. This conjugate offered an enhanced

stability of enzyme against temperature and pH and proposed to have potential in preparation of clinical diagnostic kits and biosensors (Altikatoglu et al. 2010).

Another example is coupling of bovine pancreatic trypsin with dextran wherein after coupling 53% of the esterase activity of trypsin was retained, but the conjugate had only 7% of the caseinolytic activity of the native enzyme. The modified trypsin showed greater resistance than the native enzyme to inactivation by heat treatment, autodigestion, or denaturing agents, and was also more resistant to inhibition by trypsin inhibitors, particularly ovomucoid (Marshall and Rabinowitz 1976).

6.1.2 Dextran Small-Molecule Complexes

Dextran sulphate (DS), diethylaminoethyl dextran, and carboxymethyl dextran (CMD) are among the charged dextran derivatives that are responsible for forming complexes with various chemical moieties (Fig. 2).

DS in the form of drug-coated balloons (DCBs) to deliver antiproliferative agent paclitaxel was reported to be a promising platform for controlled release (Lamichhane et al. 2016). DS has also been used successfully in combination with cross-linked chitosan for ophthalmic delivery of ciprofloxacin. In particular, owing to its multiple functional groups allowing for facile chemical modification, CMD has been extensively used to develop polymeric conjugates as the nanomedicines (Shin et al. 2018).

6.1.3 Dextran Metal Complexes

Dextran complexes with heavy metals like iron and antimony are quite easily synthesized by reacting salts of these metals with the aqueous solution of polymer following

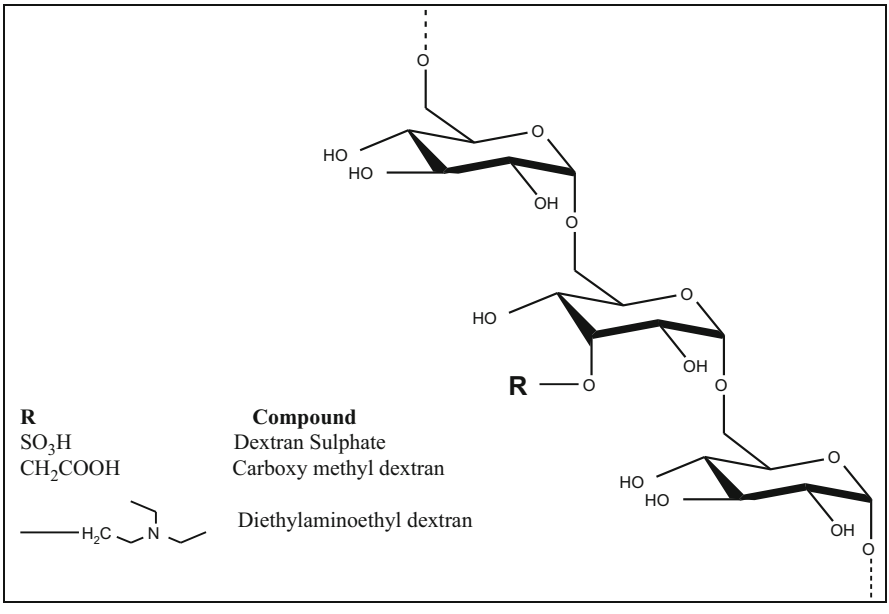


Fig. 2 Dextran small-molecule complexes

precipitation of the macromolecular derivatives with alcohol. Application of iron dextran as a thermosensitizer in radiofrequency hyperthermia for cancer treatment has been reported (Chung et al. 2019). Parenteral iron dextran has been used for the rapid regeneration of the red cell mass in anemia and in patients intolerant of oral forms of iron. Human interferon β (IFN β) was passively targeted to choroidal neovascularization by combining it with dextran, based on metal (Zn^{2+}) coordination, and an enhanced antiangiogenic effect was achieved (Yasukawa et al. 2002). Pentavalent antimonials are regarded as highly effective chemicals in the treatment of leishmaniasis but possess major drawback of rapid excretion in urine and thus short duration of action. Complexing with dextran helps maintain adequate concentration in blood and thus suppresses the *Leishmania donavani* infection effectively (Mikhail et al. 1975). Dextran complex with bioactive copper(II) has also been reported to have considerably lower acute toxicity (Cakić et al. 2008). *Cis*-platinum (II) has been complexed with carboxymethyl dextran and is reported to be cytotoxic in vitro against 5 murine- and 2 human-derived tumor cell lines (Wang et al. 2015).

6.2 Reversible Dextran Conjugates/Prodrugs

Dextrans are often selected as a carrier for macromolecular prodrugs on account of the ideal characteristics that they possess. These polysaccharides improve the stability of the therapeutics enabling also the control of their release, via either the parenteral and/or oral routes. Advantages of dextrans as drug carriers may be summarized as: (i) polymeric chemical structure with repetitive monomers, (ii) highly water solubility, (iii) high stability to acidic or alkaline conditions due to stable glucosidic bonds that are not hydrolyzed easily except under extreme pH ranges, (iv) the possibility of derivatization on numerous reactive hydroxyl groups present in its structure, (v) availability of different molecular weights, (vi) inert nature due to low toxicity and pharmacological activity, (vii) protection of conjugated drugs from biodegradation, and (viii) an apparent passive targeting of this soluble macromolecules to solid tumors due to EPR effect.

7 Modes of Covalent Drug Attachment to Dextran

Dextran is often attached as a carrier to the drug to form a prodrug by a number of techniques namely direct linkage, attachment through intercalated spacer arm, use of modulator ligand, and tissue-specific receptor ligand as shown in Fig. 3.

7.1 Dextran Conjugates for Lowering Ulcerogenicity of Nonsteroidal Anti-Inflammatory Drugs

Nonsteroidal anti-inflammatory drugs (NSAIDs) have been reported to possess major drawback of causing ulcerogenicity due to the presence of acidic group. Dextran conjugates of ketorolac and flurbiprofen were synthesized and characterized

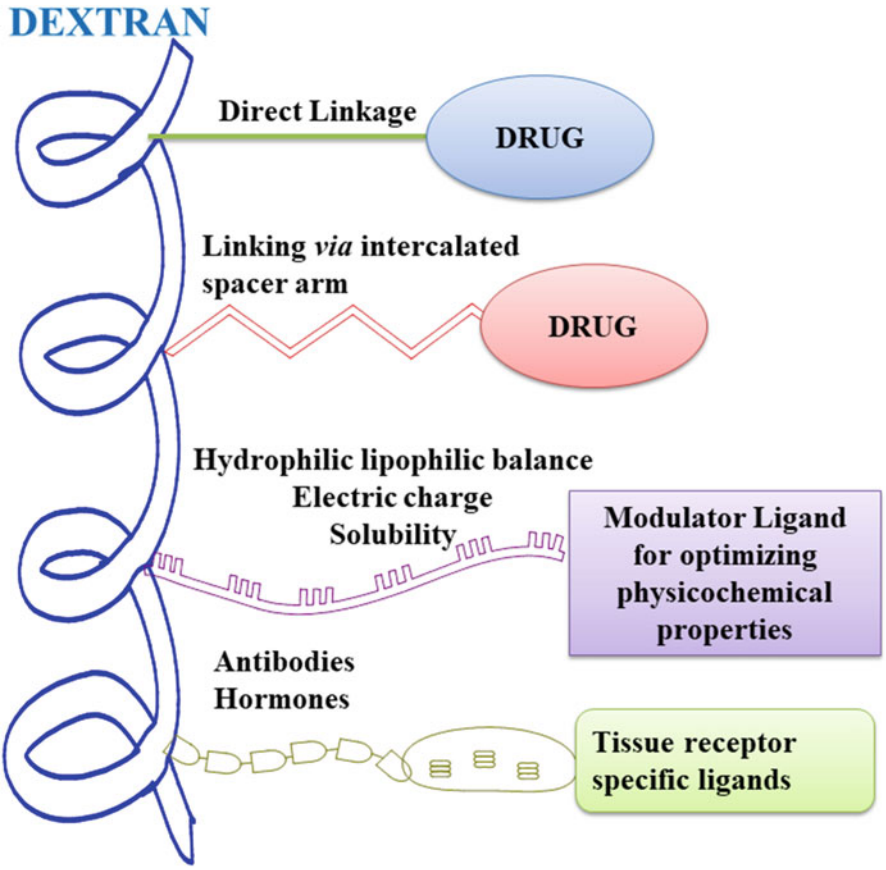


Fig. 3 Modes of covalent drug attachment to dextran

to improve ketorolac aqueous solubility and reduce gastrointestinal side effects. An *N*-acylimidazole derivative of ketorolac or flurbiprofen was condensed with dextran as a carrier polymer. In vivo biological screening in mice and rats indicated that conjugates retained analgesic and anti-inflammatory activities with significantly reduced ulcerogenicity compared to the parent drug (Vyas et al. 2007; Shrivastava et al. 2003).

7.2 Dextran Prodrugs for Enhancement of Water Solubility

Many of the antiviral and anticancer agents are poorly water soluble and their passive or active targeting is not possible unless they are solubilized. In such cases, application of dextran conjugates in solubilization of these poorly water-soluble drugs is quite beneficial. Cyclosporin A, acyclovir, and paclitaxel are some examples of drugs that are solubilized by linking with dextran (Francis et al. 2003; Tu et al. 2004; Nakamura et al. 2010).

7.3 Dextran Prodrugs in Colon-Specific Delivery

On account of their low tissue toxicity and high enzymatic degradability at desired sites, dextran prodrugs have been often considered as a potential matrix system for colon-specific delivery and/or controlled release of bioactive agents. Colon targeting of drugs like budesonide, dexamethasone, and flufenamic acid was achieved by conjugating them to dextran. The colon-specific polymeric conjugates of celecoxib have also been prepared with dextran using succinic acid as linker between the drug and dextran. 5-Aminosalicylic acid (5-ASA) prodrugs of dextrans were developed with an aim of treating Crohn's disease effectively (Varshosaz et al. 2010; Zhou et al. 2003; Shrivastava and Shrivastava 2010).

7.4 Liver-Specific Drugs Using Dextran as a Carrier

It has been reported that dextrans may serve to be of great importance in hepatic tissue targeting. Dextran conjugate of glutathione shows selective transport to hepatic cells and is intracellularly hydrolyzed to free form and protects mice from hepatotoxicity of acetaminophen. The plasma and tissue disposition of two novel dextran prodrugs of methylprednisolone (MP) containing one or five amino acids as linkers were studied in rats. The study concluded that conjugates with one amino acid may be more suitable than five for targeting immunosuppression to the liver and spleen (Mehvar et al. 1994; Penugonda et al. 2010).

7.5 Anticancer Dextran-Drug Conjugates

Conjugation of natural and synthetic polymers, proteins, and polysaccharides that have been covalently coupled with cytotoxic and other types of drugs is one of the progressive approaches. Daunorubicin and DOX were covalently coupled to dextrans and were found to reduce the toxicity in mice. Various other anticancer agents such as methotrexate and mitomycin C and 5-fluorouracil or cisplatin have been covalently coupled to dextrans. Conjugation of adriamycin to dextran has been done by peptide spacer of Gly-Leu-Gly and galactosamine also has been anchored to it as a targeting moiety. This conjugate generated the best therapeutic effect (Bisht and Maitra 2009; Mitra et al. 2001).

8 Drug Delivery Systems of Dextran Conjugates

8.1 Lymphocytokinin-Dextran Conjugate

The most recent studies on chemotherapeutic drugs for lymphatic metastasis are polymer-targeted antitumor drugs. Dextran and an antitumor small molecule drug mitomycin C were coupled through a spacer to form a dextran-targeted antitumor drug. Such conjugates are taken up by the lymphocyte's specific uptake of a

conjugate of a certain molecular size, which in turn aggregates the conjugate in the target tissue region (Yan and Zhong 1999).

8.2 Dextran-Magnetic Layered Composite Hydroxide Fluorouracil Targeting Lipid

Fluorouracil (FU) was inserted into the magnetic layered composite hydroxide (MLDH), a new type of dextran MLDH-FU with slow release and magnetic targeting was formed under the protection of dextran. Due to this strategy the cancer cells were directly killed while increasing the chance of tumor tissue necrosis due to ischemia (Shi et al. 2007).

8.3 Dextran Hydrogels

Hydrogels form a specific class of polymeric biomaterials. Hydrogels are defined as two- or multicomponent systems consisting of a three-dimensional network of polymer chains and water that occupies the space between macromolecules. Hydrogels hold a significant place in the fields like biomedical, biotechnological, and pharmaceutical, especially in controlled release of drugs, and even in the field of environment. Dextran is a suitable polymer employed for the development of hydrogels. Dextran hydrogels are prepared by several diverse approaches, different chemical compositions, and cross-linking agents: glycidyl acrylate, 1,6-hexanediisocyanate in DMSO, and epichlorohydrin. Kim et al. have described the preparation of acrylated and methacrylated derivatives of dextran (Kim et al. 1999). Allyl isocyanate dextran and lactide diacrylate were used for preparation of biodegradable dextran–polylactide hydrogel networks. This hydrogel was used in controlled release of indomethacin (Zhang and Chu 2002). In gel filtration columns, cross-linked dextran finds application as a packing material. Enzymatic cross-linking of dextran-tyramine (Dex-TA) conjugates in presence of horseradish peroxidase and hydrogen peroxide was successively applied in the preparation of hydrogels used for encapsulation of chondrocytes. The Dex-TA hydrogels are promising 3D scaffolds for cartilage tissue engineering applications (Jin et al. 2010). These dextran-based hydrogels are promising for use as injectable systems for applications in biomedical field including protein delivery and tissue engineering. Dextran hydrogels containing NSAIDs as pendant agents through ultraviolet irradiation of solutions of dextran functionalized with methacrylic groups in the presence of the drug derivatized in the same way have also been prepared.

8.4 Bioadhesive Oral Delivery Systems of Dextran

The bioadhesive properties of poly(methyl vinyl etherco- maleic anhydride) nanoparticles (NP) associated with two hydroxyl-functionalized dextrans and one amino derivative of dextran have been demonstrated by Porfire et al. (Porfire et al. 2010). The in vivo bioadhesion study has demonstrated significantly higher adhesive

interactions with the gastrointestinal tract of rats for all types of dextran-associated NP compared with control NP. The results encourage further use of these systems for oral delivery of drugs.

8.5 Conjugates of Dextran in Production of Micelles

Polymeric micelles using amphiphilic macromolecules are encouraging vehicles for antitumor targeting. A block copolymer composed of dextran and poly(DL-lactide-co-glycolide) (PLGA) was prepared by Jeong et al. (2011a) for antitumor drug delivery of doxorubicin (DOX). In an antiproliferation study, the polymeric micelles showed higher cytotoxicity to doxorubicin-resistant HuCC-T1 cells than free doxorubicin, indicating that the polymeric micelles were effectively engulfed by tumor cells, while free doxorubicin hardly penetrated the tumor cell membrane. Since aggregation states of amphotericin B (AmpB) are related to intrinsic cytotoxicity, prevention of AmpB aggregation in aqueous solution will provide low cytotoxicity and increased antimicrobial activity for the infectious disease. For this reason, AmpB was encapsulated in polymeric micelle of PLGA-grafted dextran copolymer (Bang et al. 2008). AmpB-incorporated polymeric micelle prepared from methanol/water mixture showed low cytotoxicity and favorable antimicrobial activity. Another type of micelle obtained from conjugation of dextran is stearate-g-dextran (Dex-SA). Dex-SA could self-assemble to form nanoscaled micelles in aqueous medium. Tumor cellular uptake test indicated that Dex-SA micelles had excellent internalization ability, which could deliver DOX into tumor cells and could prolong in vitro drug release to 48 h. In vivo antitumor activity results showed that Dex-SA/DOX micelles treatments effectively suppressed the tumor growth and reduced the toxicity against animal body compared with commercial DOX injection (Du et al. 2010). Enhancement of drug loading and controlled drug release may be achieved by production of interpenetrating micellar networks. Stimuli responsive micelles are another type of copolymers of dextran which can control drug release by the environmental stimuli. One of these systems is reduction-responsive biodegradable micelles that were developed from disulfidelinked dextran-b-poly(“-caprolactone) diblock copolymer (Dex-SS-PCL) and applied for triggering release of DOX in vitro and inside cells (Sun et al. 2010). DOX could be efficiently loaded into the micelles with a drug loading efficiency of about 70%. Notably, the in vitro release studies revealed that Dex-SS-PCL micelles released DOX quantitatively in 10 h.

A series of amphiphilic copolymers of dextran-graft-methoxypolyethylene glycol/poly(caprolactone) (Dex-g-mPEG/PCL) with great potential as drug carriers in biomedical fields were also synthesized by grafting both PCL and mPEG chains to dextran (Qiu et al. 2009). The prepared copolymers self-assembled into nanosized spherical micelles in aqueous solution and the critical micellar concentration (CMC) of the graft copolymers could be adjusted by controlling the degree of substitution of Mpeg and PCL. Deoxycholic acid (DA)-conjugated dextran (DexDA) micelles are another type of dextran conjugates which were synthesized for DOX delivery to

DOX-resistant CT26 colon carcinoma cells (Jeong et al. 2011b). When screened by in vitro cytotoxicity test, higher antitumor activity was obtained with DOX-incorporated nanoparticles compared to free DOX.

Targeted micelles of dextran are also prepared for active drug delivery to malignant cells. These micelles are promising in reduction of resistance to cytotoxic drugs. A novel folate (FA)-targeted amphiphilic derivative of *all-trans* retinoic acid dextran (ATRA-grafted Dex) was synthesized successfully using carbonyldiimidazole and dimethylaminopyridine for tumor-targeted drug delivery (Varshosaz et al. 2011). Zeta potential of these nanomicelles was −15.9 mV, indicating good stability of the micelles. The cytotoxicity of micelles was evaluated by MTT assay on KG-1 cell line in different concentrations of DOX. The folate-targeted micelles of DOX were cytotoxic on KG-1 cell line (acute leukemia) in half concentration of DOX and seem promising in reducing the dose of this drug in acute leukemia.

8.6 Functionalized Dextran Aldehyde-Drug Conjugate

In a study reported by Oliver et al. dextran aldehyde was successfully functionalized with quercetin, creating a water-soluble conjugate with enhanced stability and good antioxidant properties. The conjugate displayed significant anticancer activity in neuroblastoma cells (Oliver et al. 2018).

9 Common Methods for Synthesis of Dextran Conjugates

9.1 Direct Esterification

Many ester prodrugs of dextran have been developed by its conjugation with acidic drugs to prolong drug release like most of the NSAIDs. This type of linkage is formed by conjugation of hydroxyl groups of dextran in the presence of *N*, *N*-dicyclohexylcarbodiimide with carboxylic acid drugs. Another type of dextran ester is dextran ester-olefin compound copolymer. Multifunctional dextran esters with varying degrees of substitution by furoyl-, pyroglutamyl-, propyl-, and acetyl moieties are able to self-assemble into regular nanospheres.

9.2 Carbonyldiimidazole Activation Method

Activation of dextran hydroxy groups may be done with carbonyldiimidazole, and then amino groups are introduced by attaching ethylenediamine, reacting amino groups with a succinimidyl-activated derivative of other hydroxyl-containing substances like PEG (Lukyanov et al. 2004). Another example of use of this type of activation is in conjugation of cromoglycic acid with dextran.

9.3 Carbonate or Carbamate Ester Method

Drugs containing a hydroxyl or amine group can be coupled to dextran in the form of carbonate or carbamate ester linkage, respectively. First, hydroxyl groups of dextran are activated by phosgene, and then the alcoholic or amine drug is added to the activated dextran.

9.4 Periodate Oxidation Method

Enzymes and proteins are attached to dextrans and other polysaccharides by periodate oxidation of dextran which produces dialdehyde dextran. Then Schiff bases are produced from its condensation with amino compounds. The conjugate is then stabilized subsequently by reduction with sodium borohydride.

9.5 Cyanogens Bromide Activation Method

Amine-containing drugs and proteins may be attached to dextran or other polysaccharides by the cyanogens bromide activation of dextran.

9.6 Etherification of Dextran

Ethers of dextran are made by irreversible nucleophilic substitution using aliphatic or aromatic halides, sulfates or epoxides, whereas reversible etherification is achieved via Michael addition of α, β-unsaturated reagents such as acrylonitrile, acrylamide, and methyl vinyl sulfone.

10 Applications of Dextran and Its Derivatives

Dextran, composed of 1,6-linked D-glucopyranose, is a hydrophilic polysaccharide. Dextran is achieved from sucrose and maltodextrins with dextransucrase and dextrinase, respectively. Dextran is degraded using dextranase which is available in mammalian tissue. Dextran is a biocompatible and nontoxic polysaccharide that is extensively employed for its pharmaceutical and biomedical applications. Dextran acts as a scavenger of reactive oxygen species and surplus platelet activation reducer and has been widely used to reduce inflammatory responsive vascular thrombosis and hinder ischemia–reperfusion damage in organ transplantation. It is reported that dextran can be utilized as blood supplement in emergency cases. Moreover, dextran coating can be utilized for improving the substrates biocompatibility. Dextrans are utilized as heparin substitutions for anticoagulant treatment as well as for manufacturing blood plasma extenders, and sephadex gel bead–based separation of proteins.

10.1 Medical Uses

10.1.1 Antithrombotic Effect

Dextran has an ability to decline vascular thrombosis. Dextran exerts its antithrombotic effects by binding to thrombocytes, erythrocytes, and vascular endothelium, amplifying their electronegativity and thereby declining the platelet adhesiveness and erythrocytes aggregation. Dextran also suppresses platelet functions by reducing the VIII-Ag Von Willebrand factor and causing lysis of the formed clots. The thrombolytic activity is exhibited by inhibiting alpha 2-antiplasmin and acting as a plasminogen activator. Larger dextrans, which are unable to penetrate or cross the blood vessels, act as potent osmotic agents and can be utilized to treat hypovolemia. As dextran causes hemodilution and volume expansion, it leads to improvement in blood flow, patency of microanastomoses, and reduction of thrombosis.

10.1.2 Usage in Intravenous Fluids

Dextran aids in solubilizing certain factors in intravenous fluids and also acts as a lubricant in various eye drops. In intravenous solution, dextran offers an osmotically neutral fluid that is degraded intracellularly into glucose and free water. In certain emergency situations, where blood replacement is not available, dextran can be utilized to replace lost blood but as it may result into hyponatremia or other electrolyte disturbances, it must be used cautiously.

10.1.3 DNA Transfection

Diethylaminoethyl (DEAE)-dextran-based DNA transfection technique has been developed to introduce DNAs into cultured mammalian cells. Despite its toxicity on viability of cells, this nonviral vector transferring system is highly preferred. Human growth hormone (hGH) gene expression plasmid has been successfully introduced to a guinea pig mammary gland by the DEAE-dextran method. The procedure enabled delivery of plasmid DNA into vascular smooth muscle cells that were derived from the rat thoracic aorta. The results suggested that DEAE-dextran method could be applied to ex vivo gene transfer therapy. Methyl methacylate (MMA) modification has been introduced to DEAE-dextran to generate DEAE-dextran-MMA and its utility in nonviral gene transfer was examined. In addition, 5-azacytidine enhances effect of the DEAE-dextran to deliver plasmid DNA into macrophages. These lines of evidences suggest that DEAE-dextran or its derivatives may contribute to establishing an advantageous method to transfer nonviral vectors into cells. At present, the multiple throughput DEAE-dextran based transfection method would be possibly appropriate for estimating the transcriptional ability in the cells isolated from animals, including human iPS cells (Gulick 2003).

10.1.4 Iron Dextran

Dextran is a vital preparatory material for the synthesis of iron dextran. The iron dextran solution for injection is commonly utilized for treating veterinary as well as human anemic deficiency. In one of the studies, 77 iron-deficient anemic women in the third trimester of pregnancy were treated with an intramuscular iron dextran

preparation. Of the 44 who received the entire course of treatment and were observed for four or more week before delivery, 40 demonstrated an adequate response. One serious near-fatal reaction occurred, and four other patients developed systemic symptoms within a brief time after injection of the first or a subsequent dose. The study suggested that parenteral iron as an iron dextran complex is an effective agent in the treatment of anemia resulting from iron deficiency in the last trimester of pregnancy, and that it is especially useful in a population whose members cannot be relied on to take oral preparations. A careful evaluation of any systemic symptom following injection is necessary to prevent serious, potentially fatal reaction.

10.1.5 Hysteroscopy

Dextran 70 has been utilized as a distending medium for hysteroscopy since 1970. The major advantages of Dextran 70 are its excellent optical qualities and its high viscosity, which prevents it from mixing with blood and makes it ideal during procedures in which bleeding is common. Resection of space-occupying lesions such as submucous myomas have been described using Hyskon, a nonpyrogenic solution of dextran 70 (32% W/V) in dextrose (10% W/V). Intrauterine pressures of 10–160 mmHg and manual injection pressures of 0.5 and 3.9 atmos during routine manual injection of Hyskon were measured. The high pressure may result in continuous injection of the distending medium from the uterine cavity into the circulation. High serum levels of Hyskon in excess of 1000 mg.d1^{-1} have been demonstrated 30 minutes after intrauterine infusion of at least 300 ml of Hyskon. This corresponds to an absorbed volume of up to 211 ml of Hyskon. The extent of tissue trauma, the injection pressure, the duration of infusion as well as the seal of the hysteroscope around the cervix determines the amount of Hyskon absorbed. Fluid overload and not the injury to pulmonary capillary endothelium is attributed to the mechanism of pulmonary oedema after absorption of Hyskon. The hemodilution and blood volume expansion results in the hematological effects. However, certain case studies reported that a syndrome similar to disseminated intravascular coagulation can be associated with dextran 70. Both anaphylactoid and anaphylactic allergic responses are also reported. It is suggested that hysteroscopy with Hyskon should be restricted to 45 rain, and that all probable measures should be employed in order to minimize the tissue bleeding and trauma. As coagulopathy and pulmonary oedema have been reported even with lesser amount, the Hyskon volume should be restricted to not more than 500 ml. Continuous monitoring of the cumulative volume of Hyskon as well as any signs of impending pulmonary oedema in the patient should be done frequently.

10.1.6 Antimicrobial Activity

Dextran-binding receptors in humans include the dendritic cell–specific intercellular adhesion molecule 3-grabbing nonintegrin (DC-SIGN) family receptors: DC-SIGN (CD209) and L-SIGN (the liver and lymphatic endothelium homologue of DC-SIGN), the mannose receptor (CD206), and langerin. These receptors play a vital role in the uptake of pathogens by macrophages and dendritic cells and may also participate in the modulation of immune responses, mostly shown to be

beneficial for pathogens per se rather than host(s). It is logical to predict that owing to receptor-specific interactions, dextran or its derivatives can interfere with these immune responses and improve infection outcome. In a study, dextran was considered as a promising molecule for the development of lectin–glycan interaction-blocking molecules (such as DC-SIGN inhibitors) that could be applied in the treatment of diseases including tuberculosis, influenza, hepatitis B and C, human immunodeficiency virus infection, and AIDS. Dextran derivatives certainly transform the pathology of infections dependent on DC-SIGN and mannose receptors. Complete knowledge of specific dextran–lectin interactions may also be important for development of future dextran applications in biological research and medicine.

In another research study, cationic amphiphilic derivatives of dextran were tested for their antimicrobial activities against various strains of fungi and bacteria. These derivatives had a long alkyl group that was fixed to the reductive end of the main dextran and quaternary ammonium groups were also attached as pendent groups to the polysaccharide backbone. The derivatives exhibited significant antimicrobial activity against various reference strains of fungi, gram-positive and gram-negative bacteria, as well as against other clinical pathogens. The best antifungal activity was exhibited against *Candida parapsilosis*, whereas the finest antibacterial activity was observed against *Staphylococcus aureus*. The antibacterial activity against *S. aureus* was expressively improvised by reducing dextran number-average molar masses (Mn) from 8000 to 4500, due to increase of hydrophobic group relative content. The results demonstrated that these cationic polymers have potential for application as broad-spectrum external biocides (Tuchilus et al. 2017).

10.1.7 Anticoagulant Activity

Chemically synthesized sulphuric esters of polysaccharides exhibit anticoagulant action. The anticoagulant activity is reported to be dependent upon the minimum sulphate groups per glucose units but are not dependent upon the molecular weight. The small molecular weight dextran sulphates, like heparin, had no effect on platelets in vivo or in vitro, nor, when administered to experimental animals did they give rise to deposits in reticuloendothelial cells. With dosages up to 500 mg/kg body weight, no toxic effects were observed in the mouse, rat, rabbit, dog, or monkey. With certain batches, transient reactions were observed in the guinea pigs but these did not prove fatal. Careful histological examination of the liver, spleen, kidneys, central nervous system, skeletal and heart muscle, skin, bone marrow, and lymph nodes showed no parenchymal damage though this was looked for after single injections, repeated injections, and over periods from 1 hour to 3 months after the last injection. Intravenous administration produced no effect on the blood pressure, pulse rate, or respiratory rate. At anticoagulant concentrations, a negligible effect was produced on the erythrocyte sedimentation rate, and since the material has no osmotic effect on the red cells, it was observed that the ordinary hematological estimations were not interfered with. It seems probable that dextran sulphate owes its anticoagulant properties to the strong electronegative charge upon its acidic groups, since, like heparin, its action is opposed by strongly basic substances such as protamine. For its action, dextran sulphate, like heparin, requires the presence of a

co-factor in serum or plasma. In the presence of this co-factor its action is that of an antithrombin.

Clinically dextran is accessible in form of dextran 1, 40, 60, or 70. Injection solutions of dextran 40, 60, and 70 are utilized in clinical practice for replacing blood loss, plasma substitution, volume expansion, thrombosis prophylaxis, as well as rheological improvement. Before injecting dextran 40, 60, or 70, dextran 1 is administered in order to reduce the adverse reactions significantly. These dextrans are considered as the safest substitute for plasma in clinical use. They can also be utilized for various reasons like solutions for storing organs for transplantation, cryopreservation, as well as carriers in vaccines (Drozd et al. 2017).

10.1.8 Volume Expansion

Dextran is both osmotically active and too large to pass through the uninjured vessel. These characteristics, combined with its high oncotic properties, make this agent an ideal plasma expander, and is utilized in hypovolemic shock. However, the data regarding the use of 6% dextran 70 in combination with 7.5% NaCl (HSD) are conflicting. HSD is a hypertonic-hyperosmotic solution that is utilized as a plasma expander for treating hemorrhagic hypotension. In experimental animal models of controlled hemorrhage there is enough evidence to prove the superiority of HSD compared to crystalloids in equal volumes. However, its benefits in human studies have not been clearly demonstrated. Recently, a randomized, double-blinded study represented improved survival in patients with penetrating trauma to the torso who received initial fluid resuscitation with HSD as compared to normal saline. In contrast to controlled hemorrhage, in animal models of uncontrolled hemorrhage, HSD has been shown to increase and/or accelerate mortality. Its role in uncontrolled hemorrhage remains controversial.

10.1.9 Antioxidant Properties and Immunomodulatory Potential

Dextrans D10, D40, and D147 differ only in their molecular weights. They are free of protein and phenolic contaminants and are basically formed by glucose in the alpha configuration. The identical dextran entities – but having dissimilar molecular weights – exhibited diverse immunomodulatory and antioxidant activities. In one of the scientific studies, it was reported that the activity of dextran increases as per the increase in the molecular weight. However, in another test, inverse relation or no correlation has been observed between the dextran activity and its molecular weight. It was stated that dextrans exhibit antioxidant activity by specifically terminating the formation of reactive species. D40 dextrans exhibited potent antioxidant activity in comparison to the other two dextrans. D40 dextran exhibited superior hydrogen peroxide scavenging capacity and inhibited the lipid peroxidation. Also, it was the only dextran to display immunomodulatory activity. Dextran with molecular weight around 40 kDa has been reported to present remarkable antioxidant and immunomodulatory activity. However, further confirmation of this hypothesis is required. Thus, future research can be focused on the D40 dextran and other dextrans having similar molecular weights for their probable antioxidant and immunomodulatory activities.

Owing to its worst solubility in water and stability of quercetin, a conjugation of quercetin to dextran-aldehyde via a condensation reaction was performed to create a water-soluble conjugate with enhanced stability compared with native quercetin. The prepared conjugate was shown to have improved substantial free radical scavenging activity. Anticancer activity was also evaluated in vitro in a neuroblastoma cell line. The dextran–aldehyde–quercetin conjugate demonstrated to be cytotoxic to neuroblastoma cells (Soeiro et al. 2016).

10.1.10 Acute Dengue Infection

The surface glycocalyx layer, a fiber-matrix of proteoglycans, glycosaminoglycans, and plasma proteins, forms a complex with the underlying endothelial cells to regulate plasma volume within circumscribed limits. It is likely that during dengue infections loss of plasma proteins from this layer alters the permeability characteristics of the complex. The implications for resuscitation of patients with dengue shock syndrome (DSS) are potentially important. It is possible that continuous low-dose infusions of dextran may help to stabilize the permeability barrier in patients with profound or refractory shock, reducing the need for repeated boluses, and limiting the total colloid volume required (Nguyen-Pouplin et al. 2011).

10.1.11 Dextran-Based Hydrogels as Drug Delivery Systems

Dextran-based hydrogels contain moieties that may be able to interact with biomolecules. For example, many protein growth factors have domains that are known to interact with extracellular matrix elements, such as heparin. Heparin is a sulphated polysaccharide with a negative charge, and can react strongly with several growth factors, which have a heparin-binding domain such as vascular endothelial growth factor (VEGF) or the fibroblast growth factor (FGF) family. Therefore, functionalizing dextran with carboxylate, benzylamide, and sulfate groups yield a hydrogel-forming macromonomer, with a substantial increase in the negative charge, promoting higher retention rates of transforming growth factor beta (TGFß1). In addition, the aldehyde moieties generated after dextran oxidation are used to immobilize many drugs to a solitary polymer chain. The same principle is used to immobilize an antifungal agent such as amphotericin B.

Dextran hydrogels are also utilized in the organ-specific drug delivery. Hydrogels are macromolecular cross-linked biopolymers greatly employed in targeted drug delivery or cell and molecule transport. Neutral pH of dextran and its high water solubility and simple repetitive glucose units make it suitable for cross-linking. Most widely used dextran cross-linked biopolymers are 1,6-hexamethylenediisocyanate in colon-specific drug delivery; βcyclodextrin, a carrier for hydrophobic drugs; and dextran-allyl isocyanate-ethylamine (Dex-AE)-polyethylene glycol diacrylate in skin-specific angiogenic response and burn wound healing. These cross-linkers form hydrogel with dextran easily in presence of UV light and hydrogels are potent carriers for organ-specific hydrophobic drug delivery.

In another study, dextran hydrogel was prepared by cross-linking dextran and *N*, *N*-methylenebisacrylamide in aqueous sodium hydroxide solution. Highly stable and uniformly distributed silver nanoparticles were also prepared with these hydrogels as

a carrier via in situ reduction of silver nitrate without using any reducing agent. The spherical silver nanoparticle was 20–30 nm in diameter and exhibited potential antimicrobial activity against *Bacillus cereus*. Thus, dextran hydrogel can be efficiently used as an antibacterial compound.

Dextran hydrogels also serve as instructive frameworks to promote neovascularization and dermal renewal with complete skin appendages in third-degree burn wounds. A mature epithelial layer along with hair follicles and sebaceous glands was observed within 21 days in burn wounds that were treated with the hydrogel. The hydrogel also promoted growth of new hair as well as epidermal anatomy after 5 weeks of treatment that was similar to normal skin.

10.1.12 Tissue Engineering

Dextran-based hydrogels have been recently used as instructive scaffolds to promote neovascularization and skin regeneration in third-degree burn wounds. The hydrogel was based in the copolymerization of dextran-allyl isocyanate–ethylamine with polyethylene glycol diacrylate in the ratio of 80/20. When the hydrogel was placed in a skin wound, it induced more blood flow to the burn wound area than did the control hydrogel and the wound covered with only dressing. Dextran-based hydrogels seemed to accelerate the recruitment of endothelial cells to the wound area, enabling rapid neovascularization after a week of treatment. The wound-treated hydrogel resulted in skin regeneration with appendages (hair follicles and sebaceous glands).

Dextran-based hydrogels are also very useful to reduce intra-abdominal adhesions. Hydrogels formed by succinyl chitosan and dextran aldehyde significantly reduced the formation of intra-abdominal adhesions without adversely affecting wound healing. In addition, hydrogels formed by mixing hydrazide-modified carboxymethyldextran (CMDX-ADH) with aldehyde-modified dextran (DX-CHO) or carboxymethylcellulose (CMCCHO) were very effective in the prevention of abdominal adhesion (Huang and Huang 2019).

10.2 In Food Industry

Since 1950s, dextran has been extensively studied as a food constituent. Dextran is generally employed to increase viscosity in various food products, as well as emulsifying, texturing, and gelling agent. To produce dextran for its food applications, microorganisms like *Lactobacillus sanfrancisco, Leuconostoc mesenteroides, Lactobacillus plantarum,* and *Saccharomyces cerevisiae* are generally utilized. The various applications of dextran include:

10.2.1 Bakery Products

Dextran improves freshness, loaf volume, mouthfeel, softness, and shelf life in bakery products.

10.2.2 Confectionery

Dextrans augment the retention of moisture as well as help in maintaining viscosity. It is used as stabilizer and as gelling agent in candies and gum. It also inhibits sugar crystallization. Its use is also proposed in flavor extract, soft drinks, icing compositions, and milk beverages.

10.2.3 Ice Cream

As edible substances dextrans are bland, odorless, tasteless, and nontoxic, and have several advantages. Tests performed on ice cream mixes containing 2–4% dextran indicated that it conferred beneficial properties on viscosity. Dextrans act as stabilizers for frozen dairy products. The viscosity of dextran solutions (MW $<$500,000) has been found to display Newtonian behavior, i.e., the viscosity is independent of shear rate. The viscosity of dextran solutions (0–2%) is unaffected by co-solvents, salts, or changes in the pH. Dextran solutions do not form gels which is typical of many other bacterial polysaccharides.

10.2.4 Frozen and Dried Foods

The promising property of dextran is to stabilize frozen foods. Dextran film over the surface of food like meat, fish products, cheese, and vegetables can help to preserve their flavor and texture as well as help in protecting them from oxidation and other chemical changes. There is an increasing demand for the use of dextran as texture, flavor, smell enhancer as well as food preservative in frozen or dried dishes.

10.2.5 Fermented Dairy Products

Dextran maintains the texture of yogurt and yogurt-like products produced from milk. Dextran because of its water binding ability maintains the rheological properties of acidified milk gels as well as improve the creaminess, viscosity, and reduces syneresis. It is also used to replace the commercial texturizers like pectin, carrageenan, xanthan, β-glucan, and guar gum.

10.2.6 Reduced-Fat Cheese

A firm and rubbery texture of the reduced cheese is due to the high content of casein present in it. Dextran is used for producing the reduced-fat cheese by enhancing the water binding ability and the moisture content in the nonfat mass.

10.2.7 Prebiotics

Recently, prebiotics are much considered as functional foods for modulating the colonic microbiota composition that help to offer several health benefits. Prebiotic are also known to protect the host against several diseases like inflammatory bowel disease, colon cancer, obesity, coronary heart disease, osteoporosis, as well as diabetes. The (1-6) linkages present in dextran are resistant to hydrolysis by various intestinal enzymes that eventually result in its slow digestion. Furthermore, α-(1-2) linkages are also unaffected by digestive enzymes' attack. In an in vitro study of the fermentation process in the human colon, dextran has shown to augment the *Bifidobacterium* species fraction, demonstrating potent prebiotic activity [26].

Dextran with its low molecular weight and α-(1-2)-branched linkages has shown to induce the growth of several beneficial bacteria including *Bifidobacterium* and *Lactobacillus* species, exerting promising prebiotic potential.

10.3 In Photographic Industry

Highly purified dextran fractions are extensively used in the photographic industry, where the dextran biopolymers have been shown to improve the quality of silver emulsion of photographs.

10.4 In the Field of Cosmetics

Dextrans also have several valuable applications in field of cosmetics. Cationic dextran (CDC) acts as a moisturizer by making complex salts with either anionic or amphoteric surfactants that can modestly adsorb on the skin as well as on hair to form moisturizing films. CDC also acts as a promising skin and hair conditioning agent. Following effects of dextran sulfate enable its more favorable usage in cosmetic industries:

- Antiaging
- Antiwrinkle effects
- Smooth, fresh, and nonsticky feeling
- Good moisture retention
- Increased lipase activity giving weight-reducing effects and supple skin
- Anti-inflammatory and antiallergic
- Treating rough, chapped skin

Dextran sulfate has also shown to suppress the extravasation of lymphoblast in inflamed skin area, thereby exerting its anti-inflammatory effect. Dextran also helps in osmotically retaining the water in tissue that can contribute to the well-being and mechanical properties of the tissue concerned (Chen et al. 2020).

10.5 Waste Water Management

Native dextran offers application in waste water management. Dextran binds with metal ions at alkaline pH and is biodegradable. The usage is economical. It is used extensively in the waste water treatment during the flocculation process.

10.6 Laboratory Uses

Dextran can be utilized in size-exclusion chromatography matrices, e.g., sephadex. It can be employed to produce microcarriers for cell culture. Fluorescent-labeled

dextran favorably binds to endosomes and these endosomes can be easily visualized under the fluorescent microscope. Dextran is also used in immobilization in biosensors. It may also be employed as a stabilizing coat for protecting the metal nanoparticles from oxidation as well as improving biocompatibility. Dextran attached with a fluorescent molecule can help to create concentration gradients of diffusible molecules for imaging and allows subsequent characterization of gradient slope.

11 Toxic Effects of Dextran Sulphate

Dextran sulphate has pharmacological actions similar to that of heparin, but dextran exhibits much prolonged and cumulative anticoagulant effect. A clinical study reported that certain toxic effects have been observed following the treatment with dextran to 15 patients for 3 months at a total dose of 90,000 or more units. The commonest side effect observed was transient alopecia, changes in nails, diarrhoea, and reduction in platelets.

11.1 Alopecia

Fifteen patients received 90,000 or more units of dextran sulphate, and eight out of these developed severe alopecia, amounting to an almost complete loss of scalp hair in six; in addition, loss of pubic, axillary, and facial hair occurred in three patients. Three of those who suffered severe loss of scalp hair were women, one of whom had complete loss of axillary and pubic hair and partial loss of eyebrows. Two male patients reported that they had to shave much less often than normally for a few weeks. The loss of hair was diffuse, affecting the whole scalp, and was unassociated with any apparent change in the scalp itself; it was first noticed 3–8 weeks after stopping treatment. Regrowth of hair was obvious after a further 4–12 weeks, and in all cases the hair eventually returned to normal. In one other patient a small occipital bald patch was noticed for the first time 10 days after starting treatment with dextran sulphate. This area of baldness remained unchanged during the following year. In this patient the type of alopecia was different, and it appeared much earlier in the course of treatment than in the other cases, so it is doubtful whether it was related to the dextran sulphate therapy.

11.2 Changes in Nails

In three patients who developed severe alopecia, marked changes in the fingernails occurred 2–3 months after dextran sulphate therapy, the nails becoming brittle and transversely ridged. In two of these patients all fingernails split transversely, the distal parts of the nails being shed. In a fourth patient who did not develop any alopecia, transverse ridging of all fingernails was noticed 6 weeks after stopping treatment. In all cases the nails eventually regrew normally.

11.3 Diarrhoea

Severe diarrhoea with the passage of blood in the stools occurred in two patients after treatment with dextran sulphate for 14 days. The total doses were 140,000 and 185,000 units; in fact, these two patients received a larger average daily dose than any other patients in this series. Sigmoidoscopic examination showed that the mucosa was diffusely congested, but neither ulceration nor any other local lesion was seen. Diarrhoea persisted for 7 days after stopping dextran sulphate. Two other patients had mild diarrhoea, without blood in the stools, after receiving total doses of 90,000 and 130,000 units of dextran sulphate. There was no apparent correlation between the occurrence of diarrhoea and of alopecia. Of the eight patients with severe loss of hair, only two had diarrhoea, which was mild in both cases.

11.4 Reduction in Platelets

Repeated platelet counts were determined in four patients during treatment with dextran sulphate. A substantial fall in the platelet count occurred in two of these patients, both of whom subsequently developed a severe degree of alopecia. In the other two, of whom one suffered alopecia and the other developed changes in the nails without alopecia, no significant change occurred in the platelet count during treatment with dextran sulphate. Single platelet counts were performed 3–15 days after starting treatment in four other patients, two of whom subsequently developed alopecia. These platelet counts were all in the range of 200,000–350,000 so that thrombocytopenia was not detected during therapy.

11.5 Hydrocephalus

Dextrans have been used extensively as medical therapies and labeling agents in biomedical research to investigate the blood-brain barrier and CSF flow and absorption. Adverse effects from dextrans include anaphylactic reaction and dilation of the cerebral ventricles due to administration into the subarachnoid space. One retrospective study described that 51 rhesus macaques (*Macaca mulatta*) received dextran intrathecally. The purpose of intrathecal administration was to enable detection of long-lived, dextran-labeled macrophages and to study monocyte–macrophage turnover in the CNS of SIV- or SHIV-infected and uninfected animals by using immunofluorescence. Of the 51 dextran-treated macaques, 8 that received dextran diluted in saline-developed hydrocephalus; 6 of these 8 animals exhibited neurologic signs. In contrast, none of the macaques that received intrathecal dextran diluted in PBS developed hydrocephalus. These data suggest the use of saline diluent and the duration of dextran exposure as potential factors contributing to hydrocephalus after intrathecal dextran in rhesus macaques (Dufour et al. 2018).

12 Conclusion

Dextrans are polymers belonging to the class of carbohydrates having noteworthy multiplicity in chain length as well as physicochemical properties which result due to difference in degree of branching in the glucose backbone. They are extensively employed for their pharmaceutical applications, chiefly as macromolecular carriers, and drug conjugate or drug delivery systems. These polysaccharides improve the stability of therapeutics administered via oral/parenteral route. The versatility of the dextran or its conjugates can overcome many practical constraints in delivering chemotherapeutics, genes, and other drugs which arise due to the surface nature including biocompatibility, degradability, and size/dimension, are all adjustable. Moreover, the simple and inexpensive nature of this polymer can also satisfy strict demands from an economic point of view. Dextran is a commercially accessible branched polysaccharide that possesses several health benefits. Dextran is also known for its emulsifying, viscosifying, and stabilizing attributes in food applications. Prebiotic oligosaccharide production by hydrolysis of dextran is a relatively new field, garnering industrial and research attention. In view of its multiple unique and exclusive functional characteristics, dextran will continue to find important applications in pharmaceutical and biotechnological industries.

References

Altikatoglu M, Basaran Y, Arioz C, Ogan A, Kuzu H. Glucose oxidase-dextran conjugates with enhanced stabilities against temperature and pH. Appl Biochem Biotechnol. 2010;160(8):2187–97.

Bang JY, Song CE, Kim C, et al. Cytotoxicity of amphotericin B-incorporated polymeric micelles composed of poly(DL-lactide-coglycolide)/dextran graft copolymer. Arch Pharm Res. 2008;31(11):1463–9.

Bhavani AL, Nisha J. Dextran: the polysaccharide with versatile uses. Int J Pharma Bio Sci. 2010;1(4):569–73.

Bisht S, Maitra A. Dextran-doxorubicin/chitosan nanoparticles for solid tumor therapy. Nanomed Nanobiotechnol. 2009;1(4):415–25.

Cakić M, Mitić Ž, Nikolić GS, Ilić L, Nikolić GM. The investigations of bioactive copper (II) complexes with reduced low-molar dextran. Spectroscopy. 2008;22(2–3):177–85.

Chen F, Huang G, Huang H. Preparation and application of dextran and its derivatives as carriers. Int J Biol Macromol. 2020;145:827–34. https://doi.org/10.1016/j.ijbiomac.2019.11.151.

Chung HJ, Kim HJ, Hong ST. Iron-dextran as a thermosensitizer in radiofrequency hyperthermia for cancer treatment. Appl Biol Chem. 2019;62(1):1–9.

De Belder AN, Norrman B. The substitution patterns of *O*-(2-hydroxyethyl) starch and *O*-(2-hydroxyethyl) dextran. Carbohydr Res. 1969;10(3):391–4.

Drozd NN, Logvinova YS, Torlopov MA, et al. Effect of sulfation and molecular weight on anticoagulant activity of dextran. Bull Exp Biol Med. 2017;162:462–5. https://doi.org/10.1007/s10517-017-3640-2.

Du YZ, Weng Q, Yuan H, Hu FQ. Synthesis and antitumor activity of stearate-g-dextran micelles for intracellular doxorubicin delivery. ACS Nano. 2010;4(11):6894–902.

Dufour JP, Russell-Lodrigue KE, Doyle-Meyers L, Falkenstein KP, Blair RV, Didier ES, Slisarenko N, Williams KC, Kuroda MJ. Hydrocephalus after intrathecal administration of

dextran to rhesus macaques (*Macaca mulatta*). Comp Med. 2018;68(3):227–32. https://doi.org/10.30802/AALAS-CM-17-000096.
Francis MF, Lavoie L, Winnik FM, Leroux JC. Solubilization of cyclosporine A in dextran-g-polyethyleneglycolalkyl ether polymeric micelles. Eur J Pharm Biopharm. 2003;56(3):337–46.
Gulick T. Transfection using DEAE-dextran. Curr Protoc Cell Biol. 2003;20:Unit 20.4. https://doi.org/10.1002/0471143030.cb2004s19.
Heinze T, Liebert T, Heublein B, Schubert S. Functional polymers based on dextran. 2006. https://doi.org/10.1007/12_100.
Huang S, Huang G. The dextrans as vehicles for gene and drug delivery. Future Med Chem. 2019;11(13):1659–67. https://doi.org/10.4155/fmc-2018-0586.
Jeong YI, Kim DH, Chung CW, et al. Doxorubicin-incorporated polymeric micelles composed of dextran-b-poly (DL-lactide-co-glycolide) copolymer. Int J Nanomedicine. 2011a;6:1415–27.
Jeong YI, Chung KD, Choi KC. Doxorubicin release from self-assembled nanoparticles of deoxycholic acid-conjugated dextran. Arch Pharm Res. 2011b;34(1):159–67.
Jin R, Moreira Teixeira LS, Dijkstra PJ, et al. Enzymatically crosslinked dextran-tyramine hydrogels as injectable scaffolds for cartilage tissue engineering. Tissue Eng Part A. 2010;16(8):2429–40.
Kim SH, Won CY, Chu CC. Synthesis and characterization of dextran based hydrogels prepared by photocrosslinking. Carbohydr Polym. 1999;40(3):183–90.
Klotz U, Kroemer H. Clinical pharmacokinetic considerations in the use of plasma expanders. Clin Pharmacokinet. 1987;12(2):123–35.
Kothari D, Das D, Patel S, Goyal A. Dextran and food application. Polysaccharides. Springer; 2014.
Lamichhane S, Anderson J, Remund T, Kelly P, Mani G. Dextran sulfate as a drug delivery platform for drug-coated balloons: preparation, characterization, in vitro drug elution, and smooth muscle cell response. J Biomed Mater Res B Appl Biomater. 2016;104(7):1416–30.
Lukyanov AN, Sawant RM, Hartner WC, Torchilin VP. PEGylated dextran as long-circulating pharmaceutical carrier. J Biomater Sci Polym Ed. 2004;15(5):621–30.
Marshall JJ, Rabinowitz ML. Preparation and characterization of a dextran-trypsin conjugate. J Biol Chem. 1976;251(4):1081–7.
Mehvar R, Robinson MA, Reynolds JM. Molecular weight dependent tissue accumulation of dextrans: in vivo studies in rats. J Pharm Sci. 1994;83(10):1495–9.
Mikhail JW, Mansour NS, Khayyal MT. Leishmania donovani: therapeutic and prophylactic action of antimony dextran glycoside (RL-712) in the golden hamster. Exp Parasitol. 1975;37(3):348–52.
Mitra S, Gaur U, Ghosh PC, Maitra AN. Tumour targeted delivery of encapsulated dextran-doxorubicin conjugate using chitosan nanoparticles as carrier. J Control Release. 2001;74(1–3):317–23.
Nakamura J, Nakajima N, Matsumura K, Hyon SH. Water-soluble taxol conjugates with dextran and targets tumor cells by folic acid immobilization. Anticancer Res. 2010;30(3):903–9.
Nguyen-Pouplin J, Pouplin T, Van TP, et al. Dextran fractional clearance studies in acute dengue infection. PLoS Negl Trop Dis. 2011;5(8):e1282. https://doi.org/10.1371/journal.pntd.0001282.
Oliver S, Yee E, Kavallaris M, Vittorio O, Boyer C. Water soluble antioxidant dextran-quercetin conjugate with potential anticancer properties. Macromol Biosci. 2018;18(4):1700239.
Patel S, Kasoju N, Bora U, Goyal A. Structural analysis and biomedical applications of dextran produced by a new isolate Pediococcus pentosaceus screened from biodiversity hot spot Assam. Bioresour Technol. 2010;101(17):6852–5.
Penugonda S, Agarwal HK, Parang K, Mehvar R. Plasma pharmacokinetics and tissue disposition of novel dextran-methylprednisolone conjugates with peptide linkers in rats. J Pharm Sci. 2010;99(3):1626–37.
Porfire AS, Zabaleta V, Gamazo C, et al. Influence of dextran on the bioadhesive properties of poly (anhydride) nanoparticles. Int J Pharm. 2010;390(1):37–44.
Qiu F, Feng J, Wu DQ, et al. Nanosized micelles self-assembled from amphiphilic dextran graft methoxypolyethylene glycol/poly(epsilon-caprolactone) copolymers. Eur Polym J. 2009;45(4):1024–31.

Shi SJ, Xu W, Li ZF, et al. Determination of 5-Fu in 5-Fu magnetic microspheres and mouse tissues by RP-HPLC method. Chin Pharm. 2007;18:497–9.
Shin JM, Song SH, Rao NV, Lee ES, Ko H, Park JH. A carboxymethyl dextran-based polymeric conjugate as the antigen carrier for cancer immunotherapy. Biomater Res. 2018;22(1):1–7.
Shrivastava PK, Shrivastava SK. Dextran carrier macromolecule for colon specific delivery of celecoxib. Curr Drug Deliv. 2010;7(2):144–51.
Shrivastava SK, Jain DK, Trivedi P. Dextrans-potential polymeric drug carriers for flurbiprofen. Pharmazie. 2003;58(6):389–91.
Soeiro VC, Melo KR, Alves MG, Medeiros MJ, Grilo ML, Almeida-Lima J, Pontes DL, Costa LS, Rocha HA. Dextran: influence of molecular weight in antioxidant properties and immunomodulatory potential. Int J Mol Sci. 2016;17(8):1340. https://doi.org/10.3390/ijms17081340.
Sun H, Guo B, Li X, et al. Shell-sheddable micelles based on dextran-ss-poly(epsilon-caprolactone) diblock copolymer for efficient intracellular release of doxorubicin. Biomacromolecules. 2010;11(4):848–54.
Tsuchiya HM. Dextransucrase. Bull Soc chim biol. 1960;42:1777–88.
Tu J, Zhong S, Li P. Studies on acyclovir-dextran conjugate: synthesis and pharmacokinetics. Drug Dev Ind Pharm. 2004;30(9):959–65.
Tuchilus CG, Nichifor M, Mocanu G, Stanciu MC. Antimicrobial activity of chemically modified dextran derivatives. Carbohydr Polym. 2017;161:181–6. https://doi.org/10.1016/j.carbpol.2017.01.006. Epub 2017 Jan 6.
Varshosaz J. Dextran conjugates in drug delivery. Expert Opin Drug Deliv. 2012;9(5):509–23.
Varshosaz J, Emami J, Fassihi A, et al. Effectiveness of budesonide-succinate-dextran conjugate as a novel prodrug of budesonide against acetic acid-induced colitis in rats. Int J Color Dis. 2010;25(10):1159–65.
Varshosaz J, Hassanzadeh F, Sadeghi H, et al. Synthesis and characterization of folate-targeted micellar nanocapsules of dextran/retinoic acid. In: Proceedings of 2nd international conference on nanotechnology: fundamentals and applications. Ottawa, Ontario, Canada; 2011.
Virnik AD, Khomyakov KP, Skokova IF. Dextran and its derivatives. Russ Chem Rev. 1975;44(7):588.
Vyas S, Trivedi P, Chaturvedi S. Ketorolac-dextran conjugates: synthesis, in vitro and in vivo evaluation. Acta Pharma. 2007;57(4):441–50.
Walker GJ. Dextrans. Int Rev Biochem. 1978;16:75–126.
Wang X, Wang X, Guo Z. Functionalization of platinum complexes for biomedical applications. Acc Chem Res. 2015;48(9):2622–31.
Wileman TE. Properties of asparaginase-dextran conjugates. Adv Drug Deliv Rev. 1991;6(2):167–80.
Yan ZQ, Zhong YG. Synthesis and biological properties of lymphoblastic anticancer drug mitomycin-dextran conjugate research. Chin J Med Chem. 1999;9:240–4.
Yasukawa T, Kimura H, Tabata Y, Kamizuru H, Miyamoto H, Honda Y, Ogura Y. Targeting of interferon to choroidal neovascularization by use of dextran and metal coordination. Invest Ophthalmol Vis Sci. 2002;43(3):842–8.
Zhang Y, Chu CC. Biodegradable dextran-polylactide hydrogel networks: their swelling, morphology and the controlled release of indomethacin. J Biomed Mater Res. 2002;59(2):318–28.
Zhou SY, Mei QB, Liu L, et al. Characteristics of drug-release in vitro of different dextran-dexamethasone conjugates. Yao Xue Xue Bao. 2003;38(5):388–91.

Polysaccharides in Cancer Therapy

32

Banani Kundu, Rui L. Reis, and Subhas C. Kundu

Contents

Abstract

Over the past decade, mounting evidences pointing to the substantial contributions of polysaccharides in biological phenomenon like cell-cell recognition, inflammation, immune response, and metabolism lead to further investigations to reveal the "sweet side" of the polysaccharides in pathophysiological conditions, such as cancer. Taking into account the rising trend of polysaccharide-based cancer research, this chapter provides an overview to different classic polysaccharide-based cancer therapeutic approaches with their key mechanisms

B. Kundu (✉) · R. L. Reis · S. C. Kundu
3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Zona Industrial da Gandra, Barco, Guimarães, Portugal

ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães, Portugal
e-mail: bkundu@i3bs.uminho.pt; kundu@i3bs.uminho.pt

J. M. Oliveira et al. (eds.), *Polysaccharides of Microbial Origin*,
https://doi.org/10.1007/978-3-030-42215-8_42

underpinning their success. Following, it also includes the novel neo-therapeutic advances including immunotherapy, iminosugars, small molecules, or pro-drugs and vaccines. To co-relate the concepts of different therapeutic designs with its respective target (which may be a specific protein/enzyme, a dysfunction of cell organelle, or a gene expressed at certain stage during cancer development), a knowledge of fundamental stages of cancer is needed. Hence, the chapter has been started with a brief note of cancer initiation and metastasis, followed by how the fundamental understanding of the disease facilitates the exploitation of the novel characteristics of polysaccharides including antiangiogenic, antioxidant, mitochondrial function regulation, immunomodulation, cell cycle checkpoint antagonists, and ease in chemical modifications – all of which together trigger the paradigm shift of cancer therapies towards precision medicine. The chapter ends with a consensus statement of challenges and recommendations of use of polysaccharides in neo-chemotherapeutic development.

Keywords

Polysaccharides · Cancer · Iminosugars · Immunomodulatory carbohydrates · Carbohydrate cancer vaccines

1 Introduction

Polysaccharides are the most abundant biomacromolecules, playing critical role to fulfill the basic necessities of multicellular organism such as acting as "food," constructing the building block, that is, "house," and finally, polysaccharide is present on the surface of all mammalian cells, covering it just like "clothing," and regulates the interaction of cells with the surrounding extracellular matrix (ECM). In spite of its biological significance, polysaccharides are underappreciated compared with their counterparts such as proteins and nucleic acids which perhaps due to their structural complexity and functional redundancy (Shriver et al. 2004). Recently, attentions are directed to polysaccharides due to their selective toxicity to cancer cells while low toxicity or less adverse side effects (Khan et al. 2019). The anticancer activity of polysaccharides has first been discovered by Nauts et al. (1946). Compared to contemporary clinically used chemotherapeutic agents like doxorubicin, 5-fluorouracil, taxol, etc., which are associated with critical side effects like immunosuppression, alopecia, and anemia; polysaccharides such as chitin exert selective toxicity to cancer cells without any known side effects (Chen et al. 2013). In addition to anticancer properties, diseased cells such as cancer cells recruit carbohydrates to protect them from the therapeutic molecules. The cancer cells employ the carbohydrates to camouflage as "transparent" to body's immune system. Usually, all cells are covered by a complex layer of carbohydrate known as glycocalyx (10–100 nm thickness). The glycocalyx exhibits diverse molecular confirmations such as glycolipids, glycoproteins, glycophosphatidylinositol-linked proteins, and proteoglycans which are the determinant to pathophysiological conditions. Deciphering the

structure–function relationship of carbohydrates with the disease progression will lead the designing of structure-based chemical entities that imitate bioactive carbohydrates and introduce a neo-class of novel therapeutics. Hence, in the next section, the multistep progression of cancer and its spreading into different parts of the body, that is, metastasis, is summarized along with the expression of corresponding glycans that serve as biomarkers of disease advancement. Apart from its active role in disease progression, the versatile characteristics of polysaccharide (Sect. 3) also allow the use of it as anticancerous agent which is discussed with appropriate examples in latter sections (Sect. 4). Section 4 also includes the use of polysaccharides to augment the therapeutic activity of commercial anticancer drugs like Doxorubicin, Paclitaxel, etc. Together the current cancer research on polysaccharide-based derivatives envisions polysaccharides as multifaceted biomacromolecules with immense potential in cancer therapy from easy detection – to specific targeting the diseased cells – to cure the disease.

2 Tiny Tale of Cancer Metastasis

In body under pathophysiological conditions, the cells evolve gradually from normalcy into invasiveness (cancer cells) through a sequence of premalignant states and genetic mutation. Apart from heredity, free radicals cause damage to cells, especially to DNA, and play a role in cancer development. The most common hallmark of this transformation is unregulated cell division leading to the formation of tumor. The division of a cell into two is regulated by a multistep process, namely, cell cycle (G0–G1–S–G2–M phase). The regulators of cell cycle control the diverse metabolic pathways and functionality of organelles such as mitochondria and endolysosomes. In most animals, glucose is the chief source of energy. The metabolism of glucose is mutually regulated with cell cycle machinery. Glucose promotes the activity of cyclin-dependent kinases (CDK) such as CDK 4 and CDK 2, the latter is involved in entry of cells into S-phase. Thus, cancer cells need a large amount of glucose compared to normal cells to facilitate the rapid cell division – the hallmark of cancer. To meet the demand of large amount of glucose, the highly proliferative cancer cells exhibit increased number of glucose transporters (GLUTs) on the surface. The presence of GLUTs and lectins on membrane surface allow greater intake of glucose by cancer cells compared to normal cells – a phenomenon known as "Warburg effect" (Warburg 1956). Hence, both cell cycle checkpoints (the transition point, that is, the events that take place in cells confirming their shift from one phase to another) and GLUTs serve as potential targets in designing cancer therapy.

In solid tumors, the cancer cells reside at the site of primary lesion and termed as benign tumors. When cancer cells leave their origin and travel locally or far in the body, then the phenomenon is termed as metastasis. Metastasis accounts for approximately 90% of all cancer-related death (Obenauf and Massagué 2015). Metastasis is a dynamic process that consists of sequence of events which includes the invading of cancer cells and the employment of intrinsic factors of host (Stoletov et al. 2020). For

cancer cell to metastasize, it needs to interact with surroundings to make its way while surviving the physical stress imposed by the ECM and trick the host's immune surveillance. This happens only if a sequence of events successfully takes place such as (i) remodeling the ECM, (ii) neo-angiogenesis or formation of new blood vessels, (iii) loss of contacts with surrounding cells, (iv) formation of invadopodia and invasion into surrounding ECM, (v) intravasate through the lining of endothelial cells of vasculature to enter into circulatory system of host, (vi) travel as circulating tumor cells (CTC) in host's circulatory system, (vii) formation of CTCs-platelet aggregation, (viii) addition of CTCs to the endothelial lining of vasculature at a site far from primary tumor, (ix) extravasate through the endothelial lining of vasculature to leave the circulatory system, and (x) finally colonize in new tissue forming the secondary tumor.

Each step of this process is important for a tumor to be a metastatic cancer. During this journey, cancer cells coat them with a class of sugar, known as sialic acids which protects them from the attack of natural killer cells. However, some steps still suffer from low efficacy, which are our hope. For instance, the sustainability of CTCs in host's circulatory system is low, followed by the extravasation of CTCs at secondary site (Leong et al. 2014). Both of these can act as bottlenecks to serve ideal target for therapeutic designing (Stoletov et al. 2020). The different stages of cancer are summarized in Fig. 1. It also highlights the potential targets of polysaccharides in cancer treatment.

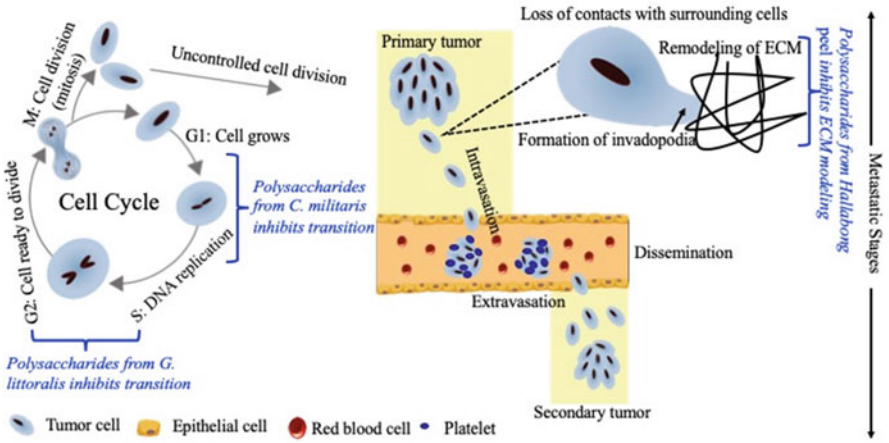


Fig. 1 Schematic summarizes the different stages of cancer metastasis intervened by polysaccharides. Cells undergo uncontrolled cell division that leads to the formation of primary tumor. Cancer cells from the primary tumor leave the site and enter into the blood circulation (intravasation). The journey of cancer cells from primary site to blood vessel involves loss of contacts with surrounding cells and formation of invadopodia followed by penetration through the blood vessel lining. The circulatory cancer cells in blood aggregate with the platelets to escape from the immune surveillance and reach at the distant site in body. The cancer cells then leave the blood vessels (extravasation) at a distant secondary site and colonize. Polysaccharides intervene at different stages of cell cycle or metastasis are also indicated

3 Polysaccharides: Properties, Modifications, and Anticancerous Mechanisms

How the knowledge of cancer biology can be useful to combat the disease? In systematic treatment regime, primary tumors when detected at early stage or benign state are excised surgically, followed by treatment using radiation or toxic chemicals. However, cancer is like weeds, getting completely rid of both of them is difficult. Toxic anticancerous chemicals or chemotherapeutics commonly employed in cancer therapy are targeted to cell-cycle check points or genetic instability of cancer cells. The US FDA has approved chemotherapeutics including doxorubicin, taxol, cyclophosphamide, 5-fluorouracil, etc., for the treatment of cancer. But the nonspecific DNA damaging properties of these chemotherapeutics effect the normal healthy cells at cancer ECM, resulting in disabling side effects. Hence, attention is paid to the anti-inflammatory, antioxidant, and anticancerous activities of natural polysaccharides (Atiq and Parhar 2020). In addition, the immunomodulatory effects of polysaccharides have been known since 1950s (Yin et al. 2019), that not only facilitates its use to improve the defense of body against cancer but also prescribe to be used in conjugation with conventional chemotherapeutic agents to reduce the immunosuppression (Bao et al. 2013).

3.1 Relation Between Structure and Anticancer Potentiality of Polysaccharides

The anticancer properties of polysaccharide depend on its molecular weight, aqueous solubility, degree of branching, three-dimensional arrangement, and composition (Sullivan et al. 2006). The bioactivity of polysaccharides can be manipulated by chemical modifications. A comprehensive overview of structure–function relationship of polysaccharide is summarized in this section with examples (also illustrated in Fig. 2).

3.1.1 Molecular Weight and Solubility

Molecular weight between 20 and 500 kDa is the determinant of the bioactivity of polysaccharides due to its well aqueous-solubility (Ferreira et al. 2015). Both low molecular weight lichenan (M.W. 20–62 kDa) and high molecular weight lentinan (M.W. 500 kDa) exhibit anticancerous activity, while other polysaccharides with molecular weight more or less than these threshold values reveal less bioactivity.

3.1.2 Bands and Branching

The nature of bonds present in the backbone of polysaccharide as well as the distribution of branches beside the backbone are also the determinants of anticancer potentiality of the polysaccharide. The (1→3) and (1→6) glyosidic bonds on glucan impart the anticancer activity. For instance, the anticancer potentiality of lentinan is due to the presence of (1→3)-β-D-glucopyronysyl bond in backbone and (1→6) β-glycosyl side branches (Ferreira et al. 2015).

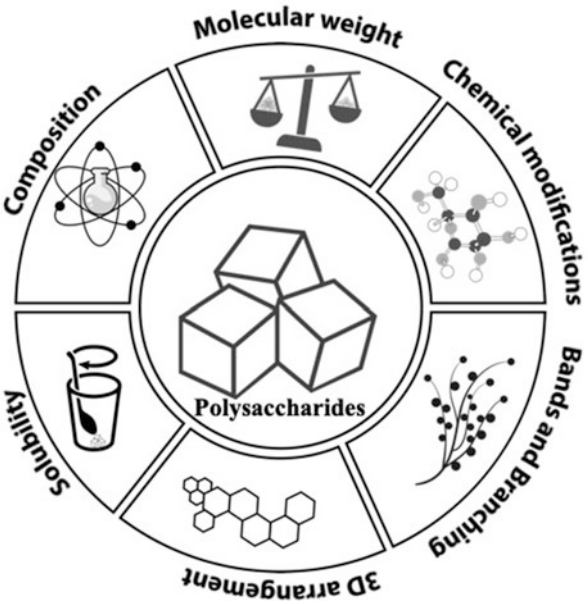


Fig. 2 Schematic illustration of versatile properties of polysaccharides that impart their anticancer potentiality

The degree of branching, that is, the number of side chains present in each saccharide, regulates its solubility – too much (more than 40%) or too little (between 1.5% and 2%) branching leads to little or no anticancer activity. The degree of branching between 20% and 33% reveals maximum anticancer potency, except lentinan (Atiq and Parhar 2020). The degree of branching in lentinan is reported around 40% but it is still bioactive.

3.1.3 3D Arrangement

Usually, polysaccharides exhibit two types of molecular configurations – α and β. Polysaccharides with α-configuration reveal poor bioactivity compared to their β-counterparts (De silva et al. 2012). Triple stand helical chain structure of polysaccharide exhibits greater anticancer performance over random coil (Meng et al. 2020). The polysaccharides readily lose their bioactivity with the transition to single chain conformation.

3.1.4 Composition

The composition of monosaccharides present in the polysaccharide also impacts their anticancer activity. The simplest one possesses two different monosaccharide units, while the most complex one contains up to 10 monosaccharide units of different types. The polysaccharides with reported anticancer activity usually possess 4–6 different types of sugars. Among monosaccharides such as glucose, galactose, and arabinose, at least one of the former always pairs with fucose, mannose, rhamnose, ribose, and xylose in different molecular ratios to exhibit anticancer

activity. In addition, some of the polysaccharides also contain proteins, sulfated sugars, uronic acids, and glucosamine that regulate their functions or serve as biomarkers under pathophysiological conditions.

3.2 Chemical Modifications

Chemical modifications like methylation, carboxylation, sulfation, and hydroxylation lead to improved anticancer performance. For example, the sulfated fucodian serves as anticancer therapeutic molecule for a wide range of cancer types (Atashrazm et al. 2015). The methylated or ethylated derivatives of polysaccharides serve as cancer suppressors while the hydroxylated derivatives stimulate the macrophages. The activation of macrophages causes the production of TNF α and nitric oxide that modulate the immune behavior (Meng et al. 2016). The sulfated derivatives of polysaccharides from *Enteromorpha intestinalis* (Wang et al. 2014a) or *Russula virescens* (Sun et al. 2009) initiate the apoptosis in cells by inducing the expression of Bax and Bad, the key apoptotic regulators. The sulfated derivatives of some polysaccharides present an extended chain in solution that stimulates the expression level of Bax while reduces the expression of Bcl-2, thus inducing cell apoptosis. Further, the sulfated groups may affect the carbon backbone of the polysaccharides that may alter their anticancer activity. The degree of substitution also influences the anticancer activity. The degree of substitution is generally greater than 1 to observe or expect the change in potentiality (Zhang et al. 2012). However, the particular relation between the substitution degree and anticancer activity is still debatable. Modifications such as phosphorylation of glucan from *Poria cocos mycelia* converts the water insoluble polysaccharide into water-soluble derivative with stronger anticancer activity (Huang and Zhang 2011).

3.3 Anticancer Mechanisms of Polysaccharides

The different mechanisms employed so far by the polysaccharides to exert their anticancerous activity are grouped into following: (i) arrest of cell cycle at different stages, (ii) activation of nitric oxide pathways, (iii) mitochondrial dysfunction, and (iv) immunomodulation (Khan et al. 2019). Most of the polysaccharides usually employ one mechanism, while polysaccharides from *Phaeo phycea*, *Pleurotus abalonus*, *Cyclocarya paliurus*, and *Phellinus linteus* recruit multiple pathways, including cell cycle arrest and antioxidant activity to fight against cancer. Based on the mechanism of anticancer activity, polysaccharides can be grouped into the following:

3.3.1 Polysaccharides Involve in Cell Cycle Arrest

In G1 phase of cell cycle, the cyclin-dependent kinases (CDKs) facilitate the transition of G–to–S phase by promoting the replication of DNA. Polysaccharides (from *Cordyceps militaris*) lower the expression of certain CDKs which correspond to the reduction of number of cells that subsequently moving to S-phase. This

activates the cell cycle check-points that cause the death of cells and generation of apoptotic bodies (Chen et al. 2018). Further, the polysaccharides from *Glehnia littoralis* reduce the expression of proliferating cell nuclear antigen, resulting in the arrest of cell cycle in S and G2/M phase (Wu et al. 2018). Polysaccharides (e.g., *Aconitum coreanum* polysaccharide; ACPI) are also able to inhibit the polymerization of actin cytoskeleton, the inhibition of which prevents the phosphorylation of Rac 1 (Ras-related C3 botulinum taxin substrates) activator. Rac 1 is responsible for the formation of tube in metastasis (Park et al. 2016). Hence, polysaccharides like ACPI have the potentiality to prevent metastasis.

Currently, there is no effective treatment option for triple negative breast cancer as hormonal therapy is not applicable. Polysaccharide from Hallabong peel extract (HBE) has been undergone clinical trial for the treatment of triple negative breast cancer cells due to its capability to inhibit the production of matrix metalloproteinases (MMP 9). MMPs are well-known cancer biomarkers and involve in ECM remodeling, cancer invasion, and metastasis. HBE also prevents neo-tube formation and angiogenesis, hence, limits the growth of tumor (Park et al. 2016).

3.3.2 Antioxidant Activity

Reactive oxygen species (ROS) influence the malignant transformation of cancer cells. Antioxidants scavenge ROS, therefore, have potential in cancer treatment. Polysaccharides with antioxidant properties are able to eliminate hydroxyl-free radicals and superoxide anions. The elimination of ROS inhibits the proliferation of cancer cells (Chen et al. 2015). The free radical scavenging ability of the polysaccharides is directly proportional to the degree of carboxymethylation in their derivatives (Machova et al. 2014). Apart from the free radical scavenging, polysaccharides are able to stimulate the macrophages to secrete the nitric oxide (NO) by upregulating inducible NO synthase (iNOS) activity. NO is multifunctional regulator, playing signatory role in immune modulation and vascular relaxation (Jiang et al. 2014). The increased production of NO activates caspase pathways in cancer cells leading to cell death. In addition to NO, polysaccharides (e.g., *Dimocarpus longan lour*) also induce the release of cytokines like interleukin 6 (IL 6), IL 1, and tumor necrosis factor alpha (TNF α) – together, contributing to anticancer activity (Jiang et al. 2014). Polysaccharides also facilitate the macrophage-mediated phagocytosis (Bai et al. 2012). Thus, the NO-mediated anticancerous activity of polysaccharides is not only due to the activation of iNOS but also its ability to regulate another synergistic physiological phenomenon.

3.3.3 Mitochondrial Dysfunction

In mammalian cells, mitochondria are the key player in programmed cell death or apoptosis. The apoptotic activities of cells are broadly grouped into two major pathways: (i) the intrinsic pathway or mitochondrial pathway and (ii) extrinsic pathway. Polysaccharides are able to depolarize the mitochondrial membrane resulting in the release of Cytochrome C. The release of Cytochrome C activates the caspases which in turn stimulate the process of apoptosis (Tian et al. 2016). The depolarization of mitochondrial membrane is usually estimated by means of caspase activity. Besides disrupting the membrane potential of mitochondria, polysaccharide

III of *Hirsutella sinensis* also generates the reactive oxygen species – together improve its anticancer activity (Khan et al. 2019).

3.3.4 Immunomodulation

Cancer cells escape the immune surveillance of host and metastasize to the other organs through the mechanism called "Immunosuppression." Hence, the immune system of the tumor-bearing host is needed to be boosted to restore the dynamic balance between the host immune system and cancer cells to fight against cancer. Several polysaccharides own immune-stimulant activity. Polysaccharides from *Artemisia argyi* (FAAP-02) (Bao et al. 2013) and *Epimedium koreanum* (EPS) (Wang et al. 2017) stimulate the production of cytokines like TNF α, IL 2, IL 4, IL 6, IL 12, etc. These cytokines upregulate the expression of lymphocytes or enhance the expression of surface accessory molecules on dendritic cells or resident macrophages that improve the immune response of host. The elevation in level of pro-inflammatory cytokines like ILs and TNF α also facilitates the apoptosis of cancer cells by inducing the oxidative stress and depolarization of mitochondrial membranes. The soluble polysaccharides from red wine induce the secretion of TNF α that causes the death of cells in slightly different manner by activating necroptosis pathway (Stipp et al. 2017). It recruits factors like RIP 1, RIP 3, and MLKL that generate necrosomes to rupture the cells leading to the cell death. Besides cytokines, immune cells such as CD4+ and CD8+ cells, T lymphocytes are triggered by polysaccharides obtained from *Epimedium koreanum*, *Artemisia argyi*, and *Gynostemma pentaphyllum*, thus playing critical role in activating the immune system of host. Polysaccharides from *Gonaderma atrum*, *Trametes orientalis* activate the host's macrophages leading to phagocytosis. The carboxymethylation and sulfation of polysaccharides improve their interaction with the immune cell surface receptors through hydrogen bonding and electrostatic attraction (Chen et al. 2010). The involvement of electrostatic interaction and chemical bonding results strong immune response.

3.3.5 Antiangiogenesis

Cells get their nutrients through blood vessels. The formation of new blood vessels or angiogenesis is the key of cancer cell invasion and metastasis. Hence, the inhibition of the generation of neo-blood vessels cuts the food supply to highly proliferative cancer cells, restricting their growth. Sulfated polysaccharides (polysaccharides of *Phellinus ribis*, cuttlefish or laminarin) inhibit the synthesis/expression of vascular endothelial growth factors and basic fibroblast growth factors – the two key players and essential proteins for the formation of neo-blood vessels. An exposure to sulfated polysaccharides reduces the formation of neo-capillaries and generation of vascular endothelial cells, thus inhibiting the tumor growth (Liu et al. 2009; Hoffman et al. 1996).

4 Polysaccharides in Cancer Therapy

"Therapy" means the process of treatment to heal or relief a disorder. During past decade, neo-anticancer therapeutics have emerged from our understanding of polysaccharides (popular polysaccharides with antitumor activity obtained from diverse

sources in nature are summarized in Table 1) and identification of a number of biological or molecular targets of cancer. This section provides a comprehensive overview of effective anticancerous polysaccharide compounds under clinical trials as well as working in basic biomedical sciences as prodrugs, vaccines, glycosidase inhibiting iminosugars, and early diagnostics of cancer.

4.1 Anticancerous Polysaccharides in Clinical Trial

There are very few clinical trials conducted so far on anticancer potential of polysaccharides. One of the early clinical trials has been conducted on 2009 using the extracts from *Grifola frondosa*. The extracts exhibit anticancerous activity in postmenopausal breast cancer patients with no dose-limiting toxicity (Deng et al. 2009). Genistein concentrated polysaccharide has been tested effective against prostate cancer (Zharinov et al. 2017). It prolongs the doubling time of prostate-specific antigen, the marker of tumor growth. Lentinan from *Lentinus edodes* is used in conjugation with well-established chemotherapeutic agents like paclitaxel (Zong et al. 2012). The conjugation therapy prolongs the administration period while reduces the adverse side effects in pancreatic, colorectal, hepatic, and gastric cancers. Polysaccharide K from *Trametes versicolor* is also used in combination with conventional chemotherapeutics like oxaliplatin or folinic acid (Zong et al. 2012) in colorectal and gastric cancer patients. The combination therapy possesses immunomodulatory effects that reduce the apoptosis of T cells. Further, the peptidoglycan complex of Spirulina has finished phase 1 trial on 2016 in advanced pancreatic cancer patients. However, the reports of trials are not presently available. Schizophyllan, a polysaccharide from fungus *Schizophyllum commune*, is known to have immunomodulatory effect. Phase II randomized controlled trial in patients with locally advanced head and neck squamous cell carcinoma has indicated safety and well tolerability of Schizophyllan in all patients (Mantovani et al. 1997). Schizophyllan in conjugation with chemotherapeutics has been proven to prolong the life span of recurrent gastric cancer Japanese patients without any side effects (Furue et al. 1985).

4.2 Polysaccharide-Based Prodrugs

The love of cancer cells towards sugar is exploited by conjugating the cytotoxic anticancer therapeutics like paclitaxel, docetaxel, busulfart with sugars to improve their pharmacokinetic properties including (i) short circulation time, (ii) poor solubility and reduced availability, and (iii) poor penetrability to the targeted site that results diffusion in healthy tissues leading to side effects like diarrhea, vomiting, nausea, constipation, and decreased liver function. Glycoconjugation has been proven as effective strategy for targeted delivery of chemotherapeutics. The sugar moieties used so far for the delivery of common chemotherapeutics are summarized in Table 2. After dose administration, the various intracellular glycosidases cleave

Table 1 Bioactive polysaccharides: origin and effects

Origin	Polysaccharide	Source	Effects	References
Bacteria	Fructan	*Zymomonas mobilis*, *Bacillus spp.*, *Streptococcus spp.*, *Pseudomonas spp.*, *Xanthomonas spp.*, *Aerobacter spp.*	Immunomodulator, antioxidant activity	Belghith et al. (2012)
	Curdlan	*Alcaligenes faecalis*	Immunomodulator	Zong et al. (2012)
Fungi	Heteroglucans and homoglucans	*Agaricus blazei*, *Ganoderma lucidum*, *Lentinus edodes*, *Grifola frondosa*, *Coriolus versicolor*, *Schizophyllum commune*	Anticancer and antitumor activities	El Enshasy and Hatti-Kaul (2013), Zhang et al. (2013), Wang et al. (2014)
	Glucans	*Sclerotina sclerotiorum* IFO 9395, *Schizophyllum commune*, *Grifola frondosa, Poria cocos mycelia*	Immunostimulator, antitumor activity	Vannucci et al. (2013)
	(1➞3)-β-D-glucan derivatives	*Saccharomyces cerevisiae*	Antioxidant, antitumor activity, immunostimulator	Kogan and Kocher (2007)
	Pullulan	Fermentation of liquefied starch, beet molasses, agro-industrial waste with *Aureobasidium pullulans*	Pullulan targets the asialoglycoprotein receptor on liver cancer cells	Prajapati et al. (2013)
	Lentinan	*Lentinus edodes*	Improves the availability and reduces the toxicity of chemotherapeutic paclitaxel	Atiq and Parhar (2020)
	Cordyceps polysaccharides	*Cordyceps militaris*	Arrests cell cycle and facilitate the cell death	Chen et al. (2018)
	Polysaccharide III	*Hirsutella sinensis*	Promotes reactive oxygen species activity	Khan et al. (2019)
	PRP-SI–IV	*Phellinus ribis*	Reduces angiogenesis	Liu et al. (2009)
Algae	Fucoidan	*Undaria pinnatifida*	Activation of caspase pathways	Boo et al. (2011)
		Fucus vesiculosus	Inhibited the MMP-2 and MMP-9 protein expression, and prevent the	Huang et al. (2015)

(continued)

Table 1 (continued)

Origin	Polysaccharide	Source	Effects	References
			cell migration, and invasion activities of LLC cells	
	Polysaccharide	*Sargassum fusiforme*	Inhibit VEGF-A-linked angiogenesis	Chen et al. (2017)
	Glycoprotein	*Codium decorticatum*	Induces apoptosis	Senthilkumar and Jayanthi (2016)
	d-Galactan sulfate	*Gymnodinium*	Inhibits topo I and topo II	Umemura et al. (2003)
Chinese Herbs	Saponins	*Akebia quinata*	Cytotoxic and apoptotic activity	Kang et al. (2010)
		Cynanchum paniculatum	Anticancer activity	Kim et al. (2013)
	Extract contains a mixture of saponins, flavonoids, phenolic compounds, and quinones	*Spatholobus suberectus Dunn*		Wang et al. (2013)
	α-Glucan	*Lobelia chinensis Lour*	Antimutagenic activity and anticancer activity	Li et al. (2016)
	FAAP-02	*Artemisia argyi*	Enhance the expression of surface accessory molecules on dendritic cells or resident macrophages	Bao et al. (2013)
Plants	Polysaccharide	Herb *Gynostemma pentaphyllum*	Activates immune cells to promote anticancer activity	Khan et al. (2019)
	Tea plant flower polysaccharide (TFP)	Tea plant flower	Inhibited tumor growth	Han et al. (2010)
	Safflower polysaccharide (SPS)	Safflower	Cytotoxic activity	Zong et al. (2012)
	Polysaccharide	Cactus pear fruit	Antiproliferative activity	
	SPG-56	Sweet potato	Promoting apoptosis and inhibiting metastasis	Li et al. (2019)

Animals	Sepia ink polysaccharide (SIP)	Squid *(Illex argentines, Ommastrephes bartrami),* cuttlefish (*Sepiella maindroni, Sepia esculenta ink)*	Chemotherapeutic ancillary agent	Li et al. (2018)
	SEP [D-glucan with α-(1→4)-linked backbone branched at α-(1→6)-linkage]	Worm *Sipunculus nudus* eggs	Enhances host's immune function	Zong et al. (2012)
	Water-soluble polysaccharide (WSP)	Sea snail *Hemitoma cumingii*	Arrests the cell cycle at G0/G1 phase	
	Heteropolysaccharide MAP	*Misgurnus anguillicaudatus* fish	Mitochondrial dysfunction	
	Squid ink polysaccharide TBA-1	Squid *Ommastrephes bartramii*	Inhibits angiogenesis	
	Crude polysaccharides CSPS-3	*Cyclina sinensis*	Antiproliferative activity	
	Short-chain polysaccharide (PS)	Porcine cartilage	Inducing cellular apoptosis	Liu et al. (2007)
	Sulfated polysaccharide–protein conjugate (GSPP)	*Gekko swinhonis* Guenther	Arrests the cell cycle at G2/M phase, enhances host's immune function	Zong et al. (2012)

Table 2 Glycoconjugated chemotherapeutics – sugar moieties and targeting mechanisms

Chemotherapeutics	Sugar moieties	Targeting mechanisms
Doxorubicin (Adriamycin)	Galactose (Gal)	Asialoglycoprotein receptor 1 are highly expressed on hepatocarcinoma or breast cancer cells ASGPR1 has high affinity towards Gal
	2-Amino-2 deoxy-D glucose and succinic acid (2DG-SUC)	Adriamycin has high affinity to 2DG-SUC The glucose in the derivative exploits the GLUT 1 receptor of cancer cells
Paclitaxel	Glucose	GLUT 1 receptor on cancer cell surface
5-Fluorouracil	L-rhamnose	ASGPR receptors on surface of hepatocarcinoma cells
Emodin	D-rhamnose	Rhamnose-binding lectin receptors on cancer cells such as Asialoglycoprotein receptors
Geldanamycin	Galactose (Gal) or Glucose	GLUT 1 receptors on surface of cancer cells
Ifosfamide	Glucose	
Chlorambucil	D-threose	Thymidine phosphorylase receptors are overexpressed in cells in response to stressful conditions like hypoxia, radiotherapy

Adapted from Molejon et al. (2020)

the sugar, following the release of active compound. Among various GLUTs, GLUT-1 is the most common surface receptor in cancer cells. Apart from cytotoxic anticancer compounds, the prodrugs also contain inhibitors such as phlorizin and phloretin for GLUT-1. Another popular cell surface receptor is lectin receptors. Lectins are considered as proteins that commonly linked to sugars (Molejon et al. 2020). Among diverse lectin-based cell surface receptors, the rhamnose-binding lectin receptors are overexpressed on cancer cells including human prostatic small-cell carcinoma, squamous-cell carcinoma, and breast adenocarcinoma. The rhamnosylated anticancer molecules have recently been exploited for pharmacological applications (Xu et al. 2019).

The large amount of glucose consumed by cancer cells is digested to lactic acid regardless of the availability of oxygen. The release of lactic acid turns the ECM of cancer cells into acidic, the pH of which becomes around 5.5. The acidic cancer ECM serves as a potential target to design cancer prodrugs. Pullulan is modified either by periodate oxidation and cysteamine conjugation (Scomparin et al. 2011) or hydrazide – covalent modification (Lu et al. 2009) to form nanoassemblies that exhibit rapid release of anticancer agent doxorubicin at pH 5–5.5 while slow release at pH 7.4. These prodrug nanoassemblies also improve the serum stability and water solubility of the formulation which in turn results better pharmacokinetics.

The tendency of cancer cells to utilize high amount of glucose is also exploited in diagnostic purpose. Cancer cells are served with glucose-analog, 2-deoxy-2(18F) fluoro-D-glucose (18F-FDG) and then followed/observed the cells that intake the

glycoconjugates via positron emission tomography (Herrmann et al. 2011). This is a hallmark in glycoconjugate-based therapeutic designing and opens a new therapeutic window of widespread interest of glycosyl-based prodrugs over past few years.

4.3 Immune-Therapy with Polysaccharide-Based Vaccine

Vaccines are commonly targeted to exogenous causative agents of disease but this is not the case for cancer. For cancer, the target is one's own mutated cells that are able to divide uncontrollably and invade into other tissues. Another critical phenomenon of cancer is altered glycosylation that causes the increased expression of tumor-associated carbohydrate antigens (TACAs) on surface of cancer cells compared to normal cells. An increasing amount of preclinical and clinical data indicates that antibodies against TACAs can remove the circulating tumor cells and stop micro-metastases (Slovin et al. 2005). TACAs are self-glycans and have the potency to act as potential vaccine antigen. However, to evoke antibodies against TACAs to kill selectively and effectively the malignant cells become an ambitious project due to the poor immunogenicity and microheterogeneity of glycans. This makes it difficult to extract it from unique natural sources such as globohexaosylceramide. Hence, synthetic processing of vaccine is required. Further, to stimulate the T-cell response, early vaccine designs involve carbohydrate-protein conjugates. For example, isolated TACAs are conjugated with a carrier protein like keyhole limpet hemocyanin (KLH). To further improve the immunogenicity, strong adjuvant like QS-21 is also used. Clinical trials with TACAs containing lapidated reducing end such as gangliosides GD3-based conjugates of KLH carrier and QS-21 adjuvant pair reveal to break the tolerance to TACAs (Helling et al. 1994). Alternative carrier and advent strategies are also in practice, such as a fully synthetic vaccine containing Toll-like receptor 2 agonist, a tumor-associated glycopeptide, and a promiscuous peptide helper T-cell epitope that recognizes cancer cells expressing TACAs and elicits robust antibody responses (Ingale et al. 2007).

In body, TACAs typically are linked with mucins like Tn, sTn, and Thomsen-Friedenreich (TF); hence, mimicking this presentation is expected to elicit strong immune response. Synthetic glycopeptide cluster KLH conjugates with TF, sTn, and Tn have demonstrated immunogenicity and safety (Musselli et al. 2001). Mucin 1 (MUC1) overexpressed in epithelial tumor cells like breast, lung, pancreas, colon, kidney, and ovary are also used to develop synthetic vaccine like sTn-MUC1 tandem-repeat glycopeptide conjugates (Kaiser et al. 2009). The heterogeneity of TACA in cancer cells leads to the designing of multivalent vaccines which can readily be tailored depending on cancer type.

4.4 Iminosugars for Cancer Therapy

Iminosugars are doubtlessly the most attractive carbohydrate derivate so far. Discovered in 1970s, in iminosugars, the endocyclic oxygen of sugar is replaced

by a basic nitrogen atom which gives it remarkable biological properties that facilitate its progression from laboratory to clinic. Due to the involvement in multiple signaling pathways that are engaged in cell viability and proliferation, the iminosugars serve as neo-therapeutic agents in a range of disease related to carbohydrate metabolism.

The glycans of cells are synthesized in the endoplasmic reticulum of cells involving glycosyltransferases. These enzymes are responsible for the abnormal development or modification of glycan moieties on cell surface that distinguish a cancer cells from healthy cells. Due to abnormal O-glycosylation, N-linked oligosaccharides play critical role in oncogenesis and metastasis. Hence, the development of therapeutic strategies to prevent abnormal N-glycosylation can restrict the development of cancer. Diverse naturally occurring iminosugars are able to target the biosynthesis pathway of N-glycans. The most common examples are piperidine, nojirimycin, pyrrolizidine, nontropane, and indolizidine (Hossain and Andreana 2019). Nojirimycin, isolated from *Streptomyces roseochromogenes*, one of the iminosugars with anticancer property, is available for pharmaceutical applications (Nash et al. 2011). Swainsonine, the (1S, 2R, 8R, 8aR)-1,2,8-trihydroxyindolizidine, is an effective inhibitor of Golgi α-manosidase II which inhibits the expression of β (1-6) branched N-glycans in malignant human cells. This leads to further clinical trial of swainsonine hydrochloride (GD0039) for the treatment of advanced renal cancer patients (Wrodnigg et al. 2008). GD0039 appears to prevent metastasis, activate lymphocytes, and improve the T-cell stimulation. But unfortunately, all the patients discontinue the oral GD0039 treatment due to the cytotoxicity or advancement of the disease. Pentahydroxylated pyrrolizidine is reported to act as glucosidase inhibitor with increased production of cytokines IL 2, IL 12, and IFN δ. Surprisingly, immune responses by iminosugars do not rely on glucosidase inhibition. However, most of the investigations have been carried out so far exploit plant glycosidases, while further research necessitates to uncover the potential of mammalian glycosidases in cancer research.

4.5 Polysaccharide-Based Cancer Diagnostics

Serum glycoproteins like prostate-specific antigen, alpha-fetoprotein, carcino embryonic antigen, carbohydrate antigen 19-9 (CA19-9) and 125 (CA-125) are found in serum of patients with prostate, ovarian, and colon cancers and act as biomarkers (Namikawa et al. 2018). In cancers that lack serum biomarkers mentioned above, lectins are employed in the diagnosis of cancers. Lectins from *Artocarpus integrifolia agglutinin* (AIA), *Vicia villosa* lectin (VVL), *Ulex europaeus agglutinin* I (UEA I), *Arachis hypogea agglutinin* (AHA), *Griffonia simplicifolia agglutinin* I (GSA I), and *Amaranthus caudatus agglutinin* (ACA) are able to bind selectively to the polysaccharide alterations including human epididymis secretory protein 4 antigens and TF, Tn, and STn modifications of CA125 due to abnormal glycosylation. Further, glycan

microarray analysis is used to confirm the presence of antibodies against certain antigens such as Globo H in the serum of cancer patients (Wang et al. 2008).

Apart from serum analysis, molecular imaging technique is another powerful tool in early detection of cancer. The increased concentration of 2-flurodeoxy-D-glucose in tumor cells is also investigated by positron emission tomography. The high metabolic activity of cancer cells is used in imaging probe namely metabolic oligosaccharide engineering technology. In this approach, non-natural polysaccharides like GalNACs are incorporated within the glycan chains and tagged with imaging probes using bio-orthogonal reactions. These probes are then monitored using magnetic resonance imaging.

5 Concluding Remarks and Future Perspective

In this chapter, an overview of the state of the art of polysaccharide-based cancer therapy is summarized, discussing the ongoing clinical trials and the challenges exist towards commercialization of polysaccharide-based products. In recent years, cancer therapy has been moved towards more precise and less invasive cancer treatment approaches like immunotherapy, glycoconjugates, and targeted therapy. However, there are considerable issues remain to be addressed.

Due to the fact that natural carbohydrates lack the potency to compare conventional chemotherapeutics, there is immense research scope for designing synthetic analogs of naturally occurring polysaccharides with better pharmacokinetic profiles. In addition, the structure and molecular weight of certain polysaccharides change with surrounding environment and seasons which further affect their performance as anticancerous therapeutic molecule.

In general, the mechanism that regulates the immunotolerance of polysaccharide antigens is yet to resolve. Common obstacles of polysaccharide-based vaccine development are poor immunogenicity, antigenic mimicry of self-glycans, identification and access to protective epitopes, and heterogeneity. The conjugation of polysaccharide to immunogenic protein carriers becomes a popular well-established method to improve the immunogenicity of glycans and increase the degree of immune response. In addition, approaches involving the clustering of the TACAs are getting its way in neo-vaccine development. The advancement in glycomics further facilitates the vaccine development.

Small interfering RNAs (siRNAs) that comprise of double-stranded RNAs are able to silence/suppress the activity of targeted genes. Rationally designed siRNAs can precisely block the activity of mutated genes that are involved in uncontrolled cell proliferation, metastatic invasion, antiapoptotic proteins, and cancer mutated genes. However, siRNAs are unstable under physiological condition and undergo enzymatic degradation or phagocytosed during circulation. Degradation of modified siRNAs evokes undersized side effects. siRNAs are condensed into cationic carriers like chitosan, a polysaccharide, which improve their stability and cellular uptake. Nevertheless, the critical challenges such as correct dose to individual patients,

stage-specific dose concentration, and controlled release are needed to set up the finest personalized therapy.

Moreover, altered glycosylation plays critical roles in cancer progression. Though the complexity of glycosylation in cancer is a challenge, efforts have been put to initiate personalized cancer therapy targeting the altered glycosylation. Different assays have been designed targeting various glycosyltransferases such as GlcNAcT5, ST3GaI1, ST3Gal3, FUT6 ppGaINAx-T2, and ppGaINAx.T3. However, the assays targeting the glycosylation mostly are biochemical assays which overlook the effect of the individual compounds on cells or the whole system within which it is inserted. Thus, these biochemical assays fail to interpret the toxicity and specificity of the candidate. The envision of high-throughput screening employing cellular systems may overcome these limitations. New clauses of anticancer therapeutic molecules are constantly under trials to overcome the therapeutic resistance. Hence, more comprehensive structure-function relation of polysaccharides will be helpful to develop their synthetic analogs as therapeutic agents. Additionally, polysaccharides can also perform as scaffolding materials to develop 3D *in vitro* cancer models to serve as screening platforms (Kundu et al. 2019).

Acknowledgments The work was supported by the European Union Framework Programme for Research and Innovation Horizon 2020 under grant agreement n° 668983 — FoReCaST.

References

Atashrazm F, Lowenthal RM, Woods GM, et al. Fucoidan and cancer: a multifunctional molecule with anti-tumor potential. Mar Drugs. 2015;13:2327–46.

Atiq A, Parhar I. Anti-neoplastic potential of flavonoids and polysaccharide phytochemicals in glioblastoma. Molecules. 2020;25:4895.

Bai L, Zhu LY, Yang BS, et al. Antitumor and immunomodulating activity of a polysaccharide from Sophora flavescens Ait. Int J Biol Macromol. 2012;51:705–9.

Bao X, Yuan H, Wang C, et al. Antitumor and immunomodulatory activities of a polysaccharide from Artemisia argyi. Carbohydr Polym. 2013;98:1236–43.

Belghith KS, Dahech I, Hamden K, et al. Hypolipidemic effect of diet supplementation with bacterial levan in cholesterol-fed rats. Int J Biol Macromol. 2012;50:1070–4.

Boo HJ, Hyun JH, Kim SC, et al. Fucoidan from Undaria pinnatifida induces apoptosis in A549 human lung carcinoma cells. Phytother Res. 2011;25:1082–6.

Chen X, Zhang L, Cheung PC. Immunopotentiation and anti-tumor activity of carboxymethylated-sulfated β-(1→3)-D-glucan from Poria Cocos. Int Immunopharmacol. 2010;10:398–405.

Chen Y, Hu M, Wang C, et al. Characterization and in vitro antitumor activity of polysaccharides from the mycelium of Sarcodon aspratus. Inter J Biol Macromol. 2013;52:52–8.

Chen P, Yong Y, Gu Y, et al. Comparison of antioxidant and antiproliferation activities of polysaccharides from eight species of medicinal mushrooms. Int J Med Mushrooms. 2015;17:287–95.

Chen H, Zhang L, Long X, et al. Sargassum fusiforme polysaccharides inhibit VEGF-A-related angiogenesis and proliferation of lung cancer in vitro and in vivo. Biomed Pharmacother. 2017;85:22–7.

Chen F, Ran L, Mi J, et al. Isolation, characterization and antitumor effect on DU145 cells of a main polysaccharide in pollen of Chinese wolfberry. Molecules. 2018;23:2430.

De Silva DD, Rapior S, Fons F, et al. Medicinal mushrooms in supportive cancer therapies: an approach to anti-cancer effects and putative mechanisms of action. Fungal Divers. 2012;55:1–35.
Deng G, Lin H, Seidman A, et al. A phase I/II trial of a polysaccharide extract from Grifola frondosa (Maitake mushroom) in breast cancer patients: immunological effects. J Cancer Res Clin Oncol. 2009;135:1215–21.
El Enshasy HA, Hatti-Kaul R. Mushroom immunomodulators: unique molecules with unlimited applications. Trends Biotechnol. 2013;31:668–77.
Ferreira SS, Passos CP, Madureira P, et al. Structure–function relationships of immune stimulatory polysaccharides: a review. Carbohydr Polym. 2015;132:378–96.
Furue H, Uchino H, Orita K, et al. Clinical evaluation of schizophyllan (SPG) in advanced gastric cancer (the second report) – a randomized controlled study. Cancer Chemother. 1985;12:1272–7.
Han Q, Ling ZJ, He PM, et al. Immunomodulatory and antitumor activity of polysaccharide isolated from tea plant flower. Prog Biochem Biophyic. 2010;37:646–53.
Helling F, Shang A, Claves M, et al. GD3 vaccines for melanoma: superior immunogenicity of keyhole limpet hemocyanin conjugate vaccines. Cancer Res. 1994;54:197–203.
Herrmann K, Benz MR, Krause BJ, et al. (18)F-FDG-PET/CT in evaluating response to therapy in solid tumors: where we are and where we can go. Q J Nucl Med Mol Imaging. 2011;55:620–32.
Hoffman R, Paper DH, Donaldson J, et al. Inhibition of angiogenesis and murine tumour growth by laminarin sulphate. Brit J Cancer. 1996;73:1183–6.
Hossain F, Andreana PR. Developments in carbohydrate-based cancer therapeutics. Pharmaceuticals (Basel). 2019;12:84.
Huang Q, Zhang L. Preparation, chain conformation and anti-tumor activities of water-soluble phosphated (1→3)-α-D-glucan from Poria cocos mycelia. Carbohydr Polym. 2011;83:1363–9.
Huang TH, Chiu YH, Chan YL, et al. Prophylactic administration of fucoidan represses cancer metastasis by inhibiting vascular endothelial growth factor (VEGF) and matrix metalloproteinases (MMPs) in Lewis tumor-bearing mice. Mar Drugs. 2015;13:1882–900.
Ingale S, Wolfert MA, Gaekwad J, et al. Robust immune responses elicited by a fully synthetic three-component vaccine. Nature Chem Biol. 2007;3:663–7.
Jiang J, Meng FY, He Z, et al. Sulfated modification of longan polysaccharide and its immunomodulatory and antitumor activity in vitro. Int J Biol Macromol. 2014;67:323–9.
Kaiser A, Gaidzik N, Westerlind U, et al. A synthetic vaccine consisting of a tumor-associated sialyl-T(N)-MUC1 tandem-repeat glycopeptide and tetanus toxoid: induction of a strong and highly selective immune response. Angew Chem Int Ed Engl. 2009;48:7551–5.
Kang HS, Kang JS, Jeong WS. Cytotoxic and apoptotic effects of saponins from Akebia quinata on HepG2 hepatocarcinoma cells. Korean J Food Preserv. 2010;17:311–9.
Khan T, Date A, Chawda H, Patel K. Polysaccharides as potential anticancer agents—a review of their progress. Carbohydr Polym. 2019;210:412–28.
Kim CS, Oh JY, Choi SU, et al. Chemical constituents from the roots of Cynanchum paniculatum and their cytotoxic activity. Carbohydr Res. 2013;381:1–5.
Kogan G, Kocher A. Role of yeast cell wall polysaccharides in pig nutrition and health protection. Livest Sci. 2007;109:161–5.
Kundu B, Bastos ARF, Brancato V, et al. Mechanical property of hydrogels and the presence of adipose stem cells in tumor stroma affect spheroid formation in the 3D osteosarcoma model. ACS Appl Mater Interfaces. 2019;11:14548–59.
Leong HS, Robertson AE, Stoletov K, et al. Invadopodia are required for cancer cell extravasation and are a therapeutic target for metastasis. Cell Rep. 2014;8:1558–70.
Li XJ, Bao WR, Leung CH, et al. Chemical structure and immunomodulating activities of an α-glucan purified from Lobelia chinensis lour. Molecules. 2016;21:779.
Li F, Luo P, Liu H. A potential adjuvant agent of chemotherapy: Sepia Ink Polysaccharides. Mar Drugs. 2018;16:106.
Li Z, Yu Y, Wang M, et al. Anti-breast cancer activity of SPG-56 from sweet potato in MCF-7 bearing mice in situ through promoting apoptosis and inhibiting metastasis. Sci Rep. 2019;9:146.

Liu AJ, Song W, Yang N, et al. Cartilage polysaccharide induces apoptosis in human leukemia K562 cells. Cell Biol Toxicol. 2007;23:465–76.
Liu Y, Liu C, Tan H, et al. Sulfation of a polysaccharide obtained from Phellinus ribis and potential biological activities of the sulfated derivatives. Carbohydr Polym. 2009;77:370–5.
Lu D, Wen X, Liang J, et al. A pH-sensitive nano drug delivery system derived from pullulan/doxorubicin conjugate. J Biomed Mater Res B Appl Biomater. 2009;89:177–83.
Machova E, Cızova A, Bystricky P. Effect of carboxymethylation on antioxidant properties and radical degradation of mannans and glucans. Carbohydr Polym. 2014;112:603–7.
Mantovani G, Bianchi A, Curreli L, et al. Clinical and immunological evaluation of schizophyllan (SPG) in combination with standard chemotherapy in patients with head and neck squamous cell carcinoma. Int J Oncol. 1997;10:213–21.
Meng X, Liang H, Luo L. Antitumor polysaccharides from mushrooms: a review on the structural characteristics, antitumor mechanisms and immunomodulating activities. Carbohydr Res. 2016;424:30–41.
Meng Y, Lyu F, Xu X, et al. Recent advances in chain conformation and bioactivities of triple-helix polysaccharides. Biomacromolecules. 2020;21:1653–77.
Molejon MI, Weiz G, Breccia JD, et al. Glycoconjugation: an approach to cancer therapeutics. World J Clin Oncol. 2020;11:110–20.
Musselli C, Livingston PO, Ragupathi G. Keyhole limpet hemocyanin conjugate vaccines against cancer: the Memorial Sloan Kettering experience. J Cancer Res Clin Oncol. 2001;127:R20–6.
Namikawa T, Kawanishi Y, Fujisawa K, et al. Serum carbohydrate antigen 125 is a significant prognostic marker in patients with unresectable advanced or recurrent gastric cancer. Surg Today. 2018;48:388–94.
Nash RJ, Kato A, Yu CY, et al. Iminosugars as therapeutic agents: recent advances and promising trends. Future Med Chem. 2011;3:1513–21.
Nauts HC, Swift WE, Coley BL. The treatment of malignant tumors by bacterial toxins as developed by the late William B. Coley, M.D., reviewed in the light of modern research. Cancer Res. 1946;6:205–16.
Obenauf AC, Massagué J. Surviving at a distance: organ specific metastasis. Trends Cancer. 2015;1:76–91.
Park JY, Shin MS, Kim SN, et al. Polysaccharides from Korean Citrus hallabong peels inhibit angiogenesis and breast cancer cell migration. Int J Biol Macromol. 2016;85:522–9.
Prajapati VD, Jani GK, Khanda SM. Pullulan: an exopolysaccharide and its various applications. Carbohydr Polym. 2013;95:540–9.
Scomparin A, Salmaso S, Bersani S, et al. Novel folated and non-folated pullulan bioconjugates for anticancer drug delivery. Eur J Pharm Sci. 2011;42:547–58.
Senthilkumar D, Jayanthi S. Partial characterization and anticancer activities of purified glycoprotein extracted from green seaweed Codium decorticatum. J Funct Foods. 2016;25:323–32.
Shriver Z, Raguram S, Sasisekharan R. Glycomics: a pathway to a class of new and improved therapeutics. Nat Rev Drug Discov. 2004;3:863–73.
Slovin SF, Keding SJ, Ragupathi G. Carbohydrate vaccines as immunotherapy for cancer. Immunol Cell Biol. 2005;83:418–28.
Stipp MC, de Lacerda BI, Corso CR, et al. Necroptosis mediates the antineoplastic effects of the soluble fraction of polysaccharide from red wine in Walker-256 tumor-bearing rats. Carbohydr Polym. 2017;160:123–33.
Stoletov K, Beatty PH, Lewis JD. Novel therapeutic targets for cancer metastasis. Expert Rev Anticancer Ther. 2020;20:97–109.
Sullivan R, Smith JE, Rowan NJ. Medicinal mushrooms and cancer therapy: translating at raditional practice into Western medicine. Perspect Biol Med. 2006;49:159–70.
Sun Z, He Y, Liang Z, et al. Sulfation of (1→)-β-D-glucan from the fruiting bodies of Russula virescens and antitumor activities of the modifiers. Carbohydr Polym. 2009;77:628–33.
Tian Y, Zhao Y, Zeng H, et al. Structural characterization of a novel neutral polysaccharide from Lentinus giganteus and its antitumor activity through inducing apoptosis. Carbohydr Polym. 2016;154:231–40.

Umemura K, Yanase K, Suzuki M, et al. Inhibition of DNA topoisomerases I and II, and growth inhibition of human cancer cell lines by a marine microalgal polysaccharide. Biochem Pharmacol. 2003;66:481–7.
Vannucci L, Krizan J, Sima P, et al. Immunomodulatory properties and antitumor activities of glucans. Int J Oncol. 2013;43:357–64.
Wang CC, Huang YL, Ren CT, et al. Glycan microarray of Globo H and related structures for quantitative analysis of breast cancer. Proc Natl Acad Sci U S A. 2008;105:11661.
Wang Z, Wang D, Han S, et al. Bioactivity-guided identification and cell signaling technology to delineate the lactate dehydrogenase an inhibition effects of Spatholobus suberectus on breast cancer. PLoS One. 2013;8:e56631.
Wang X, Chen Y, Wang J, et al. Antitumor activity of a sulfated polysaccharide from Enteromorpha intestinalis targeted against hepatoma through mitochondrial pathway. Tumor Biol. 2014a;35:1641–7.
Wang K, Wang J, Li Q, et al. Structural differences and conformational characterization of five bioactive polysaccharides from Lentinus edodes. Food Res Int. 2014b;62:223–32.
Wang C, Feng L, Su J, et al. Polysaccharides from Epimedium koreanum Nakai with immunomodulatory activity and inhibitory effect on tumor growth in LLC-bearing mice. J Ethnopharmacol. 2017;207:8–18.
Warburg O. On the origin of cancer cells. Science. 1956;123:309–14.
Wrodnigg TM, Steiner AJ, Ueberbacher BJ. Natural and synthetic iminosugars as carbohydrate processing enzyme inhibitors for cancer therapy. Anti Cancer Agents Med Chem. 2008;8:77–85.
Wu J, Gao W, Song Z, et al. Anticancer activity of polysaccharide from Glehnia littoralis on human lung cancer cell line A549. Int J Biol Macromol. 2018;106:464–72.
Xu L, Liu X, Li Y, et al. Enzymatic rhamnosylation of anticancer drugs by an α-L-rhamnosidase from Alternaria sp. L1 for cancer-targeting and enzyme-activated prodrug therapy. Appl Microbiol Biotechnol. 2019;103:7997–8008.
Yin M, Zhang Y, Li H. Advances in research on immunoregulation of macrophages by plant polysaccharides. Front Immunol. 2019;10:145.
Zhang J, Liu YJ, Park HS, et al. Antitumor activity of sulfated extracellular polysaccharides of Ganoderma lucidum from the submerged fermentation broth. Carbohydr Polym. 2012;87:1539–44.
Zhang Y, Kong H, Fang Y, et al. Schizophyllan: a review on its structure, properties, bioactivities and recent developments. Bioact Carbohydr Diet Fibre. 2013;1:53–71.
Zharinov GM, Bogomolov OA, Neklasova NN, et al. Pretreatment prostate specific antigen doubling time as prognostic factor in prostate cancer patients. Onco Targets Ther. 2017;4:7.
Zong A, Cao H, Wang F. Anticancer polysaccharides from natural resources: a review of recent research. Carbohydr Polym. 2012;90:1395–410.

Marine Polysaccharides in Pharmaceutical Uses

33

Chitin, Chitosan, Alginate, and Carrageenan

Rajendra Sukhjadeorao Dongre

Contents

Abstract

Marine offers varied ingenious biomolecules, like polyethers, lipoglycoproteins, and polysaccharides executing native bio-functions including tissue-receptor, cell-growth/separation, antimicrobials, antifungal, and antiviral. Marine domain is emergent via biotechnology progresses and manageable in-vitro microorganism growth

R. S. Dongre (✉)
Department of Chemistry, RTM Nagpur University, Nagpur, India

J. M. Oliveira et al. (eds.), *Polysaccharides of Microbial Origin*,
https://doi.org/10.1007/978-3-030-42215-8_43

through assorted plants, animals, and micro-organisms (bacteria, algae, fungi, sponge, seaweed). Marine algae alone are richest source of many polysaccharides: chitin, chitosan, agar, alginate, and carrageenan's. Marine polysaccharide are favored by multi-disciplinary fields including cell-science, biotechnology, biomedicals, pharmaceutics, medicines, and nano-engineering due to unique features like stability, economy, abundance, biocompatibility, biodegradability, and nontoxicity. Chitin, chitosan, agar, and carrageenan cater 50% marketed formulations such as nano-particles, scaffolds, membranes, and gels to combat various ailments, like cancers, microbial-infections, and injuries. This chapter spotlight current facts/findings with respect to production, physicochemical characteristics, bioactivity, discovery and novel utility of agar, alginate chitin/chitosan, and carrageenan in pharmaceutics. Especially focus on bio-appealing strategies like skeletal alteration, matrix-formation, and cross-linking of marine polysaccharides in pharmaceutics.

Keywords

Marine · Polysaccharide · Pharmaceutics · Chitin · Chitosan · Agar · Alginate · Carrageenan

1 Introduction

The concept of utilizing ocean environment as the latent prime resource for various natural products was outlined in the early of nineteenth century. Later in the mid of twentieth century more interests were drawn by marine organisms as a feedstock of assorted biomolecules for exploiting native bioactive properties in pharmaceuticals. As such in 1960, Nigrelli and Jakowska had extracted holothurin toxins from sea cucumber *Actinopyga agassizii* as the first bioactive chemical known for antitumor activity (Dongre 2017). Till now, myriad natural products, biomolecules, drugs, and polymers owing pharmaceutics significance are procured from many sea species. Marine becomes great hub for natural products/molecules right after modern technologies involve in study of aquatic ecosystems. Although marine R&D had started quite 70 years ago as resulted nearly 10,000 bio-derivatives including polysaccharides are obtained from varied marine organisms like sponges, mollusks, tunicates, and bacteria while a few have resulted fruitful in scientific studies (Dongre 2019). About 52 marine invertebrate-derived bio-compounds could reach real clinical trials, and mere seven isolated bioactive organics are granted till date (Dongre 2017, 2019). Varied marine-based polysaccharides offer myriad utilities in pharmaceuticals and biomedicals are mentioned in Fig. 1.

2 Marine-Derived Assorted Polysaccharides

Naturally, the glycan polysaccharide or carbohydrate exists via holding monomeric glycosidic and polymeric sugar units as coupled with varied biomolecules like amino-acids, peptides, proteins, and lipids to be used for energy-storage

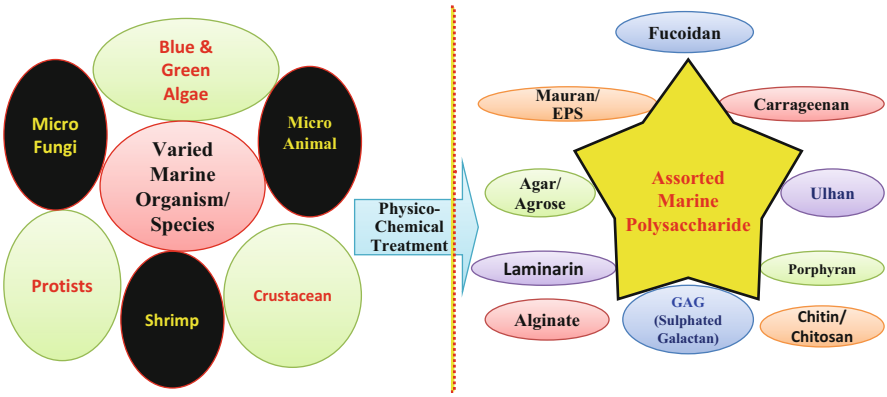


Fig. 1 Marine derived polysaccharides in pharmaceuticals and biomedical fields

along with structural supports (Joshi et al. 2019). In fact cellulose is the first and chitin is the second most abundant polysaccharides viable for structural strength in plant's cell walls (along with marine algae) and animals, respectively. Tunic chemical family includes tunicin owing proteins and complex carbohydrates is also called as the animal origin cellulose yields from marine-based tunicates-invertebrate. Marine-based brown algae species produce many polysaccharides like laminarin, fucoidan, and alginate, while ulvan is obtained from green algae (Gray et al. 2013).

Agarose and porphyran are sulfated-polysaccharides attained through red seaweeds genus. Agar polysaccharide is obtained from elidium/gracilaria red algae along with seaweed. Many marine algae are copious and cheap sources of the bioactive carrageenan's and other sulfated polysaccharides like fucoidan and laminarin. Carrageenan is galactosan with alternate 1,3- and 1,4-galactose units as subsist in cellulosic crack of seaweeds. Exopolysaccharide (EPS) is higher heteropolysaccharide owes 3/4 different monosaccharides like pentoses, hexoses, amino-sugars, and/or uronic acids [<10 units] with residual negative charge and acidic properties due to high sulfate and uronic contents aiding marine species to sustain in acute alkaline seawater conditions. Diverse marine microbes like bacteria, cyano-bacteria, action-bacteria, and fungi contain novel exopolysaccharide as a extracellular component of cell-walls owing remarkable compositions, properties, and structures to deliver native functions like adhesiveness, cell-protection, disinfectant, anti-carcinogenic, nutraceuticals, cosmetics, detergents, and pharmaceutics (Dongre 2017, 2019; Joshi et al. 2019; Gray et al. 2013).

Chitin is the second copious biopolymer in nature after cellulose being broadly distributed in marine invertebrates, insects, fungi, yeast, and crustacean but not found in higher plants/animals (Ravi Kumar 2000). Sea-based polysaccharides put a basis/template to prepare superior micro/nano-matrix contenders viable for pharmaceutic activities, viz., anticoagulant, antioxidant, anticancer, anti-inflammatory, anti-proliferative, anti-angiogenic, anti-metastatic, antiviral, antitumor, anti-parasitic, and immunomodulator (Dongre 2017; Honarkar and Barikani 2009). Various biomaterials

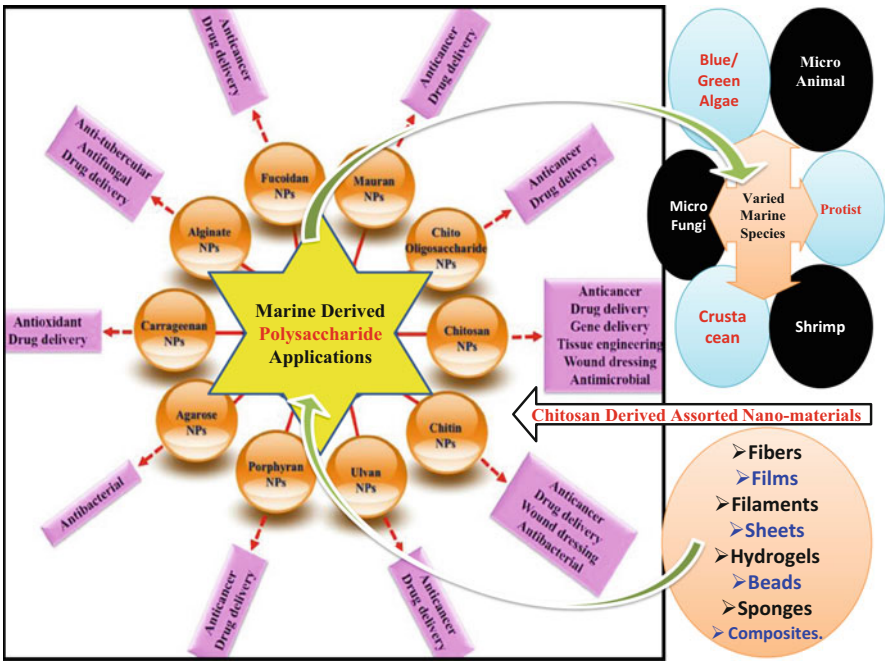


Fig. 2 Various marine biomaterials formulated prospects in biomedicals and pharmaceuticals

are derived from marine-based polysaccharides to formulate new prospects in biomedicals and pharmaceuticals as shown in Fig. 2.

The field of natural polysaccharides derived from marine resources gets quite huge and ever escalated due to never lasting market demands. Seaweeds, fish, microbes, crustaceans and fungi are prominent and profuse resources of alginates, agar/agarose, carrageenan's and chitin/chitosan Chitin is extracted from the exoskeleton of marine crustaceans and lower animals, like bristles of polychaetes and hydrozoan thecae, cyst walls of ciliates, and cell walls of bacteria. Cellulose, amylose and many other carbohydrates are extracted from the macroalga, *Ulva* found in coasts of Mediterranean Sea-Venice lagoons (Jayakumar et al. 2010a). Many polysaccharides are derived from marine-algae as market available data gain in 2003 is shown in below Table 1.

An intracellular compartmental drug delivery by encapsulated nano/microparticle is a challenging task in varied therapeutics. Rifampicin trapped in nano-alginate boosts efficacy in intracellular compartment delivery inside polymorphonuclear leukocyte and resolves bacterial infections quickly and efficiently. Drug entrapped into marine polysaccharide skeleton staid bioactive and bioactivity gets retained on its discharge. Marine polysaccharide sustained drug delivery offers unique advantageous like lessen dosing rate, low-toxicity, permit long-term action, and less-side effects (Gortari and Hours 2013).

Table 1 Marine-based Polysaccharides from macro-algae (market available data in 2003)

Sl. No.	Marine polysaccharide	Yield (tons/ annum)	Algae used (tons/annum)	Marine species/sources
1.	Alginate	30,000	126,500	*Laminaria*, *Macrocystis*, *Lessonia*, *Ascophyllum*, others
2.	Agar-agar	7630	55,650	*Gelidium* and *Gracilaria*
3.	Chitin Fibril	20,000	90,000	Cyanobacteria, green, axenic microalgae
4.	Carrageenan	33,000	168,400	*Eucheuma*, *Kappaphycus*

3 Marine Biomass: A Great Resource for Many Polysaccharides

Marine biomass appears to be raw/feedstock for biofuels as well as a good resource in order to derive numerous polysaccharides. Microalgae are mainly interesting in both sectors by virtue of its control and facile growth conditions in a bioreactor beside established biochemical diversity of such marine-derived biomolecules. Advanced biotechnology has developed better screening and selections for vital marine-based bioactive molecules including agar, agarose, alginate, chitin, and carrageenan polysaccharides (Dongre 2017; Gortari and Hours 2013). Some micro-algae like unicellular red algae *Porphyridium cruemtum* and P. aerugineum, besides blue-green algae *Chlamidomonas* spp. and *C. mexicana*, own commercial worth due to innate resources to many extracellular polysaccharides (Dongre 2017, 2019). *Porphyridium* polysaccharide has substituted current polysaccharides carrageenan in many pharmaceutics applications. Assorted marine habitats including deep-sea hydrothermal vents, cold seeps, coastal hot springs, polar-regions, and hypersaline-ponds are the vast source of unknown and uncultivated bacteria, so global attention raised toward extreme marine species (Mao et al. 2013). Many marine species including microbes/bacteria have developed special metabolism to survive and attenuate such extreme conditions, thus creates unique bioactive molecules like exopolysaccharide to defend antagonistic and other microorganism (Dongre 2017). Plentiful polysaccharides are performing structural and other functions in varied marine species, but this chapter overview vital members like agar, alginate, chitin, chitosan, and carragenan for their attracting bioactive properties and prospective in the biomedicals and pharmaceuticals (Dongre 2019). Algae, sponge, and fish marine species owe innate defense system induced by specific and potent biomolecules to endure in antagonistic and severe conditions like high salinity, pressure, temperature, darkness, beside attacks by microbes/viruses. Many marine polysaccharides are plentiful like chitin, glycosaminoglycan, agar, alginate, and carrageenan performing varied bio-functions in ocean species as well as in pharmaceutics applications in cancer therapy, drug delivery, bio-engineering, sensors/markers, wound dressing, and water purification fields. Marine polysaccharide research is vast due to native

diverse applicability like drug/cell/gene delivery, tissue engineering, cancer therapy, wound dressing, biosensor/marker, and bio-adsorbent. Several polysaccharides like fucoidan, alginate, carrageenan, agarose, porphyran, chitin/chitosan, chitooligosaccharides, ulvan, and mauran offer vital bioactivities in the marine organisms, yet not fully exploited (Dongre 2017, 2019; Joshi et al. 2019; Gray et al. 2013; Ravi Kumar 2000; Honarkar and Barikani 2009; Jayakumar et al. 2010a; Gortari and Hours 2013; Mao et al. 2013).

4 Principals of Physicochemical Modification in Biopolymeric Milieu

Assorted physicochemical modifications (Dongre 2017) are executed in marine polysaccharides to upgrade innate features like low mechanical strength, toxicity, solubility, biocompatibility, biodegradability, and designing or manufacturing as mentioned in following approaches and Fig. 3.

- Blending or chemical linkages with synthetic biopolymers
- Surface coating of micro- or nano-spheres with biocompatible synthetic polymers
- Crosslinking with different physical or chemical reagents (Pillai et al. 2009)
- Hydrophobization through various physicochemical reactions
- Derivatization via assorted chemical treatments

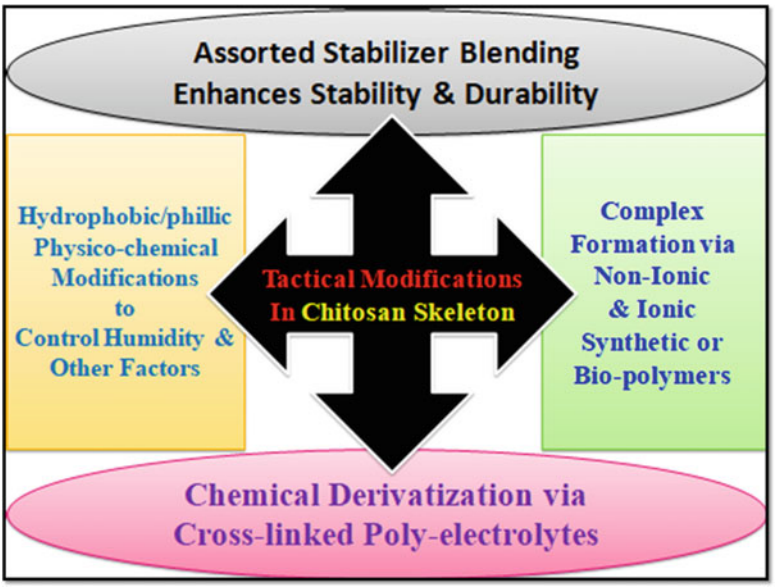


Fig. 3 Assorted physicochemical modification in polysaccharide skeleton

- Modulation of guluronic/mannuronic contents
- Degree of de-acetylation especially in chitooligosaccharides (Yaghobi and Mirzadeh 2004)
- Extent of depolymerization (in all polysaccharide)

5 Patents on Marine-Based Polysaccharides

Many marine polysaccharides and low molecular oligosaccharide-derived nano-materials offer good pharmaceutical and biomedical activities such as nano-medicine, diagnosis therapeutic diagnosis, antimicrobials, drug/gene/cell delivery, tissue engineering, cancer therapy, wound dressing biosensors, and water purifications. Till date about many patents are granted on marine polysaccharides-based nano-materials applications. Overall these patents claimed include numerous nano-composites and nano-particles grown via assorted marine stem polysaccharides, including alditol, monosaccharide, and oligosaccharide. Bioactive marine polysaccharides carry many bio-signals onto designed polymeric skeleton thus acts as efficient biomarkers and biosensors beside antimicrobials. Such nano-composite attain 3-D matrix via many synthetic alterations onto neutral/anionic/cationic polysaccharide composition wherein metallic are uniformly dispersed and stabilized. These market available patents on sea-derived polysaccharides for are used by global researchers to make numerous promising excipients in biomedicine, fabric, food, and pharmaceutical (Dongre 2017, 2019; Joshi et al. 2019; Gray et al. 2013; Ravi Kumar 2000; Honarkar and Barikani 2009; Jayakumar et al. 2010a; Gortari and Hours 2013; Mao et al. 2013).

6 Production, Applications, and Modification Approaches for Marine Polysaccharide

Varied biochemical procedures apt in intense marine environments which form the basis for breakthroughs in biotechnology being helpful to mimic the extremophiles besides explore novel bioactive formulations to be used in industries, agriculture, environment, clinical, and pharmaceutics. About 30% natural products are isolated and developed from the marine organism's derived polysaccharides. Assorted polysaccharide-based biomaterials are emerged in several purposes like tissue/scaffold revival, cartilage replacement, drug carrier/release devices, and gel-entrapping in cell immobilization. Marine-derived polysaccharides offer unique features such as controllable bio-activities, nontoxicity, biocompatibility, biodegradability, and hydrogel formation tendency. Most polysaccharides are obtained from sea sources particularly plant/algae originated kelp-derived alginates and carrageenan's along with crab shells yield chitin owe huge significance in medicine, pharmacy, and biomedicals. Recently marine polysaccharide-derived bio-materials have attributed new synthetic routes via physicochemical alterations, in order to endorse innovative bioactivities and/or to modify ultimate properties for desired applicability. Various

synthetic strategies are employed in blending/mixing such polysaccharides with other artificial and natural polymers so as to cascade the features of both milieus. Assorted advanced approaches and applications of sea-based polysaccharide-derived biomaterials and composites owing known potential significance in myriad fields of S&T (Chang et al. 2010; Jayakumar et al. 2010b; Zohuriaan-Mehr 2005).

7 Biotechnology for Marine-Based Biomolecules

Marine species adapt native bio-mechanisms to survive in severe environments also fascinated global scientists to found the basis of many superior biotechnological innovations. Advanced biotechnology is ever involved in exploring assorted new molecules from sea environments in order to cater never ending demands in pharmaceutics and medicals. Today, most of the drugs in the market are derived from carbohydrate class found in marine environments/organisms. Also, the industrialization of the marine-based microbial products and formulations are under trial conditions. Marine species are believed to be ample sources for productions of bioactive and/or new chemical molecules like oligosaccharides achieved through designed large-scale bioreactors processing (Ravi Kumar 2000). Marine biotechnology gifted several fruitful products for medicine, food, bio-energy, biofuel, nanomaterials, and biomedicals. Mere 5% of vast sea environment is explored for this purpose. In fact, biotechnology is systematic expanding zone which harness vast and hidden practical diversity of marine life owing enrich array of bio-designs beside novel biosynthetic abilities. Such quest has attributed myriad inventions including novel genes, compounds, bio-materials, and pharmaceuticals and medicines through sustainable usages and management of the earth oceans. Various modern engineering bioprocesses have developed for extraction of assorted polysaccharides and other biomolecules from marine species including lab-bioreactors batch/fed-batch and pilot implants (Dongre 2017; Ravi Kumar 2000; Böttcher et al. 2018). Certain viable pharmaceutical and biomedical applications of marine-derived polysaccharides are mentioned in Table 2.

Marine biotechnology prop up economic recovery and growths besides caters key societal challenges by delivering novel knowledge, products, and services including sustainable supply of feedstock for energy, fuel, and medical. Need of marine-based polysaccharides are ever rising day-by-day to convey rapid biotechnological progresses in pharmaceutics and biomedical. Marine biotechnology added significant values in efficiency and quality of products by exploring novel bio-species for growing physiological demands (Chiu et al. 2012). Ocean is an untapped, sustainable source of wide variety of bio-species/chemicals to cater vital confronts in marine biotechnology in the twenty-first century. Marine is a potential resource for novel drugs, innovative biomolecules, and diagnostic products for our health. Many biopolymers are obtained through marine species for the medical and biotech applications way from biocompatible plastics to food additives, pharmaceutics, wound dressings, bio-adhesives, dentistry, tissue regenerative, and 3-D bone-scaffolds (Dongre 2017; Barikani et al. 2008). Presently, about 15 natural polysaccharides are derived from marine-organisms

Table 2 Pharmaceutical and biomedical application of marine-derived polysaccharides

Sl. No.	Marine resource	Polysaccharides	Applications
1.	Brown algae	Fucoidan	Cytotoxicity, anticancer
2.	Brown algae	Alginate	Drug delivery, anti-tubercular, antifungal, antitumor
3.	Red algae	Carrageenan	Antioxidant, drug delivery
4.	Red algae	Agarose	Antibacterial activity
5.	Red algae	Porphyran	Cytotoxicity, drug delivery
6.	Shrimp and crab	Chitin/ Chitoligosaccharide	Wound dressing, antibacterial activity, tissue, drug delivery, anticancer agent
7.	Shrimp and crab	Chitosan	Bone-scaffold, dentistry-template, bio-adsorbent, antimicrobial, engineering, anticancer, dressing, gene/tissue release
8.	Mauran	Bacteria	Drug carrier
9.	Bacteria	Extracellular Polysaccharide	α-Glucosidase inhibition
10	Laminaria Jponica/ brown algae	Laminaran	Blood glucose reduction, Insulin and amylin enhancements
11.	Red Algae	Galactan	HSV-1 & 2, HIV-1 & 2 Inhibitions, Antiviral
12.	Diatom, Navicula Directa	Naviculan	HSV-1 & HSV-2 Inhibitions, Antiviral
13.	*Arthrospira Platensis*	Spirulan	Measles, mumps, polio, influenza, coaxsackie anti-activities

and put in various phases of clinical tests, mostly in oncology, with few in pipeline and more are already popularized in the market (Tsioptsias et al. 2009). Marine-based biomaterials field is quite young as sea environment is pretty unused font to find new enzymes, biopolymers, and chemicals for pharmaceuticals (Sashiwa and Aiba 2004). Marine biotechnology role served in assorted domains with futuristic aspect is summarized in Table 3.

Marine Biotechnology research addresses key societal challenges and targeted area with progressive priorities and objectives in S&T. Natural marine-derived biomolecules are widely employed multifunctional pharmaceutical excipients in the formulation of diversified forms, e.g., polysaccharides like agar, alginate, carrageenan, fucoidan, chitosan, and hyaluronan are used as binders, vehicles, careers, disintegrating agents, gelling agents, and drug release/sustainers (Böttcher et al. 2018; Dongre 2018a). Marine polysaccharide owns precise pharmacological activity for the same aliment/disease, e.g., anti-cancer active drugs. Medical and industrial biotechnological formulations offer well-distinct bio-paths and bio-receptors known for better pharmacological actions as shown in Fig. 4.

Such marine-derived pharmaceutical excipients abet many built-ups by providing creative roles as binders, diluents, wettings, fillings, disintegrates, and dissolvents, shown in Fig. 5.

Table 3 Marine biotechnology role served in assorted domains/sectors (Böttcher et al. 2018; Chiu et al. 2012; Barikani et al. 2008; Tsioptsias et al. 2009)

Sl. No.	Purpose	Viable applicability
1.	Health ingredients, foods & products	For huge nutrition from marine algae, invertebrates, fish, crustacean Develop innovative methods for bio-systems with particular breeding of aquaculture/sea-origin species Build biotechnology function/mode to retain aquaculture productions Grow optimal defensive and healing assessments Advance ecological benefits, sustainable technologies for feed supply, Expand zero-waste recirculation systems Integrate new, low environmental impact feed ingredients Improve quality of products and human health benefits
2.	Energy fields	Develop viable renewable energy products & processes by marine algae Produce an inventory of microalgae resources for biofuel production to support optimization of the most appropriate strains Gain knowledge of bio-functions to make tools for steering metabolism & to cultivate many marine microalgae Enhance photosynthetic efficiency & augment lipid productivity Procure microalgae with optimum character for mass cultivation Produce biofuels and make bio-refinery Develop efficient harvest, separation/purification for micro/macroalgae
3.	Health aspects	Progress of new drugs, treatments for health & personal care products Increase R&D in taxonomy, systematic, physiology, molecular genetics and chemical ecology of marine species and organisms Boost chances of accomplishment in discovery of novel bio-actives Improve technical aspects of bio-discovery pipeline Separate bio-actives, bio-assays to put up diverse marine materials Develop strategies and software for structure determinations Overcome crisis to provide sustainable source of new pharmaceuticals & healthcare products Advances scientific inputs in aquaculture, microbial, tissue culture, chemical synthesis, and biosynthetic fields
4.	Environments	Address key issues in biotechnology, environment, and human society Develop automated high-resolution biosensor technologies for in situ marine environmental monitoring Assess coastal water quality, prediction, and detection of harmful algal blooms and health hazards Cultivate cost-effective and nontoxic antifouling technologies Cascade novel antifouling bio-compounds and surface

(continued)

Table 3 (continued)

Sl. No.	Purpose	Viable applicability
		engineering consolidate DNA-based technologies for organism, population identify and support progressive industrial tools, and platforms for routine analysis
5.	Industrial Processes & Products	Improve marine-derived biomolecules used in industry like enzymes, biopolymers, and biomaterials Assist advance technologies for high throughput enzyme screening to express marine proteins and enzymes through dedicated hosts Make marine-based biopolymers as new competitive commercial products in food, cosmetics, drug, health, and other industries

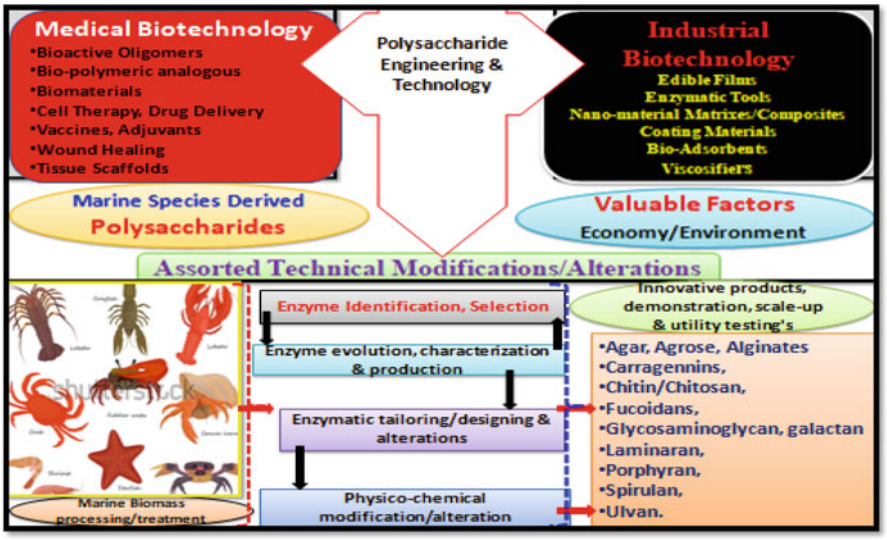


Fig. 4 Medical and industrial biotechnological modification in polysaccharide milieu

Blue biotechnology instituted marine research amid global scientific community and industry manages the new complex challenges in the domain of marine biotechnology (Böttcher et al. 2018; Dongre 2018b).

8 Compact Thrust into Marine Biotechnology Industry

In 2016, marine pharmaceutics market has reached to estimated budget of €8.6 billion with a compound annual growth rate 12.5% for the period of 2011–2016 (Dongre 2017; Böttcher et al. 2018). Global Industry Analysts of market research agency have predicted growing trend for marine biotechnologies with forecasting

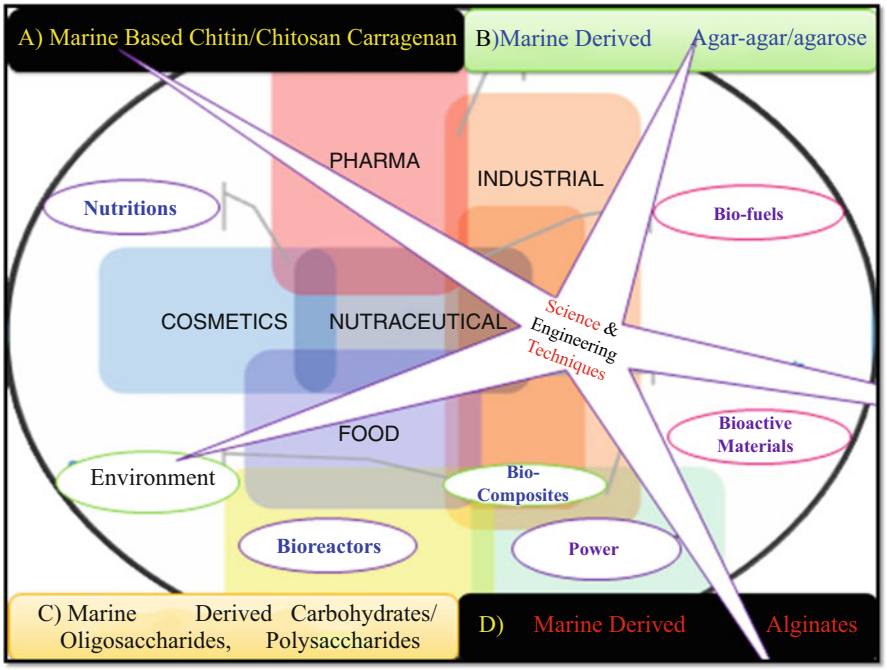


Fig. 5 Viable connectivity in the market services provided by marine polysaccharides

yearly growth rate of 4–5% and trade worth of €5 billion by 2020 span (Dongre 2018b). Marine-derived polymers offer advance bioengineered stuffs as shown in Table 4.

9 Marine Polysaccharide-Based Hydrogels

Various hydrogels are obtained from natural polysaccharides like cellulose, hyaluronic acid, and chitosan as 3-D cross-link covalent bonded polymeric frameworks being extra stable under physiological conditions. Substantial water contents (>90% of weight in swollen state) within such polysaccharide matrix make such bio-systems an exclusive amid hydrophilic gels owing peculiar features like inject ability apt for mini-invasive surgery and local therapy WS (Khotimchenko and Khotimchenko 2020). These hydrogels own massive water ratio which affects innate rheology characters through assorted orientation within its skeleton. Shear stress can be designed in such marine-derived polysaccharide-based hydrogels via water arrangement from a bound to a semi-bound status leads to shrink their mechanical properties by augmented swelling potential (Dongre 2017; Chiu et al. 2012).

Hydrogels and super hydrogels are derived from cross-linked polysaccharides of marine origin to be used for successful drug delivery and gene career besides

Table 4 Marine-polymer-derived advance bio-engineered stuffs (Dongre 2018b; Tong et al. 2011; Dudarev and Iozep 2010)

Marine-polymer	Constitutional parameters	Innate features	Beneficiary aspects
Collagen- I	α-1 & α-2 chains of 50–500 nm diameter Triple helical structure	Biopolymer Exists in variety of sources RGD binding sites	Biocompetent Biodegradable Less cytotoxic and immunogenic
Gelatin	Triple helical denatured collagens	Biopolymer Hemostatic agent Macrophages activator	Biocompatible Non-antigenic Cheap/low cost
Silk fibroin	Hydrophilic and hydrophobic co-block polymerization occurs Exist in low/heavy chain	Biopolymer RGD binding sites Slow degradation rate	Biocompatible High strength No toxic degradation products
Chitin	Amino hetero-polysaccharide	Biopolymer Hemostatic agent Varied degree of acetylation & polymerization	Biocompatible Biodegradable Bio-active Antibacterial Wound healing
Chitosan	*Poly N*-acetyl glycosaminoglycan links	Biopolymer Hemostatic agent Varied degree of acetylation & polymerization	Bioactive, biodegradable Biocompetent Antimicrobial Wound healing Bone/Tissue scaffolds Dentistry templates

making tissue engineering scaffolds. Many super-porous adsorbents can be easily formulated through unique super-porous hydrogels owing optimal swelling ratio and unified micron/millimeter pores as best employed in pollutant remediation. Specially designed interrelated porosity of super porous hydrogels aids to capture enough water/solvents being exploited in assorted functions like drug/protein delivery, fast-softening tablets, occlusion devices for aneurysm cure, cell-culture, tissue-engineered scaffolding, hygiene products, and many more uses (Campo and Carvalho 2009). Impregnated superabsorbent and super porous hydrogels can be obtained with viable elasticity through blending marine-derived carbohydrates like carrageenan, sodium alginate, chitosan, and agar with artificial polymers like polyacrylic acid and polyacrylamide (Dongre 2017; Coviello et al. 2007). Certain hybrid super hydrogels are acquired through such polysaccharides found to offer multi-functional and sustainable release of drugs by virtue of innate cross-linking density leads to super-porous and super-sorbent functions (Kurita 2001).

10 Marine-Derived Polysaccharides for Bio-Adhesives and Muco-Adhesives

Native bio-adhesives contain mixture of polymers amid proteins and polysaccharides are highly involved. Such bio-adhesives let carry assorted species like steroids, anti-inflammatory, receptive peptides, insulin, and proteins beside engage in healing or easiness of pain in the buckle void (Dongre 2019; Kurita 2001). Thriving bio-adhesive do control delivery of active ingredients for prolonged time in the biological systems under wet and moist environments. Marine-derived polysaccharides are exhaustively preferred in preparation of bio-adhesives due to certain unique features like non-toxicity, smoothness, and facile revival. Mucosal membranes own narrow permeability to therapeutics, but tenacity of marine algae grips ship/hull in sub-aqua sphere due to more waterproof adhesion. Practicable bio-adhesives are derived via marine sources offer very high cohesion and binding to the solid surfaces, as desired for surgical setting. Marine-derived chemicals are promising avenue to hunt efficient tissue adhesives for medical purpose like surgery closures and bone-glue. Various algal-based bio-adhesives in ophthalmic therapies and for human tissues are formulated via marine-derived polyphenolic-proteins and polysaccharides (Dongre 2017, 2019; Joshi et al. 2019; Gray et al. 2013; Ravi Kumar 2000; Honarkar and Barikani 2009; Jayakumar et al. 2010a). Chitosan with dextran/starch along with L-DOPA conjugated dextran/starch analogues bio-adhesives are developed to provide high adhesiveness in bone gluing of mussels. Various marine-derived bio-adhesives are preferred by virtue of unique features like components are natural, biodegradable, biocompetent, no cytotoxicity, and thus fine contender for bone/soft tissue gluing in surgery (Dongre 2017; Coviello et al. 2007; Kurita 2001).

11 Tactical Modifications in Marine-Based Polysaccharides

Marine polysaccharides are intrinsically biocompatibility and inexpensive feedstock which play pertinent function in pharmaceutics and biomedicals especially in drug delivery. Yet, crude polysaccharides many times not satisfy requisite conditions needed for specific pharmaceutical applications. So following reinforcement attained through chemical and/or physical amendments in native morphology alters physical–chemical properties of resultant matrixes (Bănică 2012).

11.1 Blending Actions

Blending tailors noticeable physic-chemical features of matrix in an organize way which is preferred for marine-derived polysaccharides to exploit its innate properties in biomedicals and pharmaceutics. Blending alters mixed properties of polysaccharides like biodegradability, bio-adhesiveness, and solubility pattern within its milieu to precise functions (Dongre 2019). Assorted advances in polysaccharide blending are achieved through following tactics:

(i) Adding a different component like natural block/graft copolymer in the parent matrix imparts compatibility amid the blended templates.
(ii) Physicochemical changes in solitary component yields precise and desired mutual interactions among the blended matrixes.

Such strategies crack crisis occurs through grim interfaces amid polysaccharide and blended polymers. Biodegradable blends classically involve mixing of natural and synthetic matrixes which gets poly-dispersed in terms of their mass/weight and thus akin biopolymers. Many marine-derived polysaccharides are blended with natural/synthetic polymers for representative pharmaceutics applications, e.g., alginate-polyvinyl alcohol blend yields apt biodegradation and biocompatibility (Bănică 2012; Sá-Lima 2010). Certain natural plasticizer like glycerol is affixed for advancement of mechanical performances and homogeneity in such alginate-biofilm blend. Chitosan's amine groups also blend with succinic anhydride so as to convey elevated solubility in water medium (Dongre 2017). Synergistic chelation is feasible in alginate-chitosan gelation offering superior mechanical properties in resultant hydrogels. Alginate/chitosan blends owing calcium sulphate in its milieu offers osteo-inductive phase as effective in bone restoration (Gåserod et al. 1998). Biocompatible polysaccharides blending with agar-agar yield reversible and brittle hydrogel to offer cooling effects under hot conditions due to great water-holding ability. Such fabricated agar-based gels own great prospective in biomedicals and pharmaceutics. Agar-alginate fusion imparts inflected water permeability as manageable with blend-compositions being best employed for dehydration of fruits in food industry (Dongre 2017, 2019; Joshi et al. 2019; Gray et al. 2013). Agar is extracted from marine seaweeds called rhodophycae found to contain mixture of agarose and agaro-pectin polysaccharides, first imparts gelling ability and latter reports ionic functions. Agar owes innate ability of reversible gelation through hydrogen bonds, thus forms assorted hydrogel blends (Dongre 2017). Agar blends are utilized in food, medicine and cosmetics industry for many making processed cheese, ice-cream, bread, soft-candy, fragrance additives, and binders in skin care products besides face wash powders. Agar-chitosan blending yield valuable hydrogels and films owing unique thermal, optical and surface properties like eco-friendliness, better puffiness, inert contact angles, and recovered wet-ability to be best employed for making biomedical and pharmaceutical formulations/products. In such blending agar addition lessens tensile strength and elongation at the crack results in peculiar features like enhanced water uptake, superior hydrophilicity, and more gelation (Dongre 2017, 2019; Joshi et al. 2019; Gray et al. 2013; Ravi Kumar 2000; Honarkar and Barikani 2009; Jayakumar et al. 2010a; Gortari and Hours 2013; Mao et al. 2013).

11.2 Physicochemical Alteration

Marine-derived polysaccharides are thoroughly hunted, for bulk and surface alterations in order to make new materials or matrixes for pharmaceutics and

biomedical. Chitin and chitosan both corresponds to fundamental matrixes in such aspects as physic-chemical modifications are quite facile (Dongre 2017). Amid viable functionalities like amino, acid, carboxylate, and amide, the hydroxy groups exist in polysaccharides which is most fragile for further chemical reactions/transformations. Hydroxyl groups undergo numerous reactions like oxidation, reduction, derivatization, and copolymerization to create newer functionality like aldehyde, ketone, ester, and anhydride; ketone alter features such as hydrophilic nature and concern solubility. Amine group is apt for certain chemical modification through concept of click chemistry (Pillai et al. 2009). Such modified polysaccharides of marine sources offered more successful applications in pharmaceutics. Several chemical modifications at native functionalities of polysaccharides impart novel characteristics with manageable properties like solubility and hydrophilicity besides make specific binding/interactive sites for many interested bio-species (Dongre 2017).

11.3 Hydrophobic Modification

Hydrophilic matrix of marine-based polysaccharides are modified by hydrophobic components like surfactant and assorted self-associated polymers to yield complex micelles being best for medical utilities like specific diagnosis, colloidal micelle, and coating to many biomolecules in immune- assays (Jayakumar et al. 2010a). General marine polysaccharide that is based on aminosugars is chitin (N-acetylated) and chitosan (N-de-acetylated) contains proactive amine/hydroxy functionalities are viable for hydrophobic variation. Some preface hydrophobic modifications in polysaccharides include treatment with hydrophilic, acidic, basic, and other active components which in turn improves innate physicochemical properties so as to cater progressive research emphasis in biomedical and pharmaceutical. Marine-based polysaccharide owes nucleophilic oxygen and nitrogen (in chitin) in its skeleton being fragile to assorted enzymatic/biological, physical, and chemical electrophilic attacks and results in formation of variety of derivatives. Chitin is charged polysaccharide gets protonated on nitrogen while alginate is a polyuronate salt gets derivatized or hydrolyzed easily and their limiting innate features like surface activities and solubility are augmented through many hydrophobic modifications. Alginate holds α-1-4-L-guluronic acids joined β-1-4-mannuronic acids while pectin own α-1–4-galacturonic acid methyl ester, both as uronic-acid monosaccharide chains available for oxidation to carboxylic acid at C-6 site also substitution by other mono-saccharides to vary innate hydrophilic/hydrophobic characters. The hydrophobic components like cholesterol and liposome can intrude in the fragile skeletons of marine polysaccharides and apt elevated drug excipient features leads to improved chemotherapy and immunotherapy outputs. Liposome coated cholesterol polysaccharide-1-aminolactose offers exclusive function due to mix mesophase formation like superior hydrophobicity and selective uptake of cancer cells in bile acid capture (Pillai et al. 2009; Zohuriaan-Mehr 2005).

11.4 Depolymerization

Adhesive-based physicochemically modified polysaccharides are fragile for depolymerization and results in curtailed molecular weight besides augmented polar end functionality being valuable for topical and transdermal superior drug delivery/release or penetration. Typical depolymerization of marine polysaccharide is achieved via many physic-chemical and biological methods such as ozonolysis, hydrolysis, radiation drive ionization, oxidation, reduction, and enzymatic degradation performed at various environmental conditions like high temperature, pressure, concentration, and degree of polymerization. De-polymerization has rediscovered polysaccharide skeleton through assorted new synthetic routes for their physicochemical variation that leads to promising new bio-activities for desired purposes. De-polymerization and other synthetic strategies are frequently employed on many marine polysaccharides like alginate, chitin, chitosan, and carrageenan in order to derive novel composites, hybrids and blends owing noticeable functions in pharmaceutics and biomedicals (Dongre 2019).

11.5 Sulfation

Natural marine-based polysaccharides own high sulphate groups available for sulfation easily. However, synthetic sulphated derivatives also acquired through assorted chemical reactions onto other fragile polysaccharide groups like primary/secondary hydroxyls and amines. Sulfation of polysaccharide functionalities impart desired functions due to targeted sites in specific pharmaceutical activities like antiretroviral, anti-malarial, and drug delivery across a mucosal membrane. Synthetic sulphated polysaccharide-based drug excipient lessens intestinal cholesterol and fatty acids absorption in human body through inhibition of pancreatic cholesterol esterase. High molecular weights (greater than 10 kDa) and sulfated at third sugar ring of marine polysaccharide found to enhance inhibition efficacy. Alginic acid, chitin/chitosan, and agar polysaccharide can be easily converted into sulfated derivatives owing unique features like high water solubility and potent pancreatic cholesterol esterase inhibition. Such sulfated polysaccharides are formulated in varied pharmaceutical forms like tablets, capsules, colloidal, liquids, and powders. Cartilage tissue regeneration and segregation of mesenchymal stem cells into chondrocytes is procured from sulphated polysaccharides of marine origin peculiarly glycosaminoglycans (GAGs). Sulfate and carboxylates of GAG interactions with heparin is helpful in designing pharmaceutical features like anticoagulant and assorted tissue revival scaffolds besides perform controlled therapeutic release (Dongre 2019; Yaghobi and Mirzadeh 2004). Exopolysaccharides (EPS) also obtained through marine source like deep-sea hydrothermal vents and bacteria also gets converted to novel GAGs-like molecules being demonstrated for various pharmaceutical and biomedical usages. Alginate is harmless; noninflammatory polysaccharide refined through brown seaweed of Phaeophyceae and being facile to process in water. Hyaluronan (HA) is a recurring linear glycosaminoglycans (GAG) unit of N-acetyl-glucosamine and

disaccharides of glucuronic acid occurred as an anionic, non-sulfated glycosaminoglycan distributed widely all over connective, epithelial, and neural tissues. Hyaluronan a non-sulfated glycosaminoglycans of plasma membrane instead of Golgi apparatus is the main components of extracellular matrix to carry out functions like cell proliferation-migration/separation, connect hateful tumor, close proteoglycan contacts, tie proteins/bio-adhesion, and depict electrostatic interactions with glycosaminoglycans (Dongre 2017). Marine-based oligosaccharides/polysaccharide derivatives are interested feedstock for tailoring many novel biomolecules owing great specificity for tissue engineering and regenerative medicines (Dongre 2017, 2019; Joshi et al. 2019).

12 Pharmaceutically Vital Polysaccharides

12.1 Alginate: $(C_6H_9O_7^-)_n$

12.1.1 Structural Features

Alginate is a monobasic salt of methyl 4-O-methyl-hexopyranosyluronic acid-(1-4)-hexopyranosiduronic acid, i.e., alginic acid/algin. In fact, algin is a bio-polysaccharide obtained from Phaeophyceae: brown seaweed which own 1->4 linked polyuronic owing three main blocks, namely, M-block containing repetitive β-D-mannuronic acid, G-block holding consecutive poly α-L-guluronic acid, and mixed MG-block having alternative pattern of both polyuronic acids in its skeleton. Alginate salts are solid in nature owing fair water solubility (Augst et al. 2006).

12.1.2 History and Chemistry

Alginic acid is also known as E400 owing molecular formula [D-ManA(β1→4) L-GulA(α1→4)]$_n$ was first extracted and patented by Edward Stanford in 1883. It is a high-molecular weight polysaccharide exists in varied kelps and bacteria, also extracellular contents in brown algae *Pseudomonas aeruginosa* and *Azetobacter vinilandii* (Drury et al. 2004). Its commercial production had started in 1927, which is expanded to about 50,000 tonnes per annum worldwide. Alginates exist as gel in the cell wall of algae and intercellular matrix to provide skeletal supportive strength and flexibility to these seaweeds. Basically alginate is anionic linear heteropolysaccharide of β-D-mannuronic acid and α-L-guluronic acid blocks with sequential distribution which found varying with species and sources based on seasonal and geographical changes. Medical and pharmaceutical fields owe great interest for alginates in particular by virtue of specific applicability such as esophageal reflux cure, dermatological calcium fibers, wound healing scaffolds, and dental filling gels. Alginates akin to pectin for effect of ionotropic gelation due to induce sol-gel-transition channel-pores as are controlled through various physicochemical factors such as sol/gel concentration, conformer nature, pH, and temperature (Lee and Mooney 2012). Alginate is over synthetic polymer by virtue of unique native

features like non-toxicity, mild pH, and temperature induced hydrogel formation, biocompatible, biodegradable, cheap and copious.

12.1.3 Property

The chemical composition of α-L-guluronic acid/G and β-D-mannuronic acid/M blocks along alginate units affects its ability of gelation in acidic pH. These blocks of high purity are obtained from algae at Madagascar coasts owing variable molecular weight distribution as per M/G acids ratio. Alginates yield through Sargassum species (SG1/SG4) are rich in α-L-guluronic acids. Every such M/G block establishes supportive inter-chain contacts via hydrogen bonding amid GG blocks at $pH < 3.41$, while MM blocks phase- gets separated at $pH \leq 1.9$. Commercial grade alginate own M/G ratio 1.3 viable for gelation at $pH < 3$ with utmost modulus G' observed at $pH = 2.28$. Thus gelation tendency of alginates in acidic conditions is mainly dependent on viability of GG blocks at junctions. The gels preformed with calcium ion are stronger along with swelling degree 50% higher than that of sodium salt of alginate gelation due to defended electrostatic repulsions in pH at intrinsic pK of acids via firm M block hydrogen bonding. The molecular weight of alginate is 216.12 g/mol and molar mass of aliginic acid is 10,000–600,000 with density 1.601 g/cm^3 and pKa is 1.5–3.5.

12.1.4 Utility of Alginates

Alginate is hygroscopic in nature so water gets quickly absorbed in its textures being beneficial and apt as an additive in many dehydrated products like slimming aids, and in paper besides textiles manufacturing. Alginate formulations are used in making waterproofing and fireproofing fabrics, thickening agent in drinks, ice cream, besides gelling agent in cosmetics and food engineering (Szekalska et al. 2016). Alginate is a vital component in varied pharmaceutics preparations like Gaviscon. Sodium alginate acts as an impression-making stuff in creating small-scale life casting, dentistry and prosthetics besides as a thickener for reactive dyes in textile screen-printing. Alginates not interact with such dyes and get washed away, dissimilar to starch thickeners. Rather alginate formulations are amenable to sterilization and storages, thus frequently used as disintegrates, tablet binder agent, cell-encapsulation, and controlled-drug delivery in biomedical and pharmaceutics (Chang 2015).

Drug Delivery by Alginates

The polyelectrolyte loaded alginate microspheres found to show zero-order release kinetics resulting 100% drug delivery/carrier phenomenon. Alginate-based drug delivery potency is controlled by means of hydration/barrier around the tablet, i.e., drug diffusion and water incursion both affects drug delivery as water soluble drugs gets released by diffusion, but poorly soluble drugs are released through erosion. However, modulated drug delivery can also be attained via pH-independent hydrocolloidal gelling agents or muco-adhesive like chitosan addition in alginate milieu. Alginate links with divalent metal ions in ionotropic manner induces gel-spheres in acidic conditions being best for drug-encapsulations. Alginates composites/

formulations found to suffer rapid erosion at neutral pH; also, tissue muco-adhesion tendency gets reduce via metal cationic cross-linking. At low pH due to hydration alginate forms highly viscous acid gels which offer unique properties over neutral macromolecules. While at neutral pH conditions, alginates suffer mild gelation viable for unique features like protein resistance, low-toxicity, oral drug delivery, and immunogenicity. Alginate amends performance of chitosan entraps microspheres in drug delivery due to innate superior adhesiveness. Amine/acid substrates can be incorporated into sodium alginate to optimize the resultant drug release via modulated parameters including erosion span, delivery rate, and great muco-adhesion. Adhesive copolymers further boost muco-adhesion and impart gastric defensive coats being helpful in heartburn and acid reflux treatments. Various alginate-based hydrogels are most promising for certain biochemical functions like tissue regeneration/creation and cell docking proliferation (Drury et al. 2004; Lee and Mooney 2012; Szekalska et al. 2016; Chang 2015).

Alginate matrixes can trap bio-species/agents, drugs, and proteins in order to carry its effective delivery or release. Relative mild gelation of alginate aids loading and release of entrap species through its matrix without loss of native bio-activity. Many pharmaceutics formulation uses impulsively in situ prepared alginate gel for physiological administration, otherwise gelling agent is to be added in formulations or separately (Wang et al. 2015; Ausili et al. 2013; Bale et al. 2001). Micro-encapsulated gels which gets denatured/degraded fast in hostile milieu are specifically made for the oral delivery of proteins. Proteins are encapsulated in polyethyleneglycol-alginate gels due to its resistance, low toxicity, and immunogenicity and control release rate besides biodegradable protection. Chitosan/PEG-alginate micro-encapsulations are used in oral delivery of macro-biomolecule like albumin peptides, and hirudin. Alginate-cyclodextrin composites own covalent polymeric chains viable for sustainable hydrophobic drug delivery due to gifted cumulative features like size/shape specificity and superior matrix/composite transportation. Many solid disposable formulations are developed through alginate for effective release of oral tablets, microcapsules, implants, and topical delivery systems (Khan et al. 2012). Monolithic uniformly dispersed tablets are obtained from alginate for its effective release by means of native viscosity assisting water penetration and also provide dispersal barrier. Micro/nano-capsulated alginate gels with size >200 μm are easy to prepare in aqueous conditions at pH < 4 using cross-linking agent $CaCl_2$. Stable microspheres of size <10 μm are obtained by adding surfactant while emulsification in ultra-sonicator followed by $CaCl_2$ treatment for efficient ionotropic gelation. Alginate scaffolds owe some limitations like quick erosion at neutral pH and low mucosal tissues adhesion can be rectified through bio-adhesive and delay residential formulations. Subsequently alginates matrix is mixed many bio-adhesives like chitosan, poly-lysine and vegetable oils in order to eradicate its limiting factors and to carry out floating drug delivery (Dongre 2018a). Floating alginate beads are obtained by treatment with foaming agent $CaCO_3$/$NaHCO_3$ in $CaCl_2$/acetic acid impart floating drug delivery due to lower density than gastric fluids (CO_2 gas stays within beads own low density and high porosity) thus delayed its residence span. Alginate frameworks are modified by treatment with

synthetic polymers like acrylic and poly-dimethylaminoethylacrylate to get sustain drug delivery, bio/mucoadhesion, and fencing irritations plus inflammations of gastrointestinal membranes (Sakai et al. 2010).

Wound Healing with Alginates

Calcium alginate is hemostatic, so preferred for dressings in extreme bleeding wounds, besides gelation tendency aids easy exclusion of dressing without pains. Calcium alginate also acts as micro-encapsulation in assorted medical products like skin injury dressings to promote healing as it is too easy to confiscate without pain compared to usual wound dressings (Wahl et al. 2015). Alginate hydrogel or bulk gels owing micro-particle gets combined with nerve growth factor to stimulate brain tissue for possible regeneration. Alginate composites offer desirable features like improved porosity, cell proliferation, and mechanical strength which boost bone reconstruction/regeneration. Dressings for wound healing are made through alginate to disinfect various secreting lesions in deeply oozing wound. Alginate hydrogel-derived dressings act as absorbent to furtive fluids from wound, so reduce further oozing and avoid infections beside sore-entrap fibers are biodegradable (Khan et al. 2012; Wahl et al. 2015). Further such alginate-based dressings can protect physiological moisture via granulation of tissues and encourage the curing effects. Alginates dressing exclusion not hinders the healing granulation tissue as it gets rinsed by saline solution and its change is easy. Calcium alginates augment certain bio-functions like propagates fibroblast, cuts, human dermal fibroblasts micro-vascular endothelial cell (HMEC) proliferation/motility and reduces keratocyte (Wahl et al. 2015). There was no significant effect of calcium alginate on the formation of capillary-like structures by HEC. An effective cell proliferation and migration by HMEC found mediated through released calcium ions. Alginate formulations act as valuable hemostatic agents for harsh cavity wounds due to good prothrombotic coagulation and platelet activating effects. Alginates dressing obtain hemostasis in an apicectomy cavity after its usage, since fibers left in situ extract stable and symptomatic adverse florid foreign body reactions. Certain crucial factors like viscosity, molecular mass, morphology, composition, calcium ratio, ionic strength, and pH decide swelling/gelling phenomenon in alginate-based scaffolds dressing. Homogeneous gelation of alginates yield via many in-situ alterations like slow calcium release, cross-linking, internal settings, sequestering with phosphate, citrates, EDTA, low soluble salts/$CaSO_4$, and insoluble electrolytes/$CaCO_3$ thus forms very regular gels. Uniform and well-controlled properties are obligatory for endorsing assorted tissue engineering, biomedical, and pharmaceutical applications (Niekraszewicz and Niekraszewicz 2009). Flux of cross-linking calcium is achieved through chemical treatments through bi-functional cross-linkers like glutaraldehyde, EDTA, sodium citrate, phosphate, citrate, and lactate. Alginate gels are stabilized by many treatments like multivalent Ti/Al ionization, sol-gel transition through sodium, magnesium, and EDTA along with maintaining Na/Ca ratio $<25:1$ for high-guluronate gelation and $<3:1$ for low-guluronate gelation (Lee and Mooney 2012).

Cell Immobilization Via Alginates

Immobilized cells are advantageous than free cells due to unique features like superior cell density, facile medium partition, incessant function, low adaptation/lag phase, good conversion, less reactive time, and control simulation. In this sense, calcium alginate beads and other alginate formulations are usually preferred for cell immobilization. Alginates own some limiting features like gel degradation, strict mass transfer, low flux strength, and large porosity causes fragile cell release from the support. But encapsulation efficiencies are optimized to avoid fast cell release and cell anchorage through chitosan and glutaraldehyde cross-linking in alginate milieu. Polymer modified alginate beads boost cell immobilization due to rewarding features like facile medium separation, reusable templates, low stains, and stable continuity without antibiotic use (contamination is problematic in free-cells). Assorted alginate-derived gel-entraps are frequently used in cell immobilization due to native suspension cells as cultivated in several bioreactors (Ausili et al. 2013). Huge dense network and hydrophilicity of alginate limits cell growths and survival for viable cell immobilization as augmented by biocompatible co-blocking with polymers like PEG-alginates. Reusable and robustness beads are developed from calcium/chitosan blending in alginate matrix *for* trapping *Saccharomyces cerevisiae* cells in tubular bio-syntheses like fermentation of glucose, sucrose fermentation, and ethanol making. In conclusion, with immobilized cells it was possible to carry out eight sequential reuse cycles, generating a stable final ethanol concentration in each cycle. In biomedical fields many alginate-based rapid, nontoxic, and versatile matrixes are made for immobilization of cells, tissues, enzymes, and hormones. Also alginates immobilized matrix are extensively employed in developing artificial organs and scaffolds besides in illness treatment like Parkinson's, chronic pain, liver failure, and hypocalcemia (Lee and Mooney 2012). Calcium alginate is used for immobilizing enzymes by entrapment phenomenon. Immobilized enzyme gets attached onto an inert, insoluble calcium alginate surface and affords augmented resistance in varying conditions of pH and temperature/heat. Alginate let enzymes to be held in place during this interaction, which simplify splitting from the products and thus reused repeatedly. Such enzyme entrapment technique is quite proficient so widely employed for enzyme catalyzed chemical reactions onto on immobilized alginate substrates. While an alternative to enzyme immobilization is the total cell immobilization which can also be performed via alginate-based substrates (Khan et al. 2012). Alginate substrates can facilitate the enzyme activities and mimics the performance of enzymes on cell walls.

12.2 Chitin: $(C_8H_{13}O_5N)_n$

12.2.1 Structural Features

Chitin is a β,1-4-linked homo-polymer of N-acetyl-glucosamine (carbohydrate) exists as a fibrous long-chain polysaccharide owing formula $(C_8H_{13}O_5N)_n$ besides act as the major ingredient of arthropod's exoskeleton and fungi's cell walls. It's the second most copious biopolymer after cellulose on this planet that embraces around 10% of the cell wall components and occurs in several organisms other than fungi (Dongre 2017; Dongre 2018a).

12.2.2 History and Chemistry

Chitin owns great interest as an underutilized resource and novel useful substance of high potential in assorted fields as its modern progress in S&T is quite remarkable (Dongre 2019). Chitin and chitosan both skeletons are viable for varied chemical modifications to offer beneficial derivatives yield via graft and ionic interactions as shown in Fig. 6.

Chitin and chitosan both offer valuable research with innovative and advanced approaches in myriad sectors. Chitin and chitosan both are noticeably resourceful besides prospective bioactive polymer in pharmaceutics and biomedicals (Dongre 2018a). In fact, chitosan is de-acetylated chitin; and both are biodegradable besides own lots of surface-active amino/hydroxy functionalities. In fact cellulose and chitin offer supportive and protective roles to organism/species besides consisting of monosaccharide-glucose bonded mutually in straight/branched way as shown in Fig. 7.

Fungi cell-walls and arthropods exoskeleton are composed of chitin (via linear repeating β-1,4-*N*-acetyl-*D*-glucosamine) but plant cell-walls consist cellulose (Dongre 2017) as swap acetamido by primary hydroxyl at carbon-2, distinct than chitin as shown in Fig. 8.

12.2.3 Occurrence and Properties

Chitin occurs in shells of arthropods (invertebrate animals) like crab, shrimp, and insect besides generated by fungi and bacteria. Partially N-deacetylated form of chitin is called as chitosan, the hetero-polysaccharide owing d-glucosamine and *N*-acetyl-d-glucosamine. Due to proactive free amine functionality chitosan is native cationic polymer which forms a basis of viable and facile derivatization (Joshi et al. 2019; Gray et al. 2013; Ravi Kumar 2000). In fact rigidity and crystallinity nature of

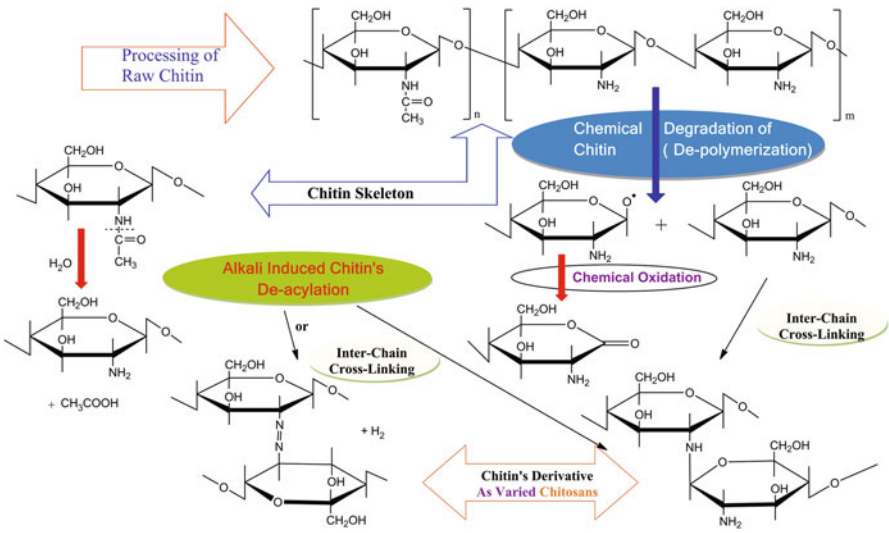


Fig. 6 Diverse beneficial derivatives of chitosan through physic-chemical treatments

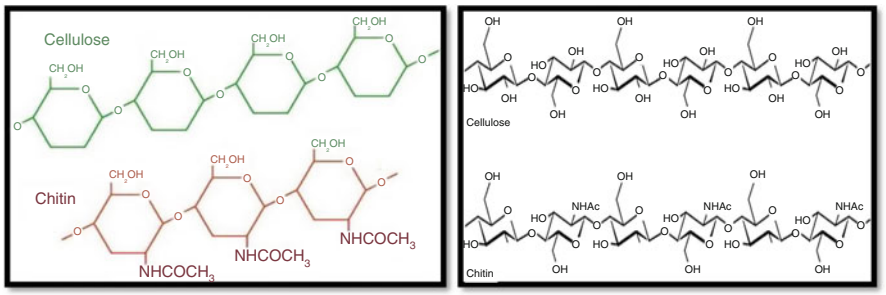


Fig. 7 Composition of cellulose & chitin (Chitin akin to cellulose but amino at C-2)

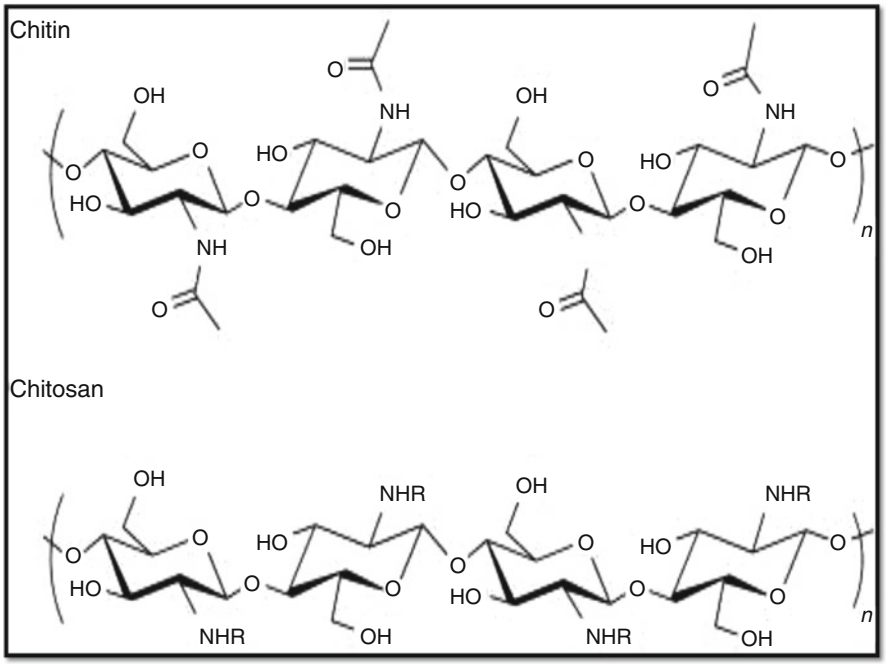


Fig. 8 Structure of Chitin *v/s* Chitosan (chitosan is de-acetylated Chitin at C-2)

chitin/chitosan is contributing to innate strength and insolubility in aqueous conditions at pH = 7 as shown in Fig. 9.

While under acidic pH chitosan gets dissolved through protonation of free amine groups, still chitin is insoluble at this condition and is chemically inert. The average molecular weight of chitin and chitosan is enormous, as high as 10^6 Da (Joshi et al. 2019). Chitin is copious, low-cost, and eco-friendly biopolymer exists on this planet. Its own low immunogenicity with no further provoke toxicity thus augments cellular

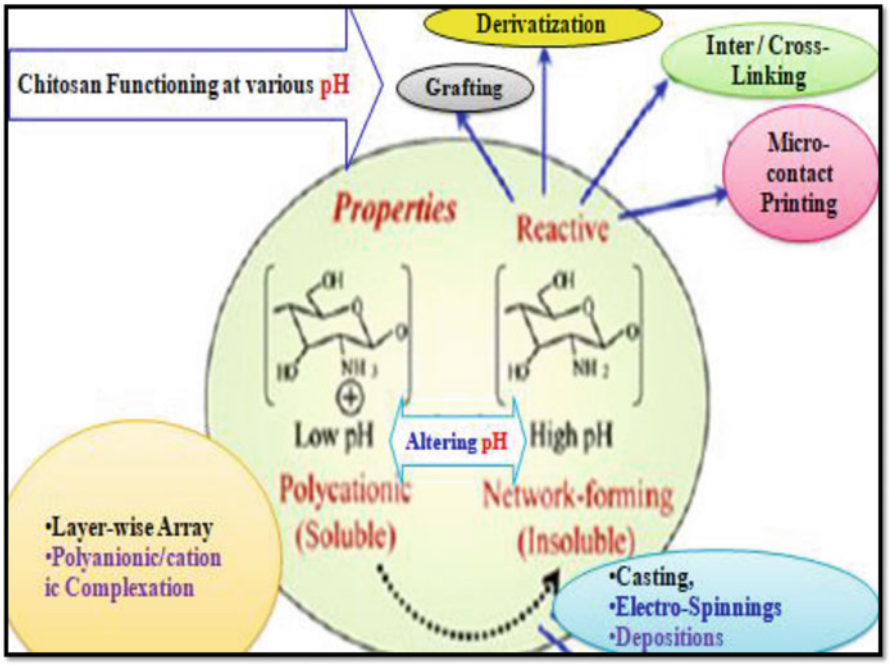


Fig. 9 Chitosan functions and properties at various pH and chemical conditions

proliferation and migration besides faster wound healings due to collagen fabrication at the repair site (Jayakumar et al. 2010a). Certain changes in its native parameters of chitin like porosity, shape/size, and thickness controls mechanical strength of the resultant matrix/scaffold. Certain factors like density, degree of de-acetylation and pH of chitin is viably control through its degradation rate of the constructed matrix/scaffold (Jayakumar et al. 2010a; Gortari and Hours 2013) as shown in Fig. 10.

Chitin gets partly absorbed and quickly decomposed by macrophages in vivo than other viable scaffolds due to innate low initial strength, thus must be supplemented with mechanically stronger matrixes to yield an effective scaffold, e.g., braided chitin-poly-e-caprolactone mixture offer ample original strength to rabbit Achilles tendon repair (Chang et al. 2010). Resorbable polysaccharides chitosan, alginate, and hyaluronan own akin properties to chitin. Chitosan, alginate, and hyaluronan found to induce least foreign matter reaction and perform variably seeded cells interactions due to low mechanical vigor and biodegradable features similar to chitin. The amelioration with other materials yields extra practical scaffold/matrix which potentially augments its innate power and cell adhesion aptitude. Chitosan-alginate blends with agarose/hyaluronan advances scaffold performance and fiber mechanical features with better adhesive linking to patellar tendon fibroblasts than pure chitosan or poly-glactin (Dongre 2017, 2019; Joshi et al. 2019; Chang et al. 2010).

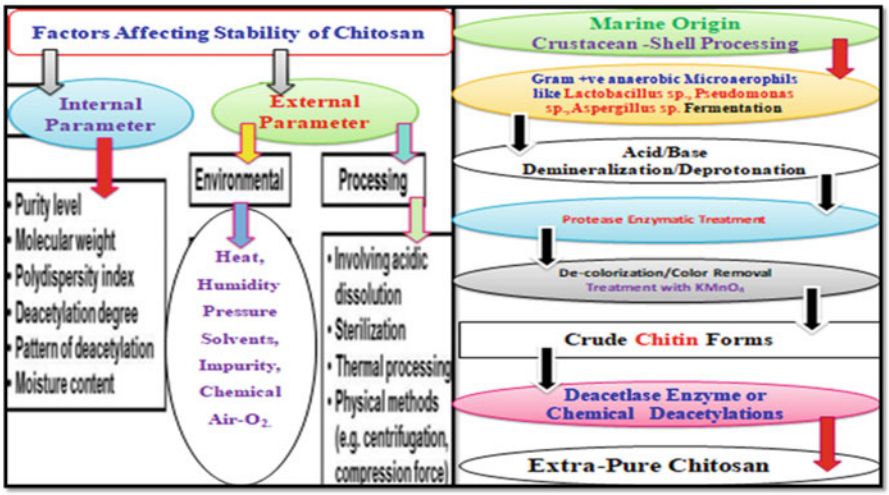


Fig. 10 Factors affecting chitosan's properties and compositions

12.2.4 Chitin/Chitosan Synthesis

Chitin also acts as minor component in the yeast wall account for 1–30% of the cell wall dry mass and imparts the innate mechanical strength. Devoid of chitin, budding hyphae be apt for lysis and figures pronounced bulges unless the osmolarity is improved. Six genes were encoding as per their sequence similarity from many yeasts and filamentous fungi viable for enzyme chitin synthases besides liable for compensatory response to cell wall stress. Generally several yeasts and filamentous fungi hold multiple chitin synthase-encoding genes. Cell wall injury occurred due to mutation in cell wall-related genes can persuade hyper-accumulation of chitin. If cells can't respond to cell wall damage, then it carries lysis, thus signifying vitality of chitin to avoid the cell's death. De-acetylated form of chitin is chitosan, also essential in the cell wall at various stages of the life cycle of fungi, but it's not openly synthesized, instead secreted chitin deacetylases (EC 3.5.1.41) changes chitin to chitosan. *Mucorale* family fungi yields chitosan during vegetative growth, whereas *Sa. cerevisiae* fungi merely produces during sporulation. Chitosan skeletally resembles to glycosaminoglycan (Chang et al. 2010).

12.2.5 Solubility Pattern

Chitosan has poly-cationic character owing higher solubility while raw chitin is neutral in nature and less soluble in any solvent (Dongre 2017, 2019). Chitosan own positively charged linear polysaccharide holding arbitrary units of more β-(1-4)-d-glucosamine and less *N*-acetyl-d-glucosamine (partial deacetylated/deaminated chitin). Positive zeta potentials subsist in chitosan due to availability of plenty amino functionalities on its surface (chitosan's pK_a is 6.5, stay protonated at acidic/neutral pH, and stabilizes drugs, polymers, cells, and nanoparticles via chemical/physical links). Skeleton of chitosan is fragile for many physiochemical

alterations depending on adjacent conditions like pH, N–P charge ratio, molecular weights, and deacetylation degree. In fact native chitosan is soluble in many aqueous organic and/or mineral acids and DMSO < pH 6.5 (Dongre 2017, 2019).

12.2.6 Utility of Chitin/Chitosan

Chitosan formulations own diverse applications including food processing, cosmetics, fabrics, water purifications, pharmaceutics, and biomedicals. Chitosan is biocompatible, biodegradable, and harmless, so employed in wound-healing and antimicrobial usages (Dongre 2018a) as shown in Fig. 11.

Chitosan formulations can be administered orally due to beneficial, falling absorption of dietary fats and cholesterols. It has innate power to impede loaded therapeutic molecules through GI-tract without enzymatic degradation, moreover aids muco-adhesion to secreted mucus with extended residual span in small intestines (Jayakumar et al. 2010a). Chitosan loaded drug-molecules are good through oral route due to empathic release at rigid junctions in para-cellular transport delivery. Many chitosan formulations are developed like trimethyl, triethyl, diethylmethyl, and dimethylethyl amino-chitosan to derive/adapt its native physiochemical properties, e.g., chitosan amines get quaternized easily to control positive zeta potentials and to recover its aqueous/water solubility. Quaternization

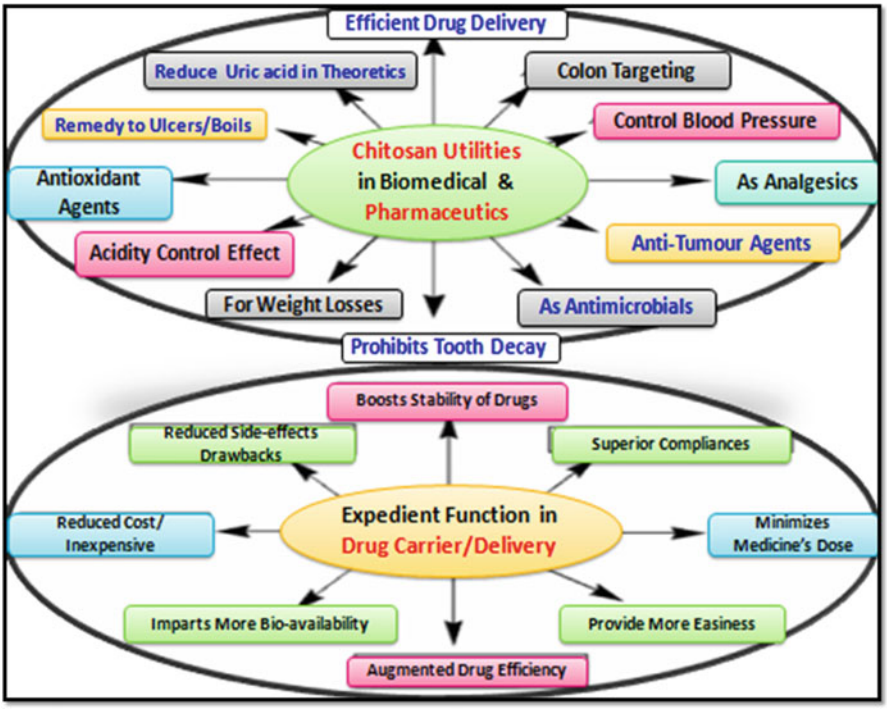


Fig. 11 Chitosan base matrixes for pharmaceutical/clinical and expedient drug delivery

degree and fewer alkylation of chitosan found to control its mucoadhesion power and enhances tight junction opening to mucus at small intestines besides permeability of nanoparticles as shown in the below order.

Trimethylchitosan>dimethylethylchitosan>diethylmethylchitosan>triethylchitosan>Chitosan

Some hydrophobic species like bile acids; fatty acids are embedding in amphiphilic chitosan in order to boost native muco-adhesiveness characters. An integrated hydrophobic species further recover muco-adhesive potential with delayed residual time in chitosan-drug conjugates. Many chitosan derivatives are made less toxic through polyethylene-glycolation, carboxylation, and chelation in order to stop enzymatic degradations and to boost stability of nano-particles. Chitosan nanoparticles attain entero-hepatic circulations via trans-cellular paths as M-cells of Peyer's patche/enterocyte aid uptake/transport akin to pathogen transfer (muco-adhesion holds mucus and advances transport). Performance of chitosan-based nano-particles mainly depend on pH as at acidic pH it stay intact and stable, but at basic pH $>$ 7 deprotonation results later aggregation/breakdown in delivery of biomolecules/drugs). Mannose-TMC-chitosan loaded siRNA beats down TNF-α expression in gut allied macrophages by improved internalization by clatherin-free mannose (Joshi et al. 2019). Polycaprolactone conjugated hyaluronic acid bind chitosan imparts better delivery of anticancer drugs like paclitaxel and doxorubicin through oral route. Chitosan coated drugs found to shield and stabilize it in acidic pH, but at pH 7.4 wrap is released to aid drug loading (Dongre 2017). Assorted chitosan matrixes own biotechnology uses as shown in Fig. 12.

Plenty amine groups of glucosamine unit of chitosan are accessible for protonation so as to attain positive charges and through electrostatic interaction gets complexed with negatively charged species like DNA. High-molecular-weight chitosan (>100 kDa) is soluble only in aqueous acids but low-molecular-weight chitosan (<22 kDa) is highly soluble in buffer solutions (Dongre 2017, 2019). Chitosan-derived poly-plexus get adsorbed at cell surface via electrostatic interactions in endosomes and internalization into cells by endocytosis (Dongre 2019) and ties DNA to guard from nuclease degradation. High-molecular-weight chitosan entraps more stable complexes with DNA through chain mess-ups (Joshi et al. 2019) than low-molecular-weight analogues. Transfixion efficiency enhances with molecular weight of chitosan polyplexes in vitro against A549 cell lines in the given orders: **213 kDa > 98 kDa > 48 kDa > 17 kDa**. Chitosan with weight 15–70 kDa and deacetylation degree 80–90% showed high transfection efficiency (Dongre 2017, 2019). Rather degree of deacetylation is vital than molecular weight in functioning the cellular uptake and cytotoxicity of DNA–chitosan polyplexes (Dongre 2019; Joshi et al. 2019). Increasing charge density of deacetylated chitosan found to convey stability and utmost transfection efficiency to resultant polyplexes in vivo. The pH 6.5–7.5 is good for achieving higher transfection efficiency for cellular uptake of chitosan polyplexes due to unhindered endosomal release, but at pH $>$ 7.5 DNA gets dissociated, so delaying

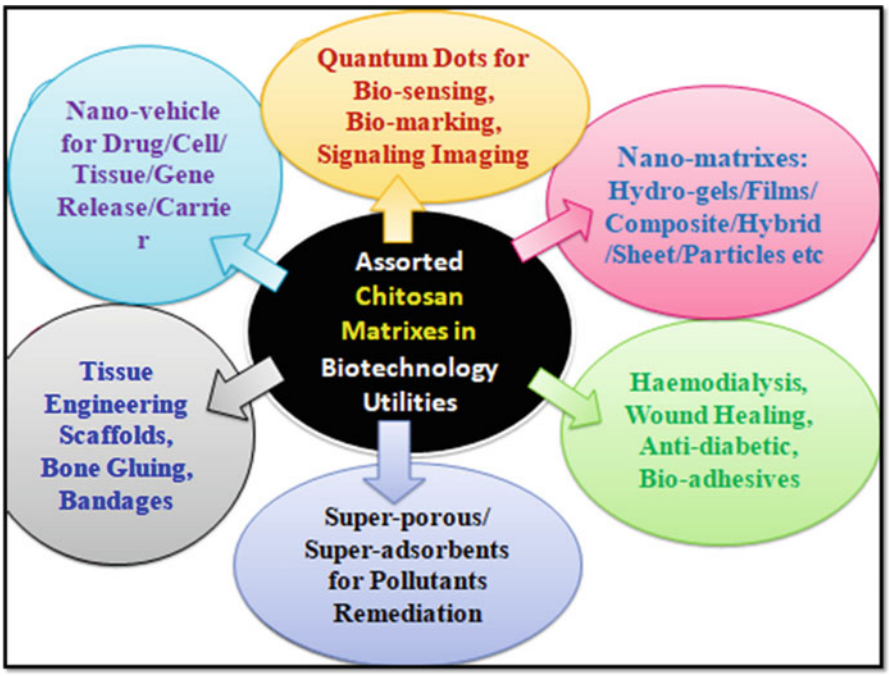


Fig. 12 Assorted chitosan matrixes for biotechnology utility

cellular uptakes in 10–20% serum than serum-free medium (Dongre 2017). These formulated chitosan polyplexes appear less cytotoxic than Lipofectin™, Lipofectamine™, and PEI in vitro for 293 cells. Chitosan formulations show variable transfection efficiency inside diverse cells in-vitro, e.g., DNA–chitosan nanoparticles exhibit top transfection rate for HEK-293 than MG3 and mesenchymal stem-cells (Dongre 2017). Skeletal N-quaternization at terminal amine groups augment cationic property and improve transfection efficiency even as more cytotoxicity in such chitosan polyplexes. Further, cytotoxicity is reduced in such chitosan polyplexes through PEG/PLL grafting in its trimethyl skeleton than untreated chitosan (Dongre 2017, 2019; Joshi et al. 2019). Stearic acid and deoxycholic acid are hydrophobic species if conjugated into chitosan then it lessens aggregation and recovers cellular uptakes besides DNA release over underivatized chitosan-polyplexes. Cell-specific and transferrin choosy marked tumor cells are targeted through conjugated chitosan polyplexes against HEK293 cells than usual DNA–chitosan (Dongre 2017). Chitosan grafted mono/disaccharide formulation show hepatocytes state asialoglycoprotein receptors empathy for glycoproteins. Galactosyl/lactosyl chitosan yields via lactobionic acid coupling marks DNA delivery to hepatocyte/HepG2 cells in liver with great transfection efficiency. Galactosylated chitosan improve transfection efficiency in HepG2 due to increasing surface charge after complexation and interparticular aggregation besides avoiding enzymatic degradation by plasma protein (Dongre 2017, 2019).

12.2.7 Chitosan in Biomedicals

Biopolymer chitosan offered unique physicochemical properties such as biocompatibility, biodegradability, nontoxic and antimicrobial, so widely exploited matrix in pharmaceutics, biomedical innovation, and regenerative medicines. Such features of chitosan compel it as potent biomaterial for developing many systems like tissue engineering scaffolds, drug delivery/career, food-packing, and wound-healing besides chelating cholesterol, fats, proteins, and metals. Chitosan-based bone implants can offer unique features like mechanically robust, cheap, bio competent, bio-electronics, antibacterial, antifungal, hemostatic, osteo-conductive, and osteoinductive besides appear structural similarity to natural bone-stuff (Joshi et al. 2019). Certain chitosan formulated hydrogels and bandages are marketed in medical usage like wound healing, skin injury, and hemodialysis as shown in below Figure. Chitosan matrix/scaffold are nontoxicity thus own admire utilities like as drug delivery/career and in dentistry to prevent tooth decay along with developing many dental care products (Dongre 2017). Chitosan is non-toxic and possess re-mineralizing power which aids hardening of tissues in tooth, so acts as desensitizer in toothpastes besides executes safe function in dentistry and oral health. Chitosan advances surgical healing of post-extraction oral wounds and causes decline in bacterial bio-film usage in dental cements. Recently progressive scientific and biomedical fields have shaped novel biomaterials that are more capable and harmless. Frequently, processing of such biomaterials is tricky and expensive method, but many marine-based biomaterial including chitosan are incessantly explored to derive successful function in surgical, orthopedic, reconstructive medicines, plastic surgery, aesthetic, and dental (Jayakumar et al. 2010b). Often marine-derived biomaterials like alginate, chitosan and collagen are beneficial due to proven outputs. Chitosan is the most desirable marine-based biomaterial due to innate ability to shape up as films, sheets, particles, and gels thus, being exploited in diverse fields of medical including dentistry, tissue engineering, etc. In traditional dentistry and surgery chitosan formulations are used to stop tooth caries/decay, involved in wound healings (Zohuriaan-Mehr 2005; Dongre 2018a). Innate antimicrobial activity is strongly influenced by formulating many materials based gels containing lactic acid, distilled water, and chlorhexidine through chitosan milieu to get desired functions performing at different pH. Such chitosan formulations are safe to use, with many prospective results in oral surgery and restorative dentistry with no side-effects (Dongre 2017; Böttcher et al. 2018). Chitosan-linked synthetic dental matrixes could improve its characteristics from a bacteriostatic to mycostatic purpose (Jayakumar et al. 2010a). Marine-derived chitin and chitosan are protagonists in gifted scientific and clinical interests being too, ascertained by their availability (Gortari and Hours 2013).

12.3 Carrageenan: $C_{42}H_{74}O_{46}S_6$

12.3.1 Structural Features

Carrageenan is a renewable and sustainable biopolymer being extracted from edible red seaweeds class of marine origin (Dongre 2017). Carrageenan is a generic name of

polysaccharides extracted through species of red seaweeds like Rhodophycea or Rhodophyta: Gigartina, Chondrus crispus, Eucheuma, and Hypnea. Carrageenan's are identified in six Greek and IUPAC prefix nomenclature as Iota (i)-, Kappa (j)-, Lambda (k)-, Mu (l)-, Nu (m)-, and Theta (h) nomenclature as per chemical classification (Khotimchenko and Khotimchenko 2020). These carrageenan types found to vary in their degree of sulfation as Kappa-carrageenan has one sulfate group per disaccharide, iota-carrageenan has two, lambda-carrageenan has three, mu as four, nu has five, and theta has six sulphate functionalities per sugar unit. In fact carrageenan's are hydrophilic linear sulphated galactan polymeric links owing alternating 3-linked b-D-galactopyranose (G-units) and 4-linked a-D-galactopyranose (D-units) or 4-linked 3, 6-anhydro-a-D-galactopyranose (DA-units). Assorted red algal found to yield varied carrageenan's during their developmental span, e.g., *Gigartina* produces mainly kappa carrageenan's during gametophytic phase while lambda carrageenan's in sporophytic phase.

12.3.2 History, Chemistry

Carrageenan or carrageenin means "little rock" a colloquial Irish carraigín family of linear sulphated polysaccharides as extracted from red edible seaweed (Dongre 2017, 2019). Gelatinous stuff was extracted from the Irish moss/seaweed called Chondrus crispus as food additives in the fifteenth century (Joshi et al. 2019). Carrageenan was used on industrial level in the 1930s, while was documented used in China around 600 B.C. and pioneered used ad Gigartina in Ireland around 400 A.D. Carrageenan own apt viscosity which is best for growing or gelling agent viable in many pharmaceuticals functions as thickening materials, cosmetics, polyelectrolyte complexes, prolonged and controlled drug delivery, wound dressing, and tissue engineering. It has limiting usages due to inherent hydrophilic property, but assorted derivatization like blending, reinforcement, and multi-layering can augment its restrictive properties beside expand prospective applications (Laurienzo 2010).

12.3.3 Property

Carrageenan is bulky and highly flexible by virtue of curling helical arrangements and textures of constituting units with molecular weight 1507.4 g/mol. This constitutional orientation aids in imparting variable gelation at NTP conditions to be used as thickening and stabilizing agents in the food, dairy, and meat industries. All the six carrageenans owe high-molecular-weight consecutive sulfated and non-sulfated forms of galactose and 3,6 anhydrogalactose units joined via alternate α-1,3 links and β-1,4 links. The quantity and location of ester sulfated functionality exists in repeating galactoses primary differentiates and control their native properties. Huge ester sulphated groups lessen the solubility temperature of carrageenan which ultimately yields loose gelation and contribute to gel inhibition. All these carrageenans are soluble in hot water, only the lambda carrageenan is soluble in cold water along with sodium salts are cold water soluble.

In food products, carrageenan owes the EU additive numbers E407 (E407a as processed eucheuma seaweed). Truly, carrageenans are considered a dietary fiber in many food products.

Carrageenan is fragile to form blends with many polymeric skeleton like nanocellulose and advances its innate tensile strength by 50%. Carrageenan films layered through polylactic acid can reduce water vapor permeability up to high extent. Carrageenan polysaccharide is extracted from marine algae appears to be top renewable biomaterial substitute for conventional synthetic plastics/polymers. Carrageenan-derived matrixes are vast used as edible and flexible films beside coatings in various pharmaceutics and biomedical utility. Hydrophilic character of carrageenan films are improved to extend suitable applications by blending co-polymeric blocks like chitin/chitosan and reinforcements through plasticizers. Low molecular weight and highly sulfated carrageenan-based matrixes are the most active against tumors (Sashiwa and Aiba 2004).

12.3.4 Utility of Carrageenan

Three main commercial classes of carrageenan, namely: *kappa, iota, and lambda* are often used in industries. *Kappa* form is mainly obtained from *Kappaphycus alvarezii* strong as rigid gels in presence of potassium ions, which can interact with proteins. *Iota* form subsists like soft gels with calcium ions as mainly obtained from *Eucheuma denticulatum*. While *lambda* carrageenan lacks gelation thus good to be used as thickening agent in assorted dairy products. Certain oligo-carrageenan blends apt G2/M phase and arrest apoptosis in cancer cells therapy besides abate immune suppression induced by antitumor drugs. Carrageenans formulations also act as adjuvant in vaccine based on dendrite cell actions. Carrageenans are known to offer anticancer and antitumor functions along with complete intracellular anti-proliferation. Novel agar/MMT hydrogels yield through cross-linking κ-carrageenan with triethyleneglycol-divinylether (TEGDE) polymer offer utmost inflammation and non-Fickian swelling capacity as per varying montmorillonite ratio. High montmorillonite reduces gelation growth, also display non-Fickian swelling capacity viable for biomedical applications (Joshi et al. 2019). Carrageenans are used in the food, dairy, and meat industry due to innate features like gelling, thickening, and stabilizing potential besides strap affinity to natural and synthetic proteins.

Gelatinous extracts of the Chondrus crispus (Irish moss) seaweed have been used as food additives since approximately the fifteenth century (Dongre 2017). Carrageenan is a vegetarian and vegan alternative to gelatin in some applications or may be used to replace collagen-derived gelatine gummy polymer in many confectionery products. Carrageenans are broadly used in food processes as thickening, gelling, and protein-suspending agents along with excipient in pills/tablets (Joshi et al. 2019). Carrageenans offer prototype bio-activities like inflammation, immunity, herpes inhibition, and prohibition of HIV/AIDS (Joshi et al. 2019; Laurienzo 2010). Carrageenan advances texture of cottage cheese, puddings, and dairy desserts through controlled viscosity besides binds/stabilize patties, sausages, and low-fat hamburgers in meat-processing. Carrageenans are also preferred in cosmetics; printing, textile, wound dressings, and toothpaste stabilization by absorbing fluids. Carrageenans own superior properties like good compatibility, huge robustness, and constant visco-elasticity during compression so apt excipients for sustained-release formulations (Dongre 2017; Joshi et al. 2019). Carrageenans own broad

bioactivity domain like antitumor, immuno-modulatory, anti-hyperlipidemic, anti-coagulant, anti-inflammatory, and antiviral properties (Dongre 2017; Laurienzo 2010). But therapeutic applications of low molecular weight carrageenans are limited due to gastrointestinal toxicity which can be removed through assorted chemical modifications (Campo and Carvalho 2009).

13 Conclusion

Many marine organisms like crabs, lobsters, shrimps, molds, seaweeds, microbes, mollusks, arthropod, crustacean, fungi, yeasts, algae, and squid pen exoskeletons are resource for deriving such polysaccharides. Marine crustacean shells are rich in chitooligosaccharides owing 20–50% calcium carbonate, 20–40% proteins, and 15–40% chitin ratios. An outline of three vital marine-based polysaccharides, namely, alginate, carrageenan, and chitin owing innate bio-activities in pharmaceutics and biomedicals are summarized including the aspects of preparation, formulation, and synthetic blending to be used for many purposes like water treatment, food, drug delivery, medicines, tissue engineering, wound healing, dentistry, cosmetics, and agriculture. Sea-based polysaccharides offer competent myriad applications due to native nontoxicity, biocompatibility, and biodegradability besides superior physico-chemical/mechanical features. These polysaccharides offer distinctive field utilities in biotechnology, nanotechnology, medicine, cosmetics, food, diary, and agriculture.

References

Augst AD, Kong HJ, Mooney DJ. Alginate hydrogels as biomaterials. Macromol Biosci. 2006;6:623–33.

Ausili E, Paolucci V, Triarico S, et al. Treatment of pressure sores in spina bifida patients with calcium alginate and foam dressings. Eur Rev Med Pharmacol Sci. 2013;17(12):1642–7.

Bale S, Baker N, Crook H, Rayman A, Rayman G, Harding KG. Exploring the use of an alginate dressing for diabetic foot ulcers. J Wound Care. 2001;10(3):81–4.

Bănică F-G. Chemical sensors and biosensors: fundamentals and applications. Chichester: Wiley; 2012. p. 576. isbn:978-1-118-35423-0.

Barikani M, Zia KM, Bhatti IA, Zuber M, Bhatti HN. Molecular engineering and properties of chitin based shape memory polyurethanes. Carbohydr Polym. 2008;4:621–6.

Böttcher S, Di Capua A, Blunt JW, Quinn RJ. Bioinspiration from marine scaffolds. In: La Barre S, Bates SS, editors. Blue biotechnology: production and use of marine molecules; 2018. p. 297. isbn:978-3-527-34138-2.

Campo VL, Carvalho I. Review carrageenans: biological properties, chemical modification & structural analysis: review. Carbohydr Polym. 2009;77:167–80.

Chang A. pH-sensitive starch-g-poly(acrylic acid)/sodium alginate hydrogels for controlled release of diclofenac sodium. Iran Polym J. 2015;24(2):161–9.

Chang PR, Jian R, Yu J, Ma X. Starch-based composites reinforced with novel chitin nanoparticles. Carbohydr Polym. 2010;80:420–5.

Chiu Y, Chan Y, Li T, et al. Inhibition of Japanese encephalitis virus infection by the sulfated polysaccharide extracts from Ulva lactuca. Mar Biotechnol. 2012;14:468–78. https://doi.org/10.1007/s10126-011-9428-x.

Coviello T, Matricardi P, Marianecci C, Alhaiqu F. Polysaccharide hydrogels for modified release formulations. J Control Release. 2007;119:5–24.
Dongre RS. Marine polysaccharides in medicine. In: Biological activities & application of marine polysaccharides, vol. 1. In-Tech Open; 2017. p. 181–206. https://doi.org/10.5772/65786. isbn:978-953-51-2860-1.
Dongre RS. Chitosan-derived synthetic ion exchangers: characteristics & applications. In-Tech Open. 2018a;1:21–42. https://doi.org/10.5772/intechopen.78964.
Dongre RS. Rationally fabricated nanomaterials for desalination and water purification. In: Book novel nanomaterials, vol. 1. In-Tech Open; 2018b. p. 348–66. https://doi.org/10.5772/intechopen.70149. isbn:978-1-78923-089-5.
Dongre RS. Chitosan formulations: chemistry, characteristics & contextual adsorption in unambiguous modernization of S&T. In: Hysteresis of composites; 2019. https://doi.org/10.5772/intechopen.83391. isbn:978-1-78984-810-6.
Drury JL, Dennis RG, Mooney DJ. The tensile properties of alginate hydrogels. Biomaterials. 2004;25:3187–99.
Dudarev VG, Iozep AA. Synthesis of carboxymethyl chitin benzylidenehydrazides. Russ J Appl Chem. 2010;83:1853–6.
Gåserod O, Smidsrod O, Skjåsk-Bræk G. Microcapsules of alginate-chitosan-I. A quantitative study of interaction between alginate & chitosan. Biomaterials. 1998;19:1815–25.
Gortari MC, Hours RA. Biotechnological processes for chitin recovery out of crustacean waste: a mini-review. Electron J Biotechnol. 2013;16:1–14.
Gray CJ, Weissenborn MJ, Flitsch SL. Enzymatic reactions on immobilised substrates. Chem Soc Rev. 2013;42(15):6378–405. https://doi.org/10.1039/C3CS60018A.
Honarkar H, Barikani M. Applications of biopolymers-I: chitosan. Monatsh Chem. 2009;140:1403–20.
Jayakumar R, Menon D, Manzoor K, Nair SV, Tamura H. Biomedical applications of chitin and chitosan based nanomaterials – a short review. Carbohydr Polym. 2010a;82:227–32.
Jayakumar R, Prabaharan M, Nair SV, Tokura S, Tamura H, Selvamurugan N. Novel carboxymethyl derivatives of chitin and chitosan materials and their biomedical applications. Prog Mater Sci. 2010b;55:675–709.
Joshi S, Eshwar S, Jain V. Marine polysaccharides: biomedical & tissue engineering applications. In: Choi A, Ben-Nissan B, editors. Marine-derived biomaterials for tissue engineering applications, Springer series in biomaterials science & engineering, vol. 14. Singapore: Springer; 2019. https://doi.org/10.1007/978-981-13-8855-2_19.
Khan S, Tøndervik A, Sletta H, et al. Overcoming drug resistance with alginate oligosaccharides able to potentiate the action of selected antibiotics. Antimicrob Agents Chemother. 2012;56(10):5134–41.
Khotimchenko M, Khotimchenko Y. Antitumor potential of carrageenans from marine red algae. Crabohydr Polym. 2020;246:116568. https://doi.org/10.1016/j.carbpol.2020.116568.
Kurita K. Controlled functionalization of the polysaccharide chitin. Prog Polym Sci. 2001;26:1921–71.
Laurienzo P. Marine polysaccharides in pharmaceutical applications: an overview. Mar Drugs. 2010;8:2435–65.
Lee KY, Mooney DJ. Alginate: properties and biomedical applications. Prog Polym Sci. 2012;37(1):106–26. https://doi.org/10.1016/j.progpolymsci.2011.06.003. PMCID: PMC3223967
Mao X, Zhang J, Kan F, Gao Y, Lan J, Zhang X, Hu Z, Li Y, Lin H. Antioxidant production and chitin recovery from shrimp head fermentation with Streptococcus thermophilus. Food Sci Biotechnol. 2013;22:1023–32.
Niekraszewicz B, Niekraszewicz A. The structure of alginate, chitin and chitosan fibres. In: Handbook of textile fibre structure. Cambridge, UK: Woodhead Publishing Limited; 2009. p. 266–304.
Pillai CKS, Paul W, Chandra P, Sharma CP. Chitin & chitosan polymers: chemistry, solubility and fiber formation. Prog Polym Sci. 2009;34:641–78.
Ravi Kumar MMV. A review of chitin and chitosan applications. React Funct Polym. 2000;46:1–27. https://doi.org/10.1016/S1381-5148(00)00038-9.

Sakai S, Hirose K, Moriyama K. Control of cellular adhesiveness in an alginate-based hydrogel by varying peroxidase and H_2O_2 concentrations during gelation. Acta Biomater. 2010;6:1446–52.

Sá-Lima H. Stimuli-responsive chitosan-starch injectable hydrogel combine encapsulated adipose-derive stromal cell for articular cartilage regeneration. Soft Matter. 2010;6(20):5184–95.

Sashiwa H, Aiba S-I. Chemically modified chitin and chitosan as biomaterials. Prog Polym Sci. 2004;29:887–908.

Szekalska M, Puciłowska A, Szymańska E, Ciosek P, Winnicka K. Alginate: current use & future perspectives in pharmaceutical and biomedical applications. Int J Polym Sci. 2016, Article ID 7697031. https://doi.org/10.1155/2016/7697031.

Tong Y, Guan H, Wang S, Xu J, He C. Syntheses of chitin-based imprinting polymers and their binding properties for cholesterol. Carbohydr Res. 2011;346:495–500.

Tsioptsias C, Tsivintzelis I, Papadopoulou L, Panayiotou C. A novel method for producing tissue engineering scaffolds from chitin, chitin-hydroxyapatite, and cellulose. Mater Sci Eng C. 2009;29:159–64.

Wahl EA, Fierro FA, Peavy TR, et al. In vitro evaluation of scaffolds for the delivery of mesenchymal stem cells to wounds. BioMed Res Int. 2015, Article ID 108571, 14 pages.

Wang X, Hao T, Qu J, Wang C, Chen H. Synthesis of thermal polymerizable alginate-GMA hydrogel for cell encapsulation. J Nanomater. 2015, Article ID 970619, 8 pages.

Yaghobi N, Mirzadeh H. Enhancement of chitin's degree of deacetylation by multistage alkali treatments. Iran Polym J. 2004;13:131–6.

Zohuriaan-Mehr MJ. Advances in chitin and chitosan modification through graft copolymerization: a comprehensive review. Iran Polym J. 2005;14:235–65.

Bacterial Polysaccharides: Cosmetic Applications

34

Sílvia Baptista and Filomena Freitas

Contents

Abstract

Bacterial polysaccharides are extensively used in cosmetic products, especially given their diverse properties and functionalities, assuming an important role in the R&D of new formulations and applications. In this chapter, the principal properties of the main polysaccharides present in this area are described and summarized. Advancements in skin biology knowledge, and the study of new formulations, support the research of active ingredients related to the maintenance of skin integrity and barrier function. Bacterial polysaccharides possess several

S. Baptista · F. Freitas (✉)
UCIBIO-REQUIMTE, Chemistry Department, Faculty of Sciences and Technology, NOVA School of Science and Technology, Caparica, Portugal

73100 Lda., Lisboa, Portugal
e-mail: a4406@fct.unl.pt

J. M. Oliveira et al. (eds.), *Polysaccharides of Microbial Origin*,
https://doi.org/10.1007/978-3-030-42215-8_45

properties (such as biocompatibility, biodegradability, film-forming, gelling, and thickening) that can provide protective effects on the skin, improving the efficacy of formulations while maintaining the skin in good conditions. Additionally, polysaccharides, which can be processed in emulsions, hydrogels, suspensions, and encapsulating structures, have been demonstrated to enhance the stability of formulations and promote sensorial properties. Data from several clinical studies revealed that the polysaccharide-based formulations promote skin health, with applications in several skin disorder treatments.

Keywords

Polysaccharides · Cosmetic · Skin · Formulation · Emulsion · Hydrogel

1 Introduction

Despite being among the most important structures in Nature, polysaccharides were often overlooked in the past. However, due to the recent global demand for a sustainable economy, the previous paradigm has shifted (Shanmugam and Abirami 2019). These natural polymers have particular physical and chemical properties, and can be found in a wide range of life forms, from microorganisms to animals and plants, being essential in several biological functions (Yildiz and Karatas 2018; Shanmugam and Abirami 2019). In fact, polysaccharides are known to be decisive for cell adhesion, to serve as reservoirs in the cytoplasm, to be part of the cell membrane or cell wall, and to be a component of the defense, regulation, and information systems of the cell. Bacterial polysaccharides are extensively used in cosmetic products, especially given their diverse properties and functionalities (Freitas et al. 2014). Cosmetics can be defined as composite multiphase arrangements, with every single different component possessing a specific function on the final product (Freitas et al. 2014). Polysaccharides can be used as natural cosmetic components due to their unique physical and chemical properties, given the fact that they are biocompatible and biodegradable biopolymers (Freitas et al. 2014).

Functional polysaccharides are claimed based on their functionalities in the cosmetics' formulation technology, such as film formers, gelling agents, thickeners, suspending agents, conditioners, and emulsifiers, that mainly rely on the physicochemical properties of the biopolymer. On the other hand, cosmetic active polysaccharides promote water loss reduction, protecting the skin barrier function, and promoting a good sensorial (Kanlayavattanakul and Lourith 2014). In this context, polysaccharides are of great interest in cosmetics for the development of stable functional formulations.

The continuous development of the cosmetic industry technology and the understanding of skin's biology allowed the emergence of several novel active ingredients used in topical formulations. Still, cosmetic ingredients research is of utmost importance to further increase the stability, safety, and efficacy of cosmetic formulations. Natural ingredients are widely present in this industry because consumers have an

interest in the benefits originated by these natural products. In this sense, polysaccharides present several interesting applications, due to their capability of creating gel structures in aqueous environments (through the hydration of the sugar building blocks), stabilizing emulsions and forming films, acting as binders, viscosity-increasing promotors, and rheology modifiers, as well as suspending agents (Freitas et al. 2014; Kanlayavattanakul and Lourith 2014). Polysaccharides interact, as active substances, with other ingredients of a cosmetic formulation, which grants them great benefits to be used, for example, as vehicles of dermatological formulations. This application is possible due to the non-reactive nature of polysaccharides when contacting the human skin. Moreover, these molecules can be metabolized and eliminated from the body using normal metabolic pathways (Ammala 2013; Freitas et al. 2014).

2 Bacterial Polysaccharides

In recent years, microbial polysaccharides have emerged as viable substitutes to animal and plant-based ingredients in many different applications, ranging from the food and agricultural industries to the pharmaceutical, medical, and cosmetic industries. This diversity in applications derives from polysaccharides' fundamental and practical properties, granting them functions as stabilizers, emulsifiers, viscosifiers, and thickeners (Shanmugam and Abirami 2019).

Therefore, the previously mentioned properties of bacterial polysaccharides contribute to their application in cosmetic formulations (Table 1), with xanthan and gellan gum being the most pertinent due to their use as cosmetic additives to control the viscosity, and as physicosensorial agents. Hyaluronic acid, bacterial cellulose, and levan are also relevant cosmetic ingredients, considering their application as bioactive components for skin regeneration/defense (Balkrishna et al. 2018; Freitas et al. 2014).

Being high molecular weight polymers that may share components with other microbial cellular carbohydrates and having fundamental intricacy and variety, bacterial polysaccharides can be classified in several groups: capsular polysaccharides (CPS) and exopolysaccharides (EPS), which are observed in all types of bacteria; lipopolysaccharides (LPS) and cell-wall teichoic acids (WTA), which are reported, respectively, in gram-negative and gram-positive bacteria (Balkrishna et al. 2018; Shanmugam and Abirami 2019; Yildiz and Karatas 2018).

CPS are extracellular polysaccharides that are important in bacteria pathogenicity due to their ability to encase microorganisms and promote adhesion and penetration of the host cell. It also acts as virulence promoters by screening the immune response, to avoid antibody reactions (an interesting feature in the advancement of vaccines) (Yildiz and Karatas 2018).

EPS are high molecular weight (10–1000 kDa) extracellular polysaccharides secreted by many prokaryotes (both eubacteria and archaebacteria) and eukaryotes (phytoplankton, fungi, and algae), which present chemical structure and composition diversification, ranging from homopolymers to heteropolymers (Balkrishna et al. 2018; Roca et al. 2015). This diversity rendered EPS auspicious in terms of

Table 1 Bacterial polysaccharides properties and their cosmetic application

Polysaccharide	Origin	Monomers	Mw	Properties	Cosmetic application	Reference
Xanthan gum	*Xanthomonas spp*	Glucose Mannose Glucuronic acid	(2.0–50) x 10^6	High viscosity Hydrocolloid	Thickener Emulsifier Rheology modifier Stabilizer	Savary et al. (2016)
Gellan gum	*S. paucimobilis*	Glucose Rhamnose Glucoronic acid	5.0 x 10^5	Gelling capacity Hydrocolloid Water soluble Film form capacity	Stabilizer Gelling agent	Freitas et al. (2014)
Dextran	*Leuconostoc mesenteriodes, Streptococcus, Weissella, Pediococcus and Lactobacillus*	Glucose	10^6–10^8	Good stability (over wide temperature and pH ranges) Newtonian fluid behavior	Moisturizer Thickener	Yildiz and Karatas (2018)
Bacterial cellulose	*Gluconacetobacter Xylinum, Agrobacterium, Alcaligenes, Rhizobium, Pseudomonas*	glucopyranose	~10^6	Insoluble in water and in most solvents High crystallinity High water-holding capacity High tensile strength moldability	Thickener Emulsifier Viscosity controller Absorbent Skin regeneration Skin moisturizer Wound healing	Alves et al. (2016), Balkrishna et al. (2018), Freitas et al. (2014), Gallegos et al. (2016), Savary et al. (2016)

Levan	*Acetobacter, Bacillus, Brenneria, Geobacillus, Halomonas, Lactobacillus*	Fructose	$3.0x10^6$	High water solubility Low viscosity Stabilizer Film form capacity	Cell-proliferating Skin moisturizing Skin irritation–alleviating effects Blending	Alves et al. (2016), Yildiz and Karatas (2018)
Hyaluronic acid	*Streptococcus sp.*	Acetylglucosamine, glucuronic acid	$2.0x10^6$	Water soluble Highly viscous hydrocolloid Viscoelastic High swelling capacity	Humectant Moisturizer Softening agent Antiaging affect Immunostimulant Angiogenic	Alves et al. (2016), Freitas et al. (2014), Savary et al. (2016)

commercial applications, in several sectors such as paper manufacturing, pharmaceuticals, cosmetics, and food. For instance, since xanthan and gellan were allowed as food additives in Europe and the US, these two EPS gained great interest and are currently widely used (Roca et al. 2015).

However, the global polymer market fraction related to bacterial EPS is still small, mainly due to production costs, in terms of substrates and bioreactors used for microbial growth, and purification procedures (Balkrishna et al. 2018).

As shown in the previous paragraphs, bacterial polysaccharides present a great variety of functional properties, which is an important feature to broaden their research, development, and commercialization (Balkrishna et al. 2018).

2.1 Main Properties

Microbial polysaccharides present hydrophilic behavior, a feature that renders them major interest for commercial purposes, due to their capacity to attract and hold water molecules, as well as to change basic properties of aqueous systems (BeMiller 2019). Moreover, microbial polysaccharides present several relevant characteristics such as high viscosity at low concentrations (1), they also have gelling properties (2) that enables them to act as thickening and stabilizing agents (3) presenting compatibility with high salt concentrations (4) and stability against temperature changes and high shear pH (5); they have high water solubility and anti-freeze behavior (6), presenting polyelectrolyte and ion exchange potential (7), having a surface-active, dispersing and flocculating capacity (8); they also present versatile adhesive and film-forming properties (9), displaying capacity and selectivity for metal ions, proteins, lipids (10), with specific biodegradability (11) (BeMiller 2019).

Microbial polysaccharides can adapt their molecular structure and rigidity by changing the intra and intermolecular interactions, which grants them unique rheological characteristics. In the cosmetics' industry, polysaccharides' capacity to present high viscosity at low concentrations is very important to allow a specific texture (of increased viscosity) to the formulation and to decrease elasticity-driven creaming of the droplets and other components of the emulsion. This ability derives from polysaccharides' inflated molecular structure in aqueous solution, with a high effective volume fraction at low concentrations (BeMiller 2019).

The main bacterial polysaccharides used in the cosmetic industry are xanthan gum, bacterial cellulose, levan, hyaluronic acid, gellan gum, and dextran because of their ability as film formers, emulsion stabilizers, binders, and viscosity increasing and skin conditioning agents (Balkrishna et al. 2018; Freitas et al. 2014; Bhavani and Nisha 2010).

2.2 Biotechnological Importance

The aforementioned features of polysaccharides (hydrophilicity, high molecular weight, and changeable molecular structure) allow them to have several

applications, mainly related to their behavior in aqueous media, in high-value markets (Roca et al. 2015). The global hydrocolloids market, in which most polysaccharides are included, was estimated at USD 9 Billion in 2020 and to be at USD 11.7 Billion by 2027, and expected to grow at a compound annual growth rate (CAGR) of 3.7% between 2020 and 2027 (Research and Markets, official site). The cosmetics and Personal Care Products segment currently accounts for a 20.8% share of the global Hydrocolloids market (Research and Markets, official site). Even though this market is dominated by plant and algal polysaccharides, xanthan gum's market value, for instance, is estimated to be USD 987.7 Million in 2020 and, expected to grow at a CAGR of 5.1% until the end of the year 2024 (Research and Markets, official site) Research and development are, therefore, important to further improve the biotechnological importance of bacterial polysaccharides. In the next paragraphs, it will be explained this importance in four pivotal areas: food, health, agriculture, and industrial sectors (Roca et al. 2015; Shanmugam and Abirami 2019).

2.2.1 Applications of Bacterial Polysaccharides in the Food Sector

Bacterial polysaccharides have several roles in functional food, for example, as additives to probiotics (Alves et al. 2016). For instance, xanthan and gellan gums can be used for microencapsulation as stabilizers and protectors, to avoid nutraceuticals degradation in two different phases (food processing and intestinal transit) thus ensuring the release in specific sites to perform the desired health effect (Alves et al. 2016).

Nowadays, specific foods demand a unique texture, viscosity, flavor, and water-control properties. For these purposes, bacterial polysaccharides are used to change food thickness, stability, and texture. For example, bacterial polysaccharides such as dextran and xanthan are used to change water-related rheology, thus altering the final product's texture (Shanmugam and Abirami 2019). Moreover, they can be used to prevent/reduce the formation of ice crystals in frozen foods (Jindal and Khattar 2018) and also play a role in the color/flavor of prepared foodstuffs (Jindal and Khattar 2018). In terms of products, microbial polysaccharides can be found in instant foods, sauces, toppings, and dairy products (Jindal and Khattar 2018; Shanmugam and Abirami 2019).

Some Food and Drug Administration (FDA) approved bacterial polysaccharides have an important role in food processing industries (Ramalingam et al. 2014). Xanthan gum is used in frozen foods (dextran also), beverages, and sauces (Ramaligam et al. Ramalingam et al. 2014; Shanmugam and Abirami 2019); gellan gum, xylinan, alginates, and curdlan polysaccharides are used as texturizers/gelling agents (Jindal and Khattar 2018; Ramalingam et al. 2014)); pullulan acts as pH stabilizer of food (Ramalingam et al. 2014). The health awareness of consumers and the multiple functions of hydrocolloids have contributed to the increased demand for these bacterial polysaccharides in the food and beverage industries (Shanmugam and Abirami 2019).

2.2.2 Applications of Bacterial Polysaccharides in the Health Sector

In the health sector, the use of bacterial polysaccharides is widespread in many applications that include drug delivery systems (Alves et al. 2016), dental impressions, absorbent dressings (Nwodo et al. 2012), anti-reflux therapies, and bioprinting (McCarthy et al. 2019). The importance of drug delivery systems has increased due to the request for pharmacologically active substances regulated distribution in specific areas of the human body. Accordingly, xanthan gum, gellan gum, hyaluronic acid, and levan have been studied as suitable for this type of formulation (Alves et al. 2016). In consequence, dextran and sulfated alginates have been reported to prevent blood disorders (Balkrishna et al. 2018) In terms of bioprinting, alginate, bacterial cellulose, hyaluronic acid, gellan, xanthan, and dextran have already been reported as appropriate for this type of application (McCarthy et al. 2019).

There are several other medical applications for bacterial polysaccharides which include their use as an anticoagulant, anticancer, anti-angiogenesis, anti-inflammatory, antithrombotic, antidiabetic, antioxidant, antiviral, antiulcer, cholesterol-lowering, and prebiotic agents (Yildiz and Karatas 2018). One of the most important influences on the global market for xanthan gum is the increase of governments' ventures in healthcare, and the key players include Danisco, Cargill, Pfizer Inc., Jungbunzlauer, Archer Daniels Midland, CP Kelco, and Fufeng Group Company Ltd. (Shanmugam and Abirami 2019).

2.2.3 Applications of Bacterial Polysaccharides in the Agricultural Sector

In the agricultural sector, bacterial extracellular polysaccharides (EPS) are among the most important due to their utilization as soil nutrition for organic matter (Shanmugam and Abirami 2019) because they have a suitable suspending nature and affinity with salt, with promising features in toxic compounds transfer by decreasing its mobility to the soil (Balkrisha et al., Balkrishna et al. 2018). Moreover, EPS plays a role in controlling plant pathogens like fungus, and enhance pesticides, herbicides, fungicides, and insecticides (Balkrishna et al. 2018; Nwodo et al. 2012). They can also be used for environmental bacterial metabolism in metal detoxification of soils (Balkrisha et al., Balkrishna et al. 2018). Essentially, EPS improve plant growth by increasing soil fertility (Shanmugam and Abirami 2019). For example, the rheological properties of xanthan were demonstrated to increase two important pesticide properties: pesticide cling and pesticide permanence (Nwodo et al. 2012).

2.2.4 Applications of Bacterial Polysaccharides in the Industrial Sector

In the industrial sector, bacterial polysaccharides applications include cosmetic, dairy, baking, textiles, and biofuel industries (Balkrishna et al. 2018). For example, microbial polysaccharides have been demonstrated to solve several problems encountered during yogurt manufacture (such as low viscosity, gel fracture, or whey separation) because they improve rheology, texture, stability, and mouthfeel of fermented milk products. Moreover, the use of EPS-producing microbial cultures during cheese manufacturing resulted in smoother, moister, and soften cheeses (Jindal and Khattar 2018). In the baking industries, in addition to their use as

stabilizing agents that decrease the staling capacity of baked products, bacterial polysaccharides also play a role in water retention, moisture, texture, and bread volume by optimizing these important parameters of baked products. In the textile industry, bacterial polymers are used, for example, in the fabrication of water-resistant products such as raincoats and vehicle covers (Balkrishna et al. 2018). In the biofuels industry, bacterial polysaccharides are proposed to improve oil recovery fluids due to its high viscosity-enhancing ability at low concentrations (Balkrishna et al. 2018).

2.3 Cosmetic Applications of Bacterial Polysaccharides

According to their role in the product, polysaccharides applied in cosmetics can be classified as functional or active polysaccharides (Freitas et al. 2014).

2.3.1 Functional Polysaccharides

Functional polysaccharides are usually classified based on their electrochemical charge in the product's structure and are commonly integrated into cosmetic products to act as gelling agents, viscosity adjusters, thickeners, and emulsifiers (according to their polymerized network ability to hold water) (Kanlayavattanakul and Lourith 2014).

Cationic Polysaccharides

Polysaccharides with a cationic charge, which is not a widespread characteristic among natural polysaccharides, are often required by the cosmetic industry (Gruber 1999). For this reason, the cosmetic industry has focused mainly on synthetically derived polyglycans, which contain the advantage to tightly bind with anionic proteins of the human skin and hair. Therefore, polyglycans properties as film-forming agents are widespread with applications in hair damage controlling, hair fixing, and skin-conditioning products. The most commercialized cationic polyglycan is chitosan, which is a partially deacetylated (>50%) version of chitin to improve its solubility (Gruber 1999; Kanlayavattanakul and Lourith 2014).

Anionic Polysaccharides

Anionic polysaccharides are very interesting, in terms of cosmetic applications, and are comprised of a group of natural materials, of which the bacterial polysaccharides xanthan gum (Gruber 1999) and gellan gum (Zia et al. 2018) are the most representative. Regarding synthetically derived polysaccharides, carboxymethylcellulose and carboxymethyl-chitin are among the most commercially used for cosmetic uses (Gruber 1999).

D-glucose, D-mannose, and D-glucuronic acid (molecular ratio of 3:3:2) building blocks linked through β-1,4 glycosidic linkages with a high number of trisaccharide side chains are the xanthan gum main constituents. With a molecular weight averaging 2000 kDa (Freitas et al. 2014; Jindal and Khattar 2018), xanthan gum is naturally synthesized, which is usually obtained via bacterial fermentation (Kanlayavattanakul

and Lourith 2014). Its applications include the utilization as a thickener and dispersing agent and emulsion stabilizer (Lochhead 2017). Xanthan gum is a hydrocolloid with the ability to dissolve in water at room temperature (Jindal and Khattar 2018), acting as a non-gelling biopolymer and providing high viscosities at low concentrations (Lochhead 2017) because of the semirigid conformation conferred by its side chains containing mannose and glucuronic acid (Dubuisson et al. 2018). Xanthan can also polymerize and crystallize which gives it the ability to emulsify suspensions, a feature that is frequently adopted in cosmetic preparations to improve the stability against freeze-thaw challenges (Kanlayavattanakul and Lourith 2014). In emulsions, xanthan presents similar rheological properties: high viscosity at low shear rates, a strong non-thixotropic shear-thinning character, and a viscoelastic behavior (Dubuisson et al. 2018). In pharmaceutical cream formulations, as in barium sulfate preparations, the suspension stability provided by xanthan is of utmost importance. In toothpaste, this feature is also an advantage, improving ingredient suspension, due to high viscosity, and facilitating brushing onto and off the teeth. The uniform dispersal of pigments, long-term stability (in terms of pH, salinity, and temperature), and its ability to thicken, xanthan grants an extensive application as shampoo base (Yildiz and Karatas et al., Yildiz and Karatas 2018). Therefore, xanthan is generally used in cosmetic systems, being produced and commercialized by major companies like CP Kelco, Merck, Pfizer, Rhone Poulenc, Sanofi-Elf, and Jungbunzlauer (Alves et al. 2016; Freitas et al. 2014).

Gellan gum is a heteropolysaccharide composed of a tetrasaccharide backbone with L-rhamnose, D-glucose, D-glucuronic acid, and D-glucose monomers and side chains of acetyl and glyceryl substituents (Alves et al. 2016). Among the main properties of gellan gum, its ability to withstand heat and acid stress in fabrication steps are most appreciated. Being a thermoresponsive, biocompatible, biodegradable, ductile, and non-toxic polysaccharide, it is used due to its gelling properties, its malleability, its texture, and its high efficiency in the final products (Zia et al. 2018). Gellan gum is mainly used in cosmetic formulations to increase viscosity and to stabilize emulsions, at low concentrations in dermal products (0.3–0.5%) and even lower in eye and hair products (0.0004%) (Freitas et al. 2014). Also, it forms gels at low concentrations (0.1%) which are available in the low acyl form (creating hard, brittle gels) and in the high acyl form (composing soft, elastic gels) (Freitas et al. 2014; Lochhead 2017). Gellan provides suspensions with low viscosity because it has significant yield stress for low viscosity ranges. So, gellan is another anionic polysaccharide used in the cosmetic industry given that it meets the requirements of the EC Cosmetics Regulation 1223/2009 (EC 2009) in which is stated that toxicological profile of all used ingredients and detailed knowledge of the product-specific exposure are required as fundamental for the safety assessment (Lochhead 2017). This polysaccharide is available with a trading name GelriteTM and KelcogelTM (Zia et al. 2018).

Nonionic Polysaccharides

Nonionic polysaccharides, such as dextran, are not charged which allows them to interact with negatively or positively charged surfactants (Gruber 1999;

Kanlayavattanakul and Lourith 2014), a feature that grants them use in the cosmetic industry as rheology modifiers and thickeners (Gruber 1999).

Dextran is a bacterial homopolysaccharide constituted by chains of D – glucose and consisting of 97–50% α-(1–6) glycosidic bonds on the main chain with the remaining α-(1–2), α-(1–3) and α-(1–4) linkages that form branches, depending on the bacteria strains (Bhavani and Nisha 2010). It is soluble in water and other solvents, in addition to being biocompatible and biodegradable. Dextran and its derivatives have several applications in cosmetics as moisturizers and thickeners, specifically cationic dextran (CDC) because of its capacity to form complex salts with anionic or amphoteric surfactants, which can adsorb to hair/skin producing moisturizing effects, making it a useful conditioning agent. Dextran sulfate is another dextran derivative used in cosmetics as an anti-aging and anti-wrinkle product. Due to its moisture retention and increased lipase activity, it presents a smooth, fresh, and non-sticky feeling which results in supple skin with reduced weight (Bhavani and Nisha 2010).

Amphoteric Polysaccharide

Polyglycans that can have cationic and anionic charges on the same chain are called amphoteric polysaccharides. In a cosmetic formulation the pH determines if the amphoteric polysaccharide is cationic, anionic, or both (Gruber 1999). Amphoteric polysaccharides are mainly produced, via modification, from natural polysaccharides, being denominated as seminatural polysaccharides. Despite having limited use in the cosmetics industry, amphoteric polysaccharides can be found in personal care products formulations, acting as surfactants. For instance, Fucogel®, which is developed by BioEurope and marketed by SOLABIA, is obtained through fermentation of a strain of *K. pneumoniae* (Roca et al. 2015). The result is a highly viscous and hydrophilic pol polysaccharide with a repeating unit of acetylated charged linear trisaccharide, fucose, galactose, and galacturonic acid. This product possesses several interesting characteristics, such as original physicosensorial qualities, moisturizing properties, and self-emulsifying properties (Guetta et al. 2003), which can explain its success in the cosmetic industry. Moreover, Fucogel® was shown to act as a skin anti-aging agent, due to the stimulation of fibroblast proliferation and survival imposed by Fucogel® oligosaccharides (Roca et al. 2015).

2.3.2 Bioactive Bacterial Polysaccharides

Many bacterial polysaccharides are also frequently used in cosmetic applications as active ingredients (Freitas et al. 2014).

Bacterial Cellulose

Cellulose is a high molecular weight, anionic, and water-insoluble biopolymer extracted from natural sources, such as plants and bacteria (*G. xylinus,* G. hansenii*, G. pasteurianus*, *Agrobacterium*, *Alcaligenes*, *Rhizobium*, *Pseudomonas,* and acetobacter) (Kanlayavattanakul and Lourith 2014; Ullah et al. 2016; Balkrishna et al. 2018; Gallegos et al. 2016) which makes it the most abundant renewable polymer. Cellulose presents a homogenous and linear structure of

D-glucopyranose sugar units connected through β linkages (Gallegos et al. 2016). Bacterial cellulose (BC) presents a porous network structure constituted by nanofibrous with high strength and low density, rendering it effective for membrane development for cosmetic products (Freitas et al. 2014). Cellulose ethers (mainly methyl and ethyl cellulose derivatives) are also observed in several cosmetic applications due to their physical and chemical properties (Kanlayavattanakul and Lourith 2014). The Hainan Guangyu Biotechnology Co. Ltd. is a major BC producer and promoter for BC applications in the cosmetic industry. Indeed, reports show the use of BC in cosmetic formulations produces stable oil-in-water emulsions which are non-irritating on the skin. Moreover, these emulsions can penetrate the skin and supply good hydration without the requirement of any surfactants (Gallegos et al. 2016; Ullah et al. 2016). BC also has other applications in components of artificial nails and fingernail polish. By presenting good water holding capacity and gas permeability, BC was also reported as an acceptable carrier in cosmetic active ingredients such as moisturizers, whitening ingredients, anti-wrinkling agents, growth factors, enzymes, or a combination thereof (Ullah et al. 2016). Additionally, BC can be used in personal cleansing formulations and contact lenses (Gallegos et al. 2016; Ullah et al. 2016. In fact, BC is a promising candidate for contact lenses production because of its transparency, light transmittance, and permeability to liquid and gases (Ullah et al. 2016).

Levan

Levan is a homopolysaccharide composed of fructose units with β-2,6-glycoside bonds with β-2,1 side branches (Alves et al. 2016; Domżał-Kędzia et al. 2019; Siqueira et al. 2020) synthesized by several bacteria (*B. subtilis, Z. mobilis,* and *E. herbicola*) and fungi (*Aspergillus sydowii* and *Aspergillus versicolor*) having suitable properties for various cosmetic applications (Domżał-Kędzia et al. 2019). Among the interesting properties of this adhesive amphiphilic polymer, its solubility in oil, low viscosity, and the ability to generate films are valorized in hair-fixing products (Domżał-Kędzia et al. 2019). Moreover, levan can be used in discoloration products because it reduces tyrosinase activity, reducing melanin production. For increased stability to oxidation, levan products can be supplemented by ascorbic acid. It can also co-create a solid polymer matrix that dissipates at skin contact (Domżał-Kędzia et al. 2019). Additionally, levan has high compatibility with salts and surfactants, presents heat stability, water retention capacity, and is nontoxic (Siqueira et al. 2020). Levan can also be used as an encapsulation agent, due to its ability to create nanoparticles in water. The polymers' biological activities (cell proliferation, skin repairing, and moisturizing) contributes to its use as part of three-dimensional artificial skin models, acting also as a protective agent against irritation. Furthermore, levan BPS diminishes skin water loss, keeping it moisturized (Balkrishna et al. 2018; Freitas et al. 2014). However, levan applications are still limited due to two major cutbacks which make its processing and preservation considerably difficult. Firstly, it has low stability in aqueous formulations (Alves et al. 2016) and secondly because, in acidic conditions or high temperatures,

the polymer hydrolyzes into fructose and oligosaccharides (Balkrishna et al. 2018; Alves et al. 2016; Freitas et al. 2014).

Hyaluronic Acid

Hyaluronic acid (HA), also known as hyaluronan, is a linear glycosaminoglycan biopolymer with high molecular weight (up to 10^8 Da) formed by repeating disaccharides D-glucuronic acid and *N*-acetyl-D-glucosamine linked by a glucuronidic β-(1–3) bond (Huynh and Priefer 2020). It plays several roles in biological processes regulation like skin repairmen, cancer diagnosis, wound healing, tissue regeneration, anti-inflammatory, and immunomodulation (Bukhari et al. 2018; Huynh and Priefer 2020). There is nearly 15 g of HA in a 70 kg human being, which can be found in the synovial fluids, umbilical cords, vitreous humor of the eye, heart valves, skin, and skeletal tissues (Huynh and Priefer 2020). This biopolymer is biodegradable and biocompatible, which allows its use in the treatment of several animal and human diseases, preventing difficulties related to non-degradable treatments. Moreover, HA can be used as prognostic molecules and is a polysaccharide with a high natural abundance (found in human and animal bodies), which can hold/trap approximately 1000 times its weight of water (Bukhari et al. 2018; Huynh and Priefer 2020). Even though commercial HA was obtained, at first, from mammalian tissues, public health concerns ignited the research of alternative sources of this biopolymer, like microorganisms and marine organisms (Alves et al. 2016). HA activity is highly conditioned by its size but there are several specific applications of each molecular weight, with many utilizations in medicine and the cosmetic industry (Freitas et al. 2014). In fact, research and development of HA and its related products have been abundant, with reports showing its effectiveness as dermal fillers, anti-wrinkle agents, and tissue regeneration agents (Huynh and Priefer 2020).

Hyaluronic acid cosmetic effects are mainly related to its performance as a soft tissue generator, a skin hydration improver, a collagen stimulator, and face rejuvenation inducer (Bukhari et al. 2018). The differences in percutaneous absorption of different molecular weight HA across the stratum corneum show that anti-wrinkle effect efficiency depends on the molecular weight of the used biopolymer (Bukhari et al. 2018). Pavicic et al., have conducted a clinical trial involving 76 females, with ages from 30 to 60 years old, who have periocular wrinkles. On these patients was applied, during 60 days (2 times a day), a 0.1% (w/w) cream formulation with different HA molecular weights (50, 130, 300, 800, 2000 kDa). The results showed tremendous enhancement in skin hydration levels and skin elasticity. Moreover, on the patients that used low HA molecular weight formulations, it was detected a reduction in periocular wrinkles (Pavicic et al. 2011).

Anti-wrinkle efficacy of HA-based topical cream formulation has also been investigated by Poetschke et al. In this study, the authors studied, for 3 months, four topical cream formulations (Balea, Nivea, Lancome, Chanel) containing HA on 20 women with periorbital wrinkles. The results showed a significant improvement in skin elasticity and tightness by 13–30%, significant reduction in wrinkle depth by 10–20%, and improved hydration level in all treatment patients (Bukhari et al. 2018; Poetschke et al. 2016).

Apart from the topical application advantages already explained, scientific research has reported the benefits of HA-based treatments, such as the intradermal injection of HA (as fillers, gels, and implants) in treating facial wrinkles and promoting skin rejuvenation. Moreover, the safety, tolerability, and patient satisfaction have contributed to the diffuse utilization of HA in cosmetics (Bukhari et al. 2018).

In terms of HA gel injection, the effects of HA in the reconstruction of deficient interdental papilla, also known as the black triangle, which is the gingival portion area between two adjacent teeth, have also been studied. The results showed a reduction in 36.5% and 45% in the triangle surface after 3 months and 6 months, respectively. (Huynh and Priefer 2020). Regarding hyaluronic acid action as filler during the congenital and acquired lip asymmetries, the results showed that one of the disadvantages of this treatment is the injections required overtime to maintain the result.

There are already several FDA-approved dermal HA fillers, which are mainly commercialized by Restylane® (Medicis, USA), Prevelle Silk® (Mentor Corp., USA), Anika® (Anika Therapeutics, Inc., MA), and Juvéderm™ (Allergan, USA) (Freitas et al. 2014). In conclusion, while dermal filler HA is indicated to treat skin aging-related issues, the topical formulation of HA is appropriate for skin treatment and to prevent skin roughness (Bukhari et al. 2018). An interesting research area to explore for hyaluronic acid could be the application of its properties in wound healing treatments/products (Huynh and Priefer 2020).

3 Skincare

3.1 Skin Structure

The skin is an integrated and dynamic organ, which accounts for 10 to 15% of our body mass, making it the largest organ of the human body. Besides serving as a barrier to the external environment, protecting our body from physical damage and pathogens, the skin is also important in maintaining the body's homeostasis by preventing water loss and due to its functions in the immune-neuroendocrine system (Lai-Cheong and McGrath 2013; Monteiro-Riviere 2006). Free radicals (endogenous and exogenous) can interact with the skin, producing negative effects that may result in skin-based diseases such as psoriasis, eczema, urticaria, and skin cancer (Lai-Cheong and McGrath 2013). Therefore, the skin has a unique defense mechanism based on endogenous (derived from melanin) and exogenous (orally and topically administrated) antioxidants, in order to avoid these adverse effects of free radicals. This defense mechanism is intrinsically linked to the structure and function of skin layers elements (Kusumawati and Indrayanto 2013). Basically, the human skin is constituted by four layers (from top to bottom): the nonviable epidermis (the stratum corneum), the viable epidermis (the remaining layers of the epidermis), dermis, and hypodermis (Fig. 1).

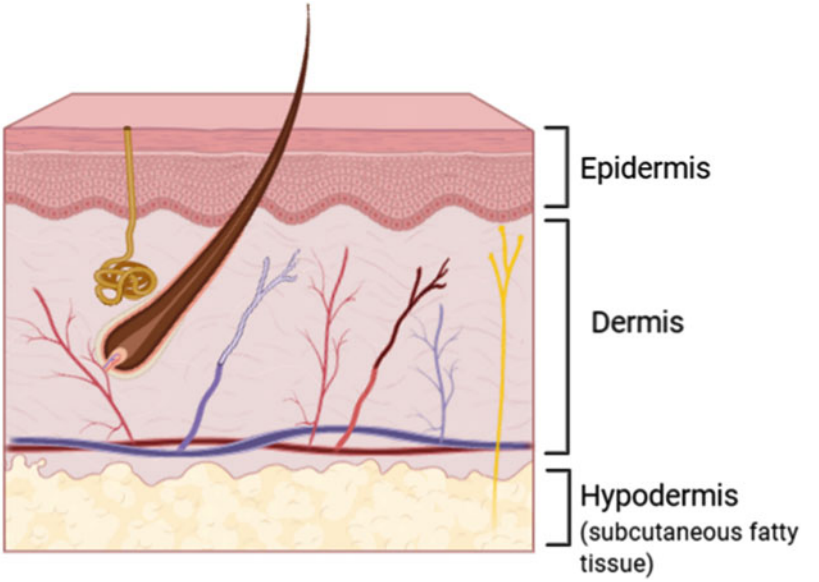


Fig. 1 Structure of the human skin

The epidermis is mainly composed of keratinocyte cells (95%), which are the producers of the protein keratin, while the remaining 5% are melanocytes, Langerhans cells, and Merkel cells (Lai-Cheong and McGrath 2013; Lawton 2019). Since this skin layer does not include blood vessels, the nutrient delivery and waste disposal are dependent on the second layer (dermis) through the basement membrane (Lawton 2019). Depending on keratinocyte differentiation, the epidermis is usually divided into four layers (Lai-Cheong and McGrath 2013; Lawton 2019): stratum corneum, stratum granulosum, stratum spinosum, and stratum germinativum/basale (Table 2) (Fig. 2) (Lai-Cheong and McGrath 2013; Lawton 2019; Kusumawati and Indrayanto 2013; Monteiro-Riviere 2006). The stratum lucidum is a fifth layer found in thick skin, such as the palms of the hands, the soles of the feet, and the digits) (Lawton 2019; Monteiro-Riviere 2006).

Keratinocyte and melanocyte cells are the main components of the stratum basale, with the former being produced exclusively in this layer via division (Lawton 2019; Kusumawati and Indrayanto 2013). During the migration of keratinocytes to the upper layers (stratum spinosum and stratum granulosum) occurs a process called keratinization, which differentiates these cells to assemble a rigid internal structure of keratin, microfilaments, and microtubules (Lawton 2019). In a 40-day long process, keratinocytes proliferate and perform terminal differentiation, leading to the establishment of the stratum corneum (Lai-Cheong and McGrath 2013). In its turn, the stratum corneum features layers of dead cells that lost their nucleus (Lawton 2019; Monteiro-Riviere 2006), which suffer desquamation, a 28-day long process (Kusumawati and Indrayanto 2013; Lawton 2019). These dead cells, also known as corneocytes, are ingrained within a

Table 2 Epidermis layers characteristics

Epidermis layers	Description
Stratum corneum	Comprised of several layers of completely keratinized flattened dead cells. Each stratum corneum cell is approximately 30 μm in diameter and 0.5 to 0.8 μm in thickness.
Stratum lucidum	Fully keratinized and homogeneous zone with dense cells without core localized between the stratum granulosum and stratum corneum. Can be found in the palms and soles, where the skin is extremely thick and lacks hair.
Stratum granulosum	Constituted for three to five layers of flattened cells and presents high lysosomal activity. This layer has lipids with a function for coating the cell membrane of the stratum corneum cells and are responsible for barrier to chemical absorption across the skin. These lipids include ceramides, fatty acids, cholesterol, and cholesterol sulphate.
Stratum spinosum	Consists of several layers of irregular polyhedral-shaped cells which are interconnected by desmosomes. Langerhans cells are mainly located in this layer.
Stratum basale	The basal cells layers of the epidermis are functionally heterogeneous. Can divide and proliferate or act as anchor cells that remain attached to the basement. It is composed by keratinocytes, which are either division phase or non-division phase. These cells contain keratin tonofibrils and melanocytes. Merkel cells are also found in the basal cell layer. These cells are a responsible for the in the skin such as touch feeling.

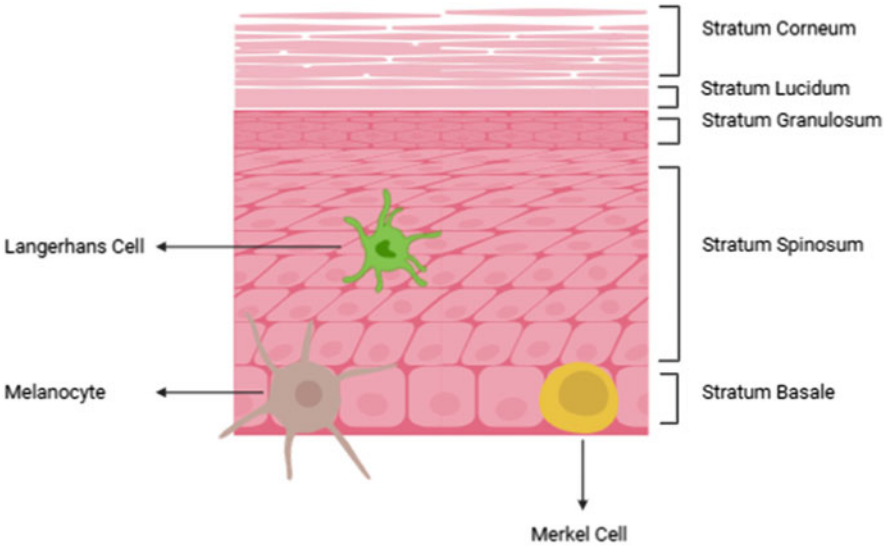


Fig. 2 Epidermis layers

hydrophobic extracellular matrix composed of lipid precursors and hydrolases, which results in the formation of fatty acids, cholesterol, and at least 10 ceramides (Lai-Cheong and McGrath 2013). Ceramide is essential for maintaining a

moisturized skin due to its function as a water retention agent, playing also a role in the maintenance of skin defense by forming lipid barriers that prevent the penetration of allergens and irritants (Kusumawati and Indrayanto 2013; Lawton 2019). Therefore, ceramides contribute to high water content in the corneocytes, which causes them to swell and keep the stratum corneum flexible, preventing skin dryness and the appearance of fissures and cracks (Lawton 2019). The loss of the epidermis moisture retention ability causes dry skin, which can only be repaired using specific treatments to improve hydration and decrease water evaporation, such as topical application of moisturizers and the utilization of occlusive masks on the skin (Freitas et al. 2014). Summing up, the stratum corneum determines the extent and ratio of percutaneous absorption (Lawton 2019), and, given its highly impermeable lipophilic layer, acts as an important protector from the external environment, being determinant for a healthy skin appearance (Kusumawati and Indrayanto 2013).

In the stratum basale, there can be found melanocytes, the synthesizers of melanin, the molecule responsible for the absorption of ultraviolet (UV) radiation (Kusumawati and Indrayanto 2013; Lawton 2019). By causing the peroxidation of the lipid matrix of the stratum corneum, which leads to loss of this structure barrier capacity, extended exposure to UV radiation can lead to mutations, causing premature skin aging or skin cancer (Freitas et al. 2014). Langerhans cells, which are bone marrow-derived, are dendritic cells with an antigen-presenting activity that can be found not only in the stratum spinosum but also in hair follicles, sebaceous glands, and apocrine glands (Lai-Cheong and McGrath 2013; Lawton 2019; Monteiro-Riviere 2006). On the other side, Merkel cells can only be found in the stratum basale and act as sensory information transmitting cells from the skin to the sensory nerves (Lawton 2019; Monteiro-Riviere 2006; Lai-Cheong and McGrath 2013).

The next skin layer is the dermis, composed of fibroblasts, collagen, elastin, and hyaluronic acid (Kusumawati and Indrayanto 2013) and containing blood vessels, nerves, glands, and hair follicles (Ammala 2013; Monteiro-Riviere 2006). The major functions of this layer are the maintenance of skin characteristics and serving as a water reservoir for the skin (Kusumawati and Indrayanto 2013). As previously stated, the fibroblast is an important component of the dermis, being the major cell type observed in this layer, acting as a synthesizer of collagen, elastin, and viscous gel (Lai-Cheong and McGrath 2013; Lawton 2019). Collagen, responsible for the toughness and strength of the skin, constitutes 70% of the dermis layer, being continuously degraded and replaced. Elastin fibers are responsible for skin elasticity (Lai-Cheong and McGrath 2013; Lawton 2019). However, these two important molecules are influenced by increasing age and exposure to UV radiation, which usually results in dips and stretches of the skin (Lawton 2019). The hypodermis, which is the deepest skin layer, provides the main structural support for the skin, consisting of adipose tissue that serves as a thermal barrier to the skin. This skin layer contains collagen and extracellular matrix and is interlaced with blood vessels and nerves (Kusumawati and Indrayanto 2013; Lawton 2019).

3.2 Skin Conditions

Skin disorders affect millions of people worldwide with physical and often traumatic psychological implications for quality of life (Long 2002). Therefore, many efforts have been made over the years to develop effective cosmetic and treatment solutions using bacterial polysaccharides.

3.2.1 Psoriasis

Psoriasis is a chronic inflammatory, autoimmune disorder of the skin (Fig. 3) that affects approximately 120 million people worldwide. In this condition of unknown etiology, it is usually observed an increased proliferation of inflammatory cells within the dermis and epidermis and an expansion of the upper dermal capillaries (Long 2002; Song et al. 2019). Nowadays, there is a great focus, in the dermatology research field, to treat and prevent this condition (Song et al. 2019). The use of emollients as a topical therapy for psoriasis is widespread due to their properties for blocking transdermal water loss, which promotes skin softness and reduces skin scaling, making the patient feel more comfortable (Long 2002). Nevertheless, there are many other topical therapies available for psoriasis treatment, such as coal tar, topical steroids, calcipotriol, anthralin, and cyclosporine (Long 2002).

In terms of bacterial polysaccharides utilization for the treatment/prevention of this condition, there are already several promising studies. For example, Song et al. (2019) investigated the use of bacterial exopolysaccharide as a moisturizer and drug-loading material. For this purpose, the authors prepared an exopolysaccharides/calcipotriol (EPS/CPT) emulsion (Fig. 4) using bacterial EPS as an emulsifier, sunflower oil as an oil phase, and CPT as the loaded drug. In vitro and in vivo animal experiments showed that the EPS/CPT emulsion could effectively treat psoriasis by increasing the accumulation of Calcipotriol in psoriatic skin lesions and reducing the levels of inflammatory cells and inflammatory factors. Additionally, the emulsion produced interesting results on reducing the side effects associated with Calcipotriol (Song et al. 2019). Xanthan gum has also been studied for the formulation of emulsions for the treatment of psoriasis (Musa et al. 2017). In that study, the active ingredient was cyclosporine, the oil phases were virgin coconut oil

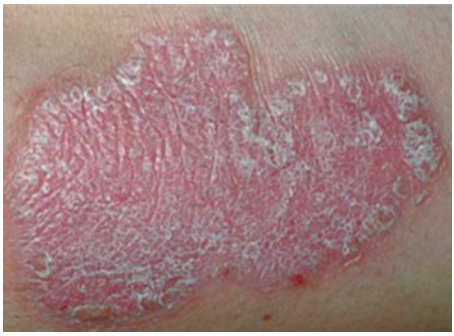


Fig. 3 Clinical manifestation of psoriasis: typical erythematous plaques with silvery scales. (Retrieved with permission from Boehncke and Schön 2015)

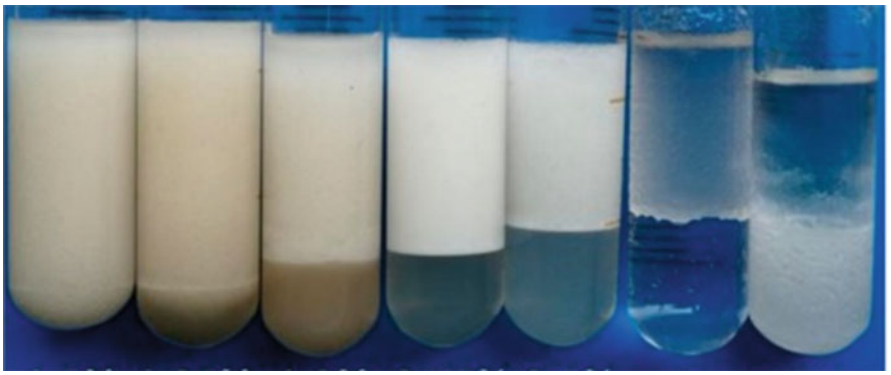


Fig. 4 EPS emulsified sunflower oil at different concentrations. From left to right: 1.5%; 1.25%; 1%; 0.75%; 0.5%; 0% and control (Song et al. 2019)

and nutmeg oil. The nanoemulsion produced significant improvements in the stratum corneum, responsible for skin hydration (Musa et al. 2017).

In another study, bacterial cellulose was combined with carboxymethylcellulose (CMC) to form a biocomposite for drug delivery systems (Fig. 5) (Lima Fontes et al. 2018). The authors loaded the BC/CMC biocomposite with methotrexate (MTX), a drug used to treat autoimmune diseases, to increase the effectiveness of the topical treatment of psoriasis (Lima Fontes et al. 2018). In a different study, hydrogels, composed of hyaluronic acid and polyvinyl alcohol, were loaded with methotrexate (Cheaburu Yilmaz et al. 2017). The resulting hydrogels revealed promising pH-responsive swelling and releasing abilities. In terms of biocompatibility, the toxicity studies performed in mice showed that the matrices are non-toxic and toxicologically safe when administered via intraperitoneal injection or topically. Therefore, these systems are a potential option for the development of novel topical formulations for psoriasis therapy (Cheaburu Yilmaz et al. 2017). Hyaluronic acid has also been reported in the development of solid microneedles (MNs) patch in an attempt to decrease the setbacks associated with MTX application in psoriasis therapy and also to increase the therapeutic effect (Du et al. 2019). That study illustrated the ability of HA to increase the solubility, biocompatibility, and biodegradability of MNs. Moreover, the presence of hyaluronic acid in the formulation conferred enough mechanic strength to the MNs to pierce the stratum corneum (Du et al. 2019).

3.2.2 Eczema

Eczema or dermatitis is a chronic inflammatory skin disease characterized by frequent spontaneous flares and remissions (Fig. 6), which manifests dry skin and intense itching in patients (Long 2002). The treatment of this condition relies mainly upon moisturizers, such as hydrogels and humectant-enriched creams and lotions formulations of oil-in-water and water-in-oil emulsions. In fact, the incorporation of humectant ingredients like HA improves skin hydration (Draelos 2011). There are

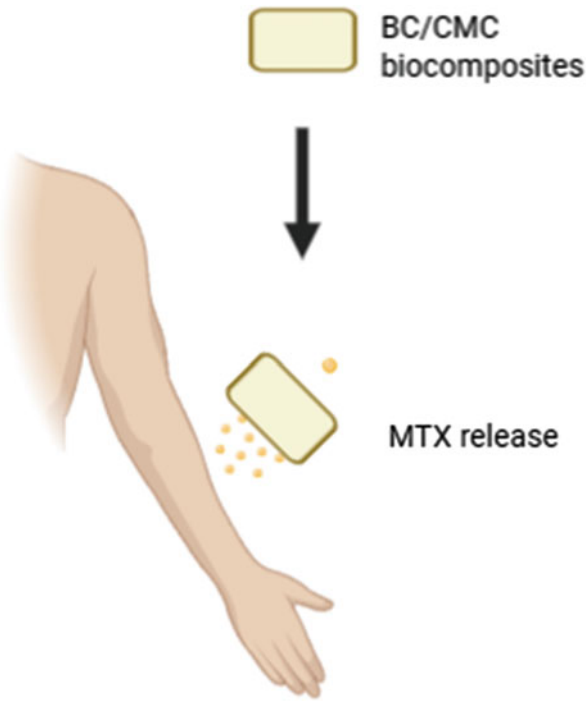


Fig. 5 BC/CMC biocomposite with methotrexate (MTX). (Adapted from Lima Fontes et al. 2018)

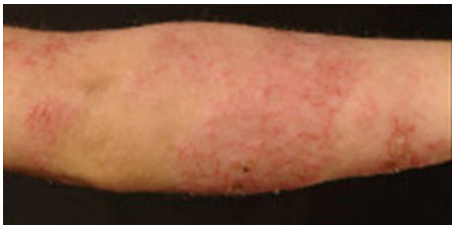


Fig. 6 Extensive atopic dermatitis (eczema). (Retrieved with permission from Mathe and Loffeld 2019)

several prescription devices already implemented to increase barrier function in atopic dermatitis patients. Those products are aimed to create an optimal healing environment, by using occlusive agents to decrease transepidermal water loss (Draelos 2011).

For that purpose, research has been made and there are reports, for example, of HA-based foam technology aiming to boost moisture and distribute transepidermal water loss (Draelos 2011). In that study, 20 patients, who presented mild/moderate symmetrical atopic dermatitis in a body surface area higher than 10%, were treated with two products: in one side of the body the hyaluronic acid-based emollient foam was applied; on the other side, a reference ceramide-containing emulsion cream was applied. The patients and the researchers rated several symptoms and signs, such as

erythema, scaling, lichenification, excoriation, itching, stinging, and burning, during the whole treatment. The results showed an overall improvement in eczema severity using HA foam, proving the effectiveness and soothing ability of the product (Draelos 2011). A different study assessed the utilization of a ceramide-HA emollient foam, in terms of short-term effectiveness, and compared the results with a pimecrolimus cream, during the treatment of atopic dermatitis. The authors reported that both products presented acceptable efficacy in patients with mild/moderate atopic eczema and that after a month of treatment, the ceramide-HA emollient foam continued to help the patients (children and adults) without side effects (Frankel et al. 2011). The HA-containing emollient can deposit ceramide proteins in the most important anatomic location, delivering the moisturizer through the stratum corneum and into the dermis (Hebert and Pacha 2012), an advantage that explains the increasing commercial availability of this safe and efficient type of product for the treatment of atopic dermatitis (Hebert and Pacha 2012). In another study, several subjects (aged between 18 and 75 years) were treated with low molecular weight HA gel, in an attempt to understand the efficiency and safety of this product for the treatment of facial seborrheic dermatitis (Schlesinger and Rowland Schlesinger and Rowland 2014). In that report, the researchers were interested in measuring scale, erythema, and pruritus, at baseline and weeks 2, 4 and 8. The results showed that HA sodium salt gel 0.2% presented improvements in the measured variables at week 4 with 76.9%, 64.3%, and 50% reductions in scale, erythema, and pruritus, respectively (Schlesinger and Rowland , Schlesinger and Rowland 2014). Resuming, HA-based medical creams are suitable for treating several dermatoses like atopic dermatitis and contact dermatitis and there are already various FDA-approved products on the market: Atopiclair® (Sinclair Pharma), HylatopicPlus® (Onset Dermatologics), and Bionect® (Innocutis Holdings).

3.2.3 Acne

Frequently present during adolescence, acne vulgaris is the most common skin condition, with 70% of the population developing recognized acne variants, which include infantile acne and occupational acne. This skin disorder is characterized by follicular dyskeratosis, increased sebum production, and inflammation induced by *Cutibacterium acnes* within the follicle (Fig. 7). Treatment for this skin condition includes the utilization of vitamin A-derived retinoids, to control the pathogenic pathways of acne, and topical applications of antibiotics such as erythromycin, clindamycin, and tetracycline. Azelaic acid is also effective in the treatment of acne (Long 2002).

Acne scarring is an important aesthetic problem because it is widely prevalent, affects the face prominently, is poorly masked by cosmetics, and begins at an early age. Research for the prevention of this problem was performed, and a low-viscosity stabilized HA dermal filler was injected at microdoses into the mid-to-superficial dermis of twelve patients (7 men and 5 women) aged between 19 and 54 years, to improve the appearance of depressed acne scars. The treatment was well tolerated by the patients and the results showed an immediate visual improvement of all lesions (Halachmi et al. 2013).

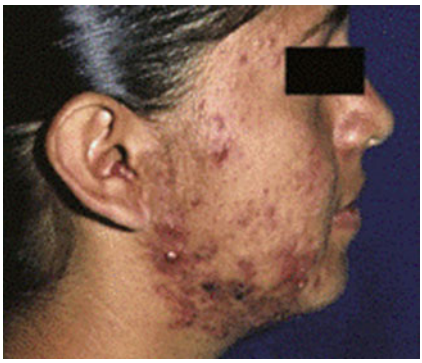


Fig. 7 Acne lesions. (Retrieved with permission from Vary 2015)

Bacterial cellulose is another bacterial polysaccharide used in acne treatment, being present, for example, in facial masks. Another example is the utilization of a bio-cellulose film (prepared from *A. xylinum*) incorporating several concentrations of *P. granatum* (pomegranate) peel extract for application as an anti-acne product. The authors of this study reported a satisfactory inhibition effect on S. aureus, S. epidermidis, and *P. acne*, when compared to antibiotics such as gentamicin and clindamycin, for BC with extracts of 5 or 10 mg/ml of pomegranate. A different study aimed to develop a system for the treatment and healing of skin prone to acne. By using a BC film loaded with natural propolis extract (with high water content), the authors expected to improve skin hydration and enhance the texture of the treated skin (Fig. 8). The product improved the skin's mechanical properties, making it an efficient way to release active substances (Amorim et al. 2020).

3.2.4 Rosacea

Rosacea, which is a chronic inflammatory skin disorder that mainly affects the facial skin, is usually characterized by skin dehydration, redness, erythema, and telangiectasia (Fig. 9). This skin condition derives from repeated environmental trauma (cold wind, ultraviolet radiation, and heat) which damages the upper dermal collagen and vasculature. Moreover, it can also be a result of prolonged use of topical steroids on the face. For the treatment of this skin disorder, topical applications of metronidazole and azelaic acid have been demonstrated to be effective, with persistent cases requiring the prescription of oral metronidazole, tetracyclines, or isotretinoin (Long 2002).

Research using bacterial polysaccharides in rosacea treatment is widely available. For instance, researchers studied the efficacy and tolerability of HA containing sodium salt cream 0.2% during clinical trials on 15 patients (Chen et al. 2018). Several clinical symptoms, such as papules, erythema, burning or stinging, and dryness, were assessed and the results showed a great efficacy of low molecular weight HA cream in the reduction of all symptoms, coupled with good tolerability by patients (Chen et al. 2018).

Fig. 8 BioMask prototype: BC film loaded with natural propolis extract (Amorim et al. 2020)

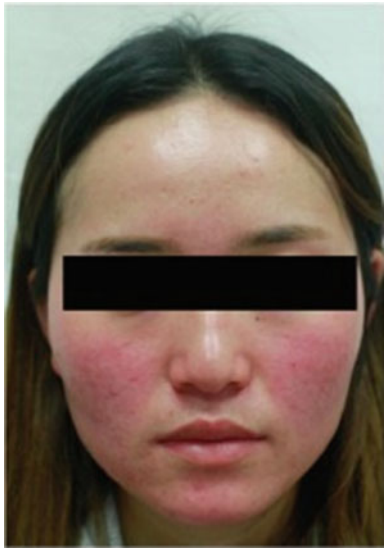


Fig. 9 Rosacea. (Retrieved with permission from Li et al. 2020)

3.2.5 Hyperpigmentation and Skin Aging

Environmental pollution and overexposure to UV radiation can lead to skin oxidative stress caused by reactive oxygen species (ROS), which in turn may lead to skin disorders such as hyperpigmentation (Fig. 10, left image) and skin aging (Fig. 10, right image). Despite possessing a defense mechanism against ROS, the skin is usually affected by these external factors, characterized by freckles, dark spots, and wrinkle formation (Jesumani et al. 2019). Excessive presence of ROS prompts melanin production via the activity enhancement of the enzyme tyrosinase, leading

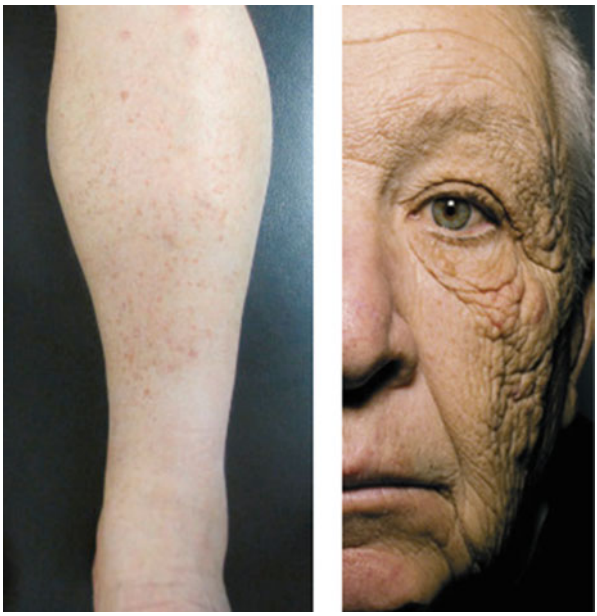


Fig. 10 Hyperpigmentation (left) and facial wrinkles (right). (Retrieved with permission from Yamada and Prow 2020, and Leijs et al. 2018, respectively)

to hyperpigmentation. Moreover, high ROS concentration has an elastase-inducing effect, which degrades elastin, resulting in skin's elasticity and strength loss, causing visible wrinkles (Jesumani et al. 2019).

HA applications in anti-wrinkle products have already been mentioned in Sect. 2.3.2.3. HA is a moisturizing active ingredient, that can be used in anti-wrinkle cosmetic formulations, such as gels, emulsions, and serums, with the ability to recover a young skin typical physiological microenvironment (Fallacara et al. 2018). This is due to its strong water binding potential, which helos the maintenance of the skin elasticity, turgor, and moisture (Pavicic et al. 2011). For instance, Fillerina® (Labo Cosprophar Suisse), an HA-based cosmetic, is known to restore skin hydration and elasticity to exert an anti-wrinkle effect (Fallacara et al. 2018). The clinical efficacy of topical nano-HA formulation to reduce facial wrinkles and erythema intensity has also been the subject of research. The results showed major improvements in skin elasticity and firmness, and a higher hydration level with the consequent reduction in erythema intensity, wrinkles, and roughness of skin (Chen et al. 2018). Recently, HA utilization as a dermal filler has been widely applied. Dermal fillers (DFs) are class III medical devices that, injected into or under the skin, restore lost volumes and correct facial imperfections such as wrinkles (Fig. 11). The reversibility of the HA dermal filler effect can explain the success of this application: the correction of wrinkles is reversible, meaning that a medical error can be fixed using an injection of HYAL (Vitrase®, ISTA Pharmaceuticals; Hylenex®, Halozyme Therapeutics). Depending on the used HA concentration, type and degree of crosslinking, and treatment area, HA DFs effects can last between 3 and 24 months

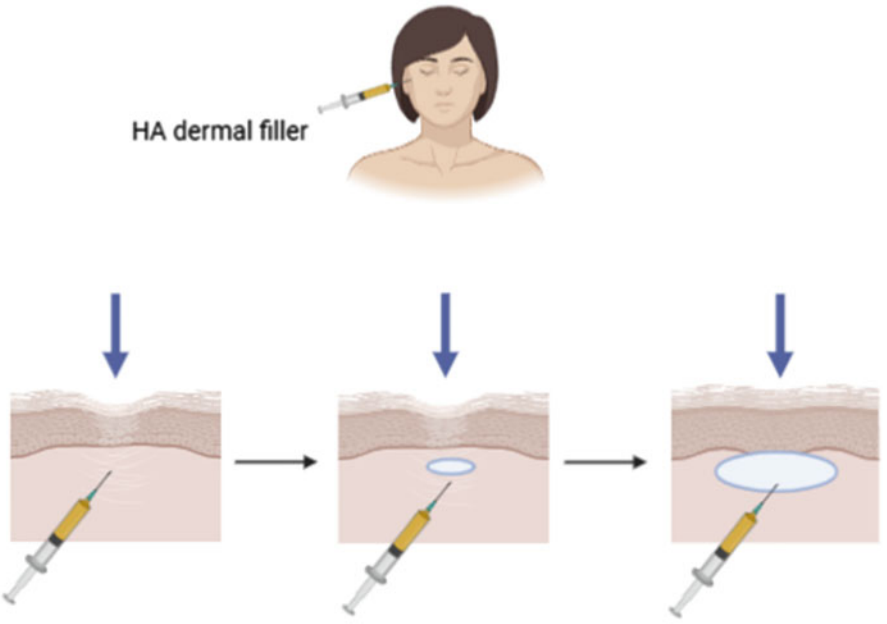


Fig. 11 HA dermal filler process for facial imperfections such as wrinkles

(Fallacara et al. 2018). Sunscreens that contain HA also assist in the maintenance of youthful skin, because HA has free radical scavenging properties, which protects the skin against negative effects of UV radiation (Fallacara et al. 2018).

As stated before, bacterial cellulose can be used in cosmetic masks. Considering its biodegradability, low toxicity, and ability to hydrate the skin, BC skin masks have been studied in the treatment and prevention of dry skin, in formulations with cosmetic substances such as sodium bicarbonate, ascorbic acid, and salicylic acid. These products have been shown to help the exfoliation and brightening of the skin, having an anti-wrinkle effect. Levan is also present in formulations because it improves skin elasticity, reduces wrinkles depth, and enhances the moisture of the skin (Kim et al. 2003). Dextran, conjugated with rosmarinic acid, was also reported in the development of a skin whitening agent, an innovative polymeric antioxidant. The studies (in vitro and in vivo) results showed that this product has a good skin whitening/lightening efficacy, being biocompatible. The cosmetic formulation has improved stability, with long-lasting effects, which means dextran is a suitable bioactive ingredient.

4 Cosmetic Products

Cosmetic and personal care products represent a massive market, providing an extensive range of properties and benefits to the consumer, with millions of consumers using cosmetics and their ingredients daily (Nohynek et al. 2010). In fact,

cosmetic products global market was valued at USD 532.43 billion in 2017, and, through an expected annual growth rate of 7.14%, it can register USD 805.61 billion by 2023 (Bilal and Iqbal 2020).

4.1 Definition and Categories of Cosmetics Products

The European Union Council regulations define cosmetic products as "any substance or mixture intended to be placed in contact with the external parts of the human body (epidermis, hair system, nails, lips, and external genital organs) or with the teeth and the mucous membranes of the oral cavity with a view exclusively or mainly to cleaning them, perfuming them, changing their appearance, protecting them, keeping them in good condition, or correcting body odors" (EC 2009, article 2.1). In general, a cosmetic product is applied for the direct treatment of the surface of the human body to maintain its good condition (1), to change the body appearance (2), to protect the body (3), or to correct the body odor (4) (Halla et al. 2018). These four functions must not affect the normal body functions or structure (Balkrishna et al. 2018; Ullah et al. 2016).

Cosmeceuticals, also known as active cosmetics, which were created in the 1980s, are cosmetic products containing biologically active ingredients with medicinal or drug-like benefits, aimed to satisfy the customer's health and beauty needs. In fact, cosmeceuticals can act as skin protectors, deodorants, whitening, tanning and anti-wrinkling (or antiaging) agents, and also possessing functions in nail and hair care products.

In consonance with their field of applications and functions, cosmetic products can be divided into seven categories, as follows. Cosmetics for personal cleansing (1) (soaps, deodorants, shampoos); cosmetics for the skin, hair, and integument care (2) (toothpaste, products for external intimate care); cosmetics for embellishment (3) (perfumes, lip colors); protective cosmetics (4) (solar products, anti-wrinkle products); corrective cosmetics (5) (beauty masks, hair dyes); maintenance cosmetics (6) (shaving cream, moisturizing creams); and active cosmetics (7) (fluoridated toothpaste, antiseptics) (Halla et al. 2018). Bacterial polysaccharides are used in most of these cosmetic products, and Table 3 illustrates its level of utilization in several applications.

Furthermore, there are several other bacterial polysaccharides with promising features for possible commercial applications. For example, Galactopol, an EPS composed of galactose, mannose, and rhamnose, has interesting properties (viscosity in an aqueous environment, emulsifier, film-forming) that could be used as alternatives to other cosmetic applications-related polysaccharides. Being anionically charged, Galactopol is usually synthesized by *P. oleovorans* and possesses a molecular weight of $1–5 \times 10^6$ Da. Another interesting EPS is FucoPol, a bacterial heteropolysaccharide constituted by fucose, glucose, galactose, and glucuronic acid. This BP has shown to form viscous solutions, with the ability to both form films and stabilize emulsions, which are interesting properties to use in the cosmetic filed. Moreover, it has already been shown its ability to encapsulate bioactive

Table 3 Bacterial polysaccharides current applications in cosmetics

Polysaccharide	Current applications in cosmetics	References
Xanthan gum	Skin care, hair care, conditioners and shampoos, aftershave, shower gel and cream, body lotion, moisturizing products, sunscreen, toothpaste.	Kanlayavattanakul and Lourith (2014), Savary et al. (2016)
Gellan gum	Skin care, hair care, lotions and creams, makeup products, face masks and packs, toothpaste, suntans and sunscreens, toothpaste.	
Dextran	Skin care, protective cosmetics, moisturizing products.	Bhavani and Nisha (2010), Kanlayavattanakul and Lourith (2014)
Bacterial cellulose	Skin care, hair care, lipstick, eyeliner, moisturizing products, masks, hair dyes and colors, bath preparation, shampoos, toothpaste, antiperspirant.	Gallegos et al. (2016), Lima Fontes et al. 2018, Savary et al. (2016)
Levan	Skin care, hair care products, whitener products.	
Hyaluronic acid	Skin care, hair care, moisturizing and hydrating products, protective products, pre/after sun lotions, sunscreen.	Fallacara et al. (2018), Pavicic et al. (2011), Savary et al. (2016)

compounds, which could be important for its application as an encapsulating matrix in cosmetics applications (Freitas et al. 2014; Lourenço et al. 2017). FucoPol (1.7×10^6–5.8×10^6 Da) is negatively charged due to the existence of glucuronic acid, succinyl, and pyruvyl in its constitution (Freitas et al. 2014).

4.2 Cosmetic Formulation

The abundant advances in studying and understanding the physicochemical properties formulation systems (and their ingredients) have contributed to the development of biologically stable products. The first generation of skincare products emphasized the relevance of free amino acids in skin hydration. In fact, water, oily substances, and humectants combinations can be efficient to keep the epidermis biological homeostasis, acting upon the physicochemical condition of the stratum corneum, since the barrier function of the skin is maintained by moisture, lipids, and natural moisturizing factors (Fig. 12) (Hosoi et al. 2017).

Any cosmetic formulation includes in their formulation base substances, active agents, and additives (Table 4) with a different function on the final product. Base substances are natural skin components; active agents are substances with specific functions such as protection, preservation, and/or improvement of the skin's natural condition; and additives are substances used to improve the product's stability, providing protection against microorganisms, temperature shifts, oxygen and light related degradation, which improves the product's shelf life (Freitas et al. 2014).

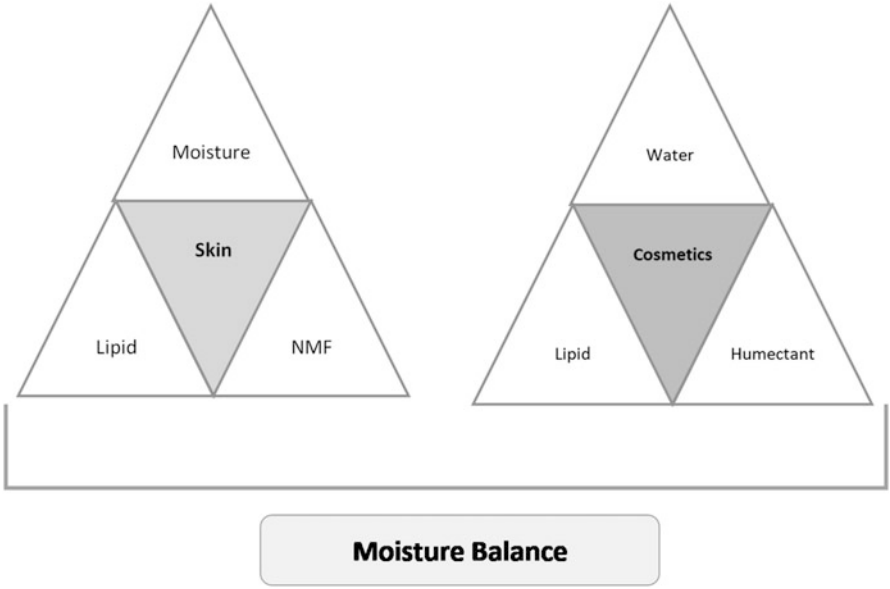


Fig. 12 Moisture balance of the skin in the first generation of skin care. NMF - natural moisturizing factors. (Adapted from Hosoi et al. 2017)

Table 4 Substances used in cosmetic formulation

Substances	Ingredients	References
Base substances	Fatty acids Mineral oil products Triglycerides Wax esters	Freitas et al. (2014)
Active agents	Hyaluronic acid L-Fucose Valproic acid Vitamins (A, B3, C, E) Lactic acid Botanical extracts (Hesperidin, tea plant, ginseng, Resveratrol) Algal extract (*B. braunii)* Ubiquinone	Freitas et al. (2014), Lautenschläger (2004)
Additives	Water Antioxidants Dyes and pigments Emulsifiers Perfumes Preservatives (Parabens, organic acids) Thickening agents (Types: lipid, naturally derived, mineral and synthetic) UV filters	Freitas et al. (2014), Lautenschläger (2004)

The active ingredients for cosmetics represent a large worldwide commercial market, projected to grow to US$1.6 Billion by the year 2025 (PRNewswire official website). These ingredients are declared using the International Nomenclature Cosmetic Ingredient (INCI) standards and are listed according to their weight ratio in decreasing order. However, aromatic principles (fragrances) are only declared as perfumes or essences. The modification of cosmetic products is a result of the overexposure to atmospheric oxygen, or due to the presence of microorganisms. Subsequently, antimicrobial and antioxidant preservatives can be added to cosmetic formulations to, respectively, inhibit microbial growth and suppress the formation of free radicals through oxidation (Halla et al. 2018).

Generally, the used additives in cosmetic formulations surpass the INCI listed base substances or active agents. Except for UV filters and consistency control substances, these additives should be averted due to their prowess to cause allergies (Freitas et al. 2014).

Given their ability to protect consumers from UV radiation, UV filters are important additives in cosmetic formulations that are controlled by international safety regulations, which are strictly evaluated before the marketing of these products. Other additives, like TiO_2 and ZnO, were also analyzed due to consumers' concerns and shown to be non-toxic and non-invasive, posing zero risk to human health (Nohynek et al. 2010).

The active ingredients used can be naturally synthesized (peptides, ceramides, most vitamins), purified from natural sources (botanicals, herbal extracts), obtained by cell culture fermentation (enzymes and cofactors, polysaccharides and proteins), or extracted from animal sources. Still, the latter reduced greatly after the bovine spongiform encephalopathy related issues (Lintner et al. 2009).

The specifications for any cosmetic ingredient are required to combine its chemical identification, in terms of structural formula, the raw material origins, the used extraction method; its physical arrangement, whether it is a powder, paste, gel or liquid; its molecular weight; its purity, with mandatory impurity characterization; its solubility in water and other relevant organic solvents; and additional specifications, like its organoleptic properties, flash point, melting/boiling point (Freitas et al. 2014).

4.3 Main Cosmetic Vehicles

In dermatology, the active substance is usually integrated with a carrier system called the vehicle, a term used to differentiate active from inactive ingredients/pharmaceuticals in the cosmetic and formulation areas (Bohnenblust-Woertz and Surber 2014; Buchmann 2001). They are tailormade for the cosmetic application that they are intended, with developers aiming to achieve stable and compatible vehicles. The vehicles' patient/consumer acceptability, usage criteria, and bioavailability are also important aspects taken into consideration (Bohnenblust-Woertz and Surber 2014).

The vehicle function is to carry and deliver the active ingredients to a specific localization in the skin or inside the human body. Despite not containing pharmacologically active substances for disease treatment, skincare cosmetic formulations

rely heavily upon vehicles for cleansing, hydration, protection, and decoration purposes (Buchmann 2001; Freitas et al. 2014). Moreover, cosmetic formulations use bifunctional compounds that can act as vehicles and also have a desired effect on the skin. Therefore, there is a great variety of cosmetic vehicles available which, depending on the application, have different physical/chemical properties (Buchmann 2001). A good vehicle should be a homogenous, cosmetically acceptable ingredient with an easy application and removal; should be nontoxic, non-irritant, and nonallergenic, being chemically stable and bacteriostatic; and should be pharmacologically inert, releasing active agents in a controlled and targeted way (Bohnenblust-Woertz and Surber 2014).

There are differences, in terms of drug absorption, between distinct cosmetic formulations for topical applications which are caused by the established interactions involving the drug, the vehicle, and the skin. In this regard, there can be three different interactions. The first is the vehicle-drug interactions determined by the drug's thermodynamic activity on the vehicle; the second is the vehicle-skin interactions that can be enhanced using vehicle components that interact with the stratum corneum improving the drug solubility/diffusion; the third is the drug-skin interactions defined by the skin's metabolism and binding of the drug in its receptors (Bohnenblust-Woertz and Surber 2014).

In cosmetic formulations, vehicle-skin and vehicle-drug are the focused interactions, with many cosmetic ingredients being responsible for decreasing the skin barrier function and increasing the partitioning of active substances from the vehicle into the stratum corneum (Bohnenblust-Woertz and Surber 2014). Polysaccharides have been widely used as vehicle systems such as in emulsions, hydrogels, encapsulating structures, and suspensions (Freitas et al. 2014).

4.3.1 Emulsions

Emulsions are the prevailing form of skincare products given their appealing feeling on the skin and ease of application when compared to waterless oils and lipids (Buchmann 2001; Epstein 2009). Lotions are flowing fluid emulsions, while creams are semisolid emulsions (Buchmann 2001; Freitas et al. 2014). An emulsion is constituted by two or more immiscible materials forming a system where one material is suspended/dispersed throughout another material. Since they are immiscible materials, the use of emulsifying agents is determinant because they act as solubilizers, spreading and dispersing the selected actives and other ingredients of the vehicle. Depending on the quantity and nature of the ingredients, an emulsion can feel oily or greasy. Emulsions can be classified into four categories. Macroemulsions (1) are simple emulsions that can be oil-in-water (O/W) or water-in-oil (W/O) like lotions and creams, with droplet size range between 0.1 and 100 μm; while nanoemulsions (2) are simple emulsions (O/W or W/O) with droplet size range between 20 and 200 nm. Thirdly, there are multiple emulsions (3) which are complex systems like oil-in-water-in-oil (O/W/O) or water-in-oil-in-water (W/O/W) with droplet sizes similar to macroemulsions; and microemulsions (4) which are water, oil, and surfactant/co-surfactant systems, constituting a single optically isotropic thermodynamically stable liquid solution, with droplet size range from 10 to 100 nm

(Epstein 2009; Savary et al. 2016). Surface-active emulsifiers can be anionic, cationic, amphoteric, non-ionic, hydrophobic, lipophilic, ethoxylated, and non-ethyloxylated. All these characteristics will determine the type of emulsion (Epstein 2009). Additionally, there are several other components used in cosmetic emulsions like emollients (sensory properties increasers) such as silicon oil and isopropyl myristate; moisturizers and humectants like glycerol and urea; active substances such as UV sunscreens and vitamins; antimicrobial agents; perfumes and coloring agents; and viscosity-increasing agents (Freitas et al. 2014).

Even though most polysaccharides are not surface-active, they increase aqueous phase viscosity and inhibit droplet movement, contributing to the stabilization of emulsions. Nevertheless, emulsifiers based on high molecular weight polysaccharides have already been demonstrated to effectively form and stabilize O/W nanoemulsions. As previously mentioned, xanthan gum is a widely used polysaccharide ingredient in cosmetic industries, acting as a thickener and stabilizer in many applications (Martins et al. 2020). In fact, xanthan gum is a beneficial hydrocolloid due to its solubility in hot/cold solutions, binding water promptly and productively (Martins et al. 2020). Xanthan gum improves the emulsification of W/O emulsions by suspending insoluble ingredients, due to its high viscosity, and can be combined with other substances to enhance texture, flow behavior, stability, and appearance of preparations (Martins et al. 2020). In fact, 1% (w/v) of xanthan gum is enough to largely increase a liquids' viscosity, with many applications using as little as 0.1% (w/v) xanthan gum in their formulations (Sight et al. 2018). Gellan gum is also used to stabilize emulsions, protecting them against temperature fluctuations, improving quality in transit and shelf-time. In addition to xanthan gum and gellan gum, bacterial cellulose and hyaluronic acid have also been applied in emulsions (Freitas et al. 2014).

4.3.2 Suspensions

Suspensions consist of active principles or functional excipients dispersed in a liquid or semisolid medium, acting as a vehicle (Buchmann 2001). If the particles are therapeutically active, the suspension is considered pharmaceutical. By using suspensions, a hydrophobic drug is effectively dispensed, avoiding cosolvents, and decreasing hydrolysis, oxidation, and microbial activity related degradation. For instance, suspensions are used for sunscreens, pigment-containing nail pearlescent lacquers, and also particle-containing shampoos or shower gels (Freitas et al. 2014; Savary et al. 2016).

The main disadvantage of suspensions is the possibility of particle sedimentation if the particles possess a higher density than the medium (Freitas et al. 2014; Savary et al. 2016). By increasing the viscosity of the medium, sedimentation can be decreased or prevented. Xanthan gum has effectively been widely applied in suspensions for that end (Freitas et al. 2014). The benefits of using xanthan gum in suspensions are related to the bacterial polysaccharides' rheological properties, the reduced viscosity at high shear rates promotes deagglomeration and mixing. In addition, gellan gum allows successful stabilization of hair products formulation

(shampoo and conditioner) due to its thickening capacity and fluid gels development (Freitas et al. 2014).

4.3.3 Hydrogels

Hydrogels are three-dimensional polymeric matrices with a hydrophilic nature, allowing the retainment of 10% of weight in water. Therefore, the combination of hydrogels with other chemicals may result in suitable cosmetic formulations (Mitura et al. 2020). A hydrogel may be classified depending on its origin (natural and/or synthetic), on its physical or mechanical properties, on the nature of its polymer side groups (ionic or non-ionic), the cross-linking type, and its response to chemical stimulation (Michelon et al. 2019). Natural hydrogels are constituted by either polysaccharide chains, like chitosan, cellulose, and hyaluronic acid, or by protein chains, like collagen. On the other hand, there are also synthetic hydrogels that are based on polymer like poly-ethylene glycol or poly-acrylamide. Hybrid hydrogels are comprised of natural and synthetic polymers combinations (Michelon et al. 2019).

Regarding cosmetic formulations, hydrogels are used for skin, hair, and oral care applications; being based on several biopolymers like collagen, hyaluronic acid, alginate, chitosan, xanthan gum, pectin, starch, and cellulose (Mitura et al. 2020).

Biopolymer based hydrogels are used for the development of new cosmetics like *beauty masks*, which promote skin hydration and restore skin elasticity, producing an anti-aging effect. Hyaluronic acid-based hydrogels have a wide variety of applications, acting as a wrinkle removal agent, treating nasolabial folds, treating skin augmentation, and promoting skin hydration and collagen stimulation (Mitura et al. 2020). Gellan gum characteristics (binding properties and gel structure) have been shown to have advantages in toothpaste formulations (Freitas et al. 2014). Levan was also demonstrated to have potential in dermal filling applications when combined, in a hydrogel, with pluronic and carboxymethylcellulose (CMC) (Choi et al. 2018). The results of this study showed that the novel levan-based hydrogel presented stability and strength enhancements, in vitro and in vivo. The product was established as a biocompatible material with the potential to stimulate dermal fibroblasts proliferation and to enhance gene expression for type I collagen production. For these reasons, the studied hydrogel was considered a valid, if not better, alternative to skin filler applications, when compared to the traditional hydrogel (based on hyaluronic acid) (Choi et al. 2018).

4.3.4 Encapsulating Structures

Encapsulation is a common process in the cosmetic industry, aimed at the preservation of active ingredients during each stage of the product fabrication (formulation, storage, application) (Faieta et al. 2019). In fact, many personal care products require encapsulation, to increase the stability of its biologically active substances. For example, vitamins are not stable, being influenced by pH, light intensity, and oxidation, and the encapsulation technique ensures they are protected against degradation. Encapsulation also promotes the release of an active substance in a specific target (Ammala 2013; Faieta et al. 2019). The use of biodegradable polymeric carriers for the encapsulation increases efficacy and bioavailability of active

ingredients, ensuring its degradation using normal metabolic pathways. The utilization of low moisture amorphous matrices is common given the high stability conferred to bioactive substances, as well as promoting the application of the encapsulated ingredients in formulated products (Faieta et al. 2019). The encapsulating materials available for cosmetic applications include polysaccharides, proteins, lipids, and natural/synthetic polymers. Moreover, second polymers can be comprised of inorganic materials (usually silicates, clays, and polyphosphates) (Casanova and Santos 2015). In skin delivery systems, the most interesting encapsulating materials are chitosan, aliphatic polyesters (e.g., poly lactic acid (PLA)), and copolymers of lactic and glycolic acids (e.g., PLGA – poly lactic-co-glycolic acid). They are natural and non-toxic materials, that do not react when contacting human tissues, which can be metabolized using normal metabolic pathways (Casanova and Santos 2015).

The most used technique for encapsulation is spray drying, due to its simple, fast, and low-cost application. The first step of spray drying consists of dissolving, dispersing, or emulsifying the active compound and the encapsulating matrix in an aqueous solution. Afterward, the mixture is submitted to atomization and drying in contact with a hot gas flow (Lourenço et al. 2017). Other examples of encapsulation techniques are lyophilization, cocrystallization, fluidized-bed coating, and extrusion (Bodade and Bodade 2020).

Nylon microparticles loaded with vitamins, sun filters, moisturizers, fragrances, and many other actives like caffeic acid ester, retinyl palmitate, D-panthenol, vitamin C, bioflavonoid, catechins, resveratrol, rosmarinic acid, vitamin P, linoleic acid, lycopene, benzoyl peroxide, tocopheryl acetate, dihydroxyacetone, salicylic acid, vitamin E, and dimethicone are among the examples of cosmetic substances encapsulated with a wide range of materials (Casanova and Santos 2015; Freitas et al. 2014). Using chitosan for encapsulation has proven to be possible, due to the increment on polymer matrix viscosity, which permitted the controlled release of the active ingredient. In terms of skin permeability, the encapsulation of retinoic acid with chitosan enabled a slower and controlled release rate of retinoic acid, when compared to free retinoic acid. Moreover, chitosan encapsulation may enhance the properties of encapsulated cosmetics while protecting against environmental agents, which could help to overcome retinoic acid and alpha-tocopherol limitations by improving their light and oxygen sensitivity, and reducing skin irritation.

Hyaluronic acid, in its pure or modified form, is also able to encapsulate proteins, allowing their controlled release for several medical treatments. Hyaluronic acid-based nanoemulsions were tested as transdermal carriers, with acceptable stability. Moreover, these emulsions were tested for encapsulation with vitamin E, and the results showed a successful carrying of lipophilic additives (Kong et al. 2011). Additionally, skin penetration and histological experiments using vitamin E-hyaluronic acid nanoemulsions were made, with the results showing there was no dermis irritation and the delivery carrier of active lipophilic ingredient was effective (Kong et al. 2011). In another study, the encapsulation and stabilization of rosmarinic acid (RA) using poly(lactic-co-glycolic acid) (PLGA) was performed. The results showed that RA-loaded PLGA microparticles exhibit antioxidant and

antibacterial activities, without releasing any harmful substance to human dermal fibroblasts, which suggests that electrosprayed RA-loaded PLGA microparticles could be used in cosmetic and pharmaceutical products. Dextran and gellan gum were also reported as materials used for microencapsulation (Bodade and Bodade 2020). FucoPol was also studied as a potential encapsulation matrix for a spray drying process. The technique produced spherical capsules with a smooth surface, which were loaded with gallic acid and oregano essential oil. The results acknowledged FucoPol's potential to encapsulate bioactive compounds for application in the cosmetic industry (Lourenço et al. 2017).

4.4 Safety Requirements and Regulation

In general, cosmetic products have presented a good safety record throughout time but there are several examples of harmful experiences for costumers. For instance, until the early twentieth century, make-up dyes had toxic heavy metals, such as lead, mercury, and cadmium oxides in their constitution. In the 1930s, depilatory products caused severe intoxications, due to the thallium present in their formulation. Another example was the 1958/1959 epidemic of photo-allergic reactions in the UK caused by halogenated salicylanilide-containing cosmetics. During a decade (1950–1960), deodorants constituted by zirconium caused an outbreak of long-lasting allergic inflammatory skin reactions in consumers in Europe and the USA (Nohynek et al. 2010). A strict and rule-guided toxicological safety evaluation has been pivotal to prevent this type of occurrence, and for instance, in both the EU and US cosmetics safety evaluation has a particular legal position where they need to be proven safe under normal use conditions (Nohynek et al. 2010).

4.4.1 The Requirements of the EU Regulation

The safety assessment of cosmetics requires a detailed toxicological profile of all used ingredients and manufacturers need to present a report regarding the effects of product-specific exposure (Hussain-Gambles 2020) in order to meet the requirements of the EC Cosmetics Regulation 1223/2009 (EC, 2009). Moreover, technical details of the ingredients and the final formulation placed on the consumer market need to be disclosed to receive EC approval (Basketter and Whitte 2016; Hussain-Gambles 2020; Nohynek et al. 2010).

Europe has been active in animal-rights related issues and companies are required to present toxicological data for hazard identification using ex vivo or in vitro methods, instead of the usual animal testing, which is strictly prohibited under the EC Cosmetic Regulation No. 1223/2009. Another particularity of the EC Regulation No. 1223/2009 is the use of the same legal text, with a binding nature in all member states, which simplifies regulations in the EU market, ensuring that all companies comply under the same rules (Basketter and Whitte 2016; Hussain-Gambles 2020).

The Scientific Committee on Cosmetic Products and Non-Food Products (SCCNFP) is responsible for the administration of Directive 76/768/EEC. SCCNFP belongs of the European Commission and is composed of knowledgeable scientists from the different Member States (Pauwels and Rogiers 2004). There are three stages

for the safety evaluation of cosmetics conducted by SCCNFP. The first stage consists of identifying hazardous substances by analyzing a specific ingredient, and usually, a newly developed ingredient is required to be presented by individual firms and/or the European Toiletry and Perfumery Association (Colipa). The second stage is the risk assessment of the toxicology particularities of an ingredient under consideration, using available literature and studies, which allows safety evaluations for consumers exposed to the finished cosmetic product. The third stage is only used when additional toxicological tests are requested to reassess the safety profile of the ingredient under consideration (Pauwels and Rogiers 2004). The toxicological requirements for cosmetic ingredients according to Cosmetic Directive 76/768/EEC are condensed in the SCCNFP Notes of Guidance (SCCNFP/0690/03, 20031) as follows: (1) Acute toxicity (if available); (2) Irritation and corrosivity; (3) Skin sensitization; (4) Dermal/percutaneous absorption; (5) Repeated dose toxicity; (6) Mutagenicity/genotoxicity; (7) Carcinogenicity; (8) Reproductive toxicity; (9) Toxicokinetics; (10) Photo-induced toxicity, and (11) Human data (Pauwels and Rogiers 2004; Nohynek et al. 2010).

To perform the safety evaluation of the finished product (Table 5), all the available information (chemical, toxicological, and technical) are compiled in the TIF of the product, which is conjugated with the human exposure studies/information. The evaluated cosmetic risk is focused on irritation (and photo-irritation if relevant) and immunobiological reactions, such as contact allergy and eventually photo-allergic reactions. Potential systemic effects are also important in case of considerable skin penetration and/or oral intake of the product. The assessment must indicate, in an undersigned document, if a certain cosmetic product can be available on the EU market, posing no human health-related risks, considering a normal utilization (Pauwels and Rogiers 2004).

Companies are also required to present a Cosmetic Product Safety Report (CPSR), which is divided into two main parts (Table 6). Part A compiles all the

Table 5 Tools in safety evaluation for cosmetic ingredients and finished products (based on Pauwels and Rogiers 2004)

Safety aspects	Tools
Hazard	Animal studies Replacement studies Human data, epidemiological studies, clinical studies
Exposure	Product type Use pattern Application site Concentration Frequency Amount applied Chemical composition Stability Microbiological purity Target population Percutaneous absorption data
Risk	Safety evaluation

Table 6 Content of cosmetic product safety report to the European Directive 93/35/EEC (based on Basketter and White 2016)

Part A- Cosmetic product safety information	Part B- Cosmetic product safety assessment
1. Quantitative and qualitative composition of the cosmetic product 2. Physical/chemical characteristics and stability of the cosmetic product 3. Microbiological quality 4. Impurities, traces, information about the packaging material 5. Normal and reasonably foreseeable use 6. Exposure to the cosmetic product 7. Exposure to the substances 8. Toxicological profile of the substances 9. Undesirable effects and serious undesirable effects	1. Assessment conclusion 2. Labelled warnings and instructions of use 3. Reasoning 4. Assessor's credentials and approval of part B

information related to product composition, microbiological quality, physical/chemical characteristics, and stability; Part B is the safety assessment conducted by a qualified EU Safety Assessor (Turnbull 2018). There are several annexes in the EU Cosmetics Regulation, detailing the required content of the CPSR (Annex I), listing the prohibited substances to use in cosmetic products (Annex II), substances subjected to restrictions (Annex III), coloring agents (Annex IV), allowed preservatives (Annex V), and allowed UV filters (Annex VI) (Basketter and Whitte 2016).

4.4.2 The Requirements of the United States

The US Food and Drug Administration (USFDA) is the government agency responsible for the safety of personal of cosmetics, designated in the U.S. Food, Drug and Cosmetic Act of 1938 (Nohynek et al. 2010). Specifically, cosmetics are regulated by a specialized branch of the USFDA called the Center for Food Safety and Applied Nutrition (CFSAN) (Turnbull 2018). Cosmetic drugs are considered to be over-the-counter (OTC) drugs, which are scoped under the definitions of both cosmetics and drugs. In fact, there are several products, listed in the EU as cosmetics, that are considered OTC drugs by the USFDA. Among these products, sunscreen products, fluoride-containing toothpastes, antiperspirant deodorants, antidandruff preparations, moisturizers, and makeup with sun-protection properties, are the most well-known cases. In these cases, if the manufacturer wants to sell the product in both the EU and US markets, they must meet both regulatory requirements (Nohynek et al. 2010; Turnbull 2018). For instance, an antidandruff shampoo is a cosmetic because it is focused on hair-cleaning, but it is also considered a drug due to the intention to control and help prevent the recurrence of dandruff symptoms, like flaking and itching (Turnbull 2018).

Cosmetic Ingredient Review (CIR) and Good Clinical Practices (GCPs)

The Cosmetic, Toiletry and Fragrance Association, now denominated the Personal Care Products Council (PCPC), determined the guidelines of the Cosmetic Ingredient Review (CIR) in 1976, a mechanism for voluntary self-regulation of the industry (Nohynek et al. 2010). The CIR has an independent expert panel (constituted by

Toxicologists, Dermatologists, and Chemists from academic institutions) that review the available data on cosmetic ingredients and decides if they are considered safe (Boyer et al. 2017; Nohynek et al. 2010). The CIR panel also includes 3 nonvoting members appointed by the USFDA, Consumer Federation of America (CFA), and PCPC. These members represent, respectively, the government, consumer, and industry interests, acting as a link between the Expert Panel and the stakeholders (Boyer et al. 2017). The minimal data required to submit a cosmetic ingredient to CIR is the used concentration, the chemistry data, the skin irritation and sensitization, the dermal absorption data, and the genetic toxicity (Nohynek et al. 2010). The safety assessments of different ingredients used in the cosmetic industry are published in the International Journal of Toxicology (Nohynek et al. 2010). Regarding bacterial polysaccharides, CIR considered hyaluronic acid (Becker et al. 2009), bacterial cellulose (Final Report of the CIR) xanthan gum, gellan, levan, and dextran (Fiume et al. 2016) to be safe ingredients for cosmetic formulations. In fact, studies showed that hyaluronic acid did not provoke adverse reactions in topical applications and for the treatment of osteoarthritis and tissue augmentation with injected hyaluronic acid did not raise safety concerns regarding its use in cosmetics (Becker et al. 2009), being cytotoxic in high concentrations (Silva et al. 2017).

Xanthan gum is also widely used in the cosmetic industry (3,470 reported uses), being present at up to 6% in leave-on formulations (Fiume et al. 2016). Moreover, xanthan gum and dextran are present pharmaceutical applications and have demonstrated to be of safe use. For example, Dextran (3,000 to 20,000 Da) cannot be absorbed through intact human skin (Fiume et al. 2016). Levan cytotoxicity has also been evaluated in human fibroblast and keratinocyte cell lines. The results showed that levan (100 mg/mL) was not cytotoxic to human fibroblasts, presenting a proliferative effect in keratinocytes, with proliferation over 30% at levan concentrations higher than 1 mg/mL (Fiume et al. 2016). Ocular irritation was also evaluated, with xanthan gum (1%) and gellan gum (0.8%) presenting no ocular irritancy in rabbits, while gellan gum (0.5%) did not have an irritation effect in humans (Fiume et al. 2016).

After the ingredients and formulation safety assessment is complete and approved, USFDA requires a stage of human subject testing. These tests are executed under the control of Good Clinical Practices (GCPs) to demonstrate human tolerance and confirm the intended use of the product. An important note is that this stage of human testing is not intended to analyze the existence of hazards, but to confirm the safety of the cosmetic product (Fig. 13).

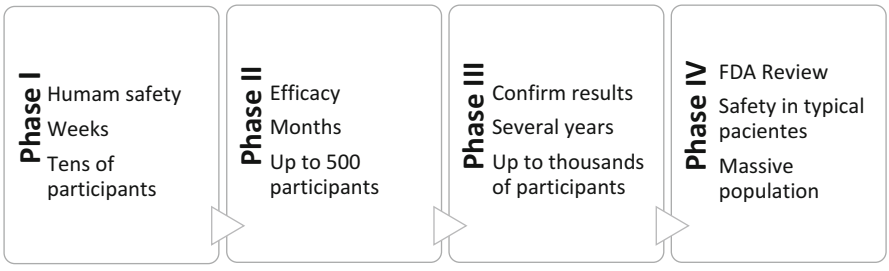


Fig. 13 Development of Good Clinical Practices steps (based on Foroughi-Heravani et al. 2020)

5 Conclusions

A growing trend toward more sophisticated cosmetics and personal care products is observed due to the desire of formulators to obtain a competitive advantage to meet consumers' expectations for product improvements and enhancements. The literature review revealed the positive effects of polysaccharide-based formulations on skin human, namely, the improvement of the skin barrier function and hydration. Moreover, polysaccharides rheological properties can increase formulation stability and enhance sensorial properties, which are important aspects of formulation technology. Accordingly, polysaccharides have been extensively used in cosmetic formulations. For example, hyaluronic acid is used as a bioactive ingredient, and in cosmetic vehicles; xanthan gum is used in cosmetic products due to its high viscosity-enhancing ability at low concentrations. Additionally, there are several promising bacterial polysaccharides, such as FucoPol and GalactoPol, which are still in the development studies to be used in cosmetic formulations. Finally, polysaccharide-based formulations are important to maintain physiological skin conditions and to prevent and treat skin disorders related to barrier function alterations.

References

Alves VD, Torres CA, Freitas F. Bacterial polymers as materials for the development of micro/nanoparticles. Int J Polym Mater Polym Biomater. 2016;65(5):211–24.

Ammala A. Biodegradable polymers as encapsulation materials for cosmetics and personal care markets. Int J Cosmet Sci. 2013;35(2):113–24.

Amorim JDP, Galdino CJS, Costa AFS, et al. Biomask, a polymer blend for treatment and healing of skin prone to acne. Chem Eng Trans. 2020;79:205–10.

Balkrishna A, Agarwal V, Kumar G, et al. Applications of bacterial polysaccharides with special reference to the cosmetic industry. In: Singh J, Sharma D, Kumar G, Sharma NR, editors. Microbial bioprospecting for sustainable development. Singapore: Springer; 2018. p. 189–202.

Basketter D, Whitte R. Legislative aspects of cosmetic safety in the European Union: the case of contact allergy. Cosmetics. 2016;3(17):1–7.

Becker LC, Bergfeld WF, Belsito DV, et al. Final report of the safety assessment of hyaluronic acid, potassium hyaluronate, and sodium hyaluronate. Int J Toxicol. 2009;28(4):5–67.

BeMiller JN. Polysaccharides: properties. In: BeMiller JN, editor. Carbohydrate chemistry for food scientists. 3rd ed. Duxford: Elsevier; 2019. p. 103–57.

Bhavani AL, Nisha J. Dextran – the polysaccharide with versatile uses. Int J Pharm Bio Sci. 2010;1(4):569–73.

Bilal M, Iqbal HMN. New insights on unique features and role of nanostructured materials in cosmetics. Cosmetics. 2020;7(2):24. https://doi.org/10.3390/cosmetics7020024.

Bodade RG, Bodade AG. Microencapsulation of bioactive compounds and enzymes for therapeutic applications. In: Pal K, Banerjee I, Sarkar P, Kim D, Deng W-P, Dubey NK, Majumder K, editors. Biopolymer-based formulations. 1st ed. Amsterdam: Elsevier; 2020. p. 381–404.

Boehncke W-H, Schön MP. Psoriasis. Lancet. 2015;386(9997):983–94.

Bohnenblust-Woertz K, Surber C. The "magic" effects of dermatologic and cosmetic vehicles. In: Barel AO, Paye M, Maibach HI, editors. Handbook of cosmetic science and technology. 4th ed. New York: Marcel Dekker Inc; 2014. p. 175–88.

Boyer IJ, Bergfeld WF, Heldreth B, et al. The cosmetic ingredient review program – expert safety assessments of cosmetic ingredients in an open forum. Int J Toxicol. 2017;36(5):5S–13S.

Buchmann S. Main cosmetic vehicles. In: Barel AO, Paye M, Maibach HI, editors. Handbook of cosmetic science and technology. New York: Marcel Dekker; 2001. p. 145–69.
Bukhari SNA, Roswandi NL, Waqas M, et al. Hyaluronic acid, a promising skin rejuvenating biomedicine: a review of recent updates and pre-clinical and clinical investigations on cosmetic and nutricosmetic effects. Int J Biol Macromol. 2018;120:1682–95.
Casanova F, Santos L. Encapsulation of cosmetic active ingredients for topical application – a review. J Microencapsul. 2015;33(1):1–17.
Cheaburu Yilmaz CNC, Pamfil D, Vasile C, et al. Toxicity, biocompatibility, pH-responsiveness and methotrexate release from PVA/hyaluronic acid cryogels for psoriasis therapy. Polymers. 2017;9(12):123. https://doi.org/10.3390/polym9040123.
Chen LH, Xue JF, Zheng ZY, et al. Hyaluronic acid, an efficient biomacromolecule for treatment of inflammatory skin and joint diseases: a review of recent developments and critical appraisal of preclinical and clinical investigations. Int J Biol Macromol. 2018;116:572–84.
Choi WI, Hwang Y, Sahu A, et al. An injectable and physical Levan-based hydrogel as a dermal filler for soft tissue augment. Biomater Sci. 2018;6(10):2627–38.
Domżał-Kędzia M, Lewińska A, Jaromin A, et al. Fermentation parameters and conditions affecting Levan production and its potential applications in cosmetics. Bioorg Chem. 2019;93:102787. https://doi.org/10.1016/j.bioorg.2019.02.012.
Draelos ZD. A clinical evaluation of the comparable efficacy of hyaluronic acid-based foam and ceramide-containing emulsion cream in the treatment of mild-to-moderate atopic dermatitis. J Cosmet Dermatol. 2011;10(3):185–8.
Du H, Liu P, Zhu J, et al. Hyaluronic acid dissolving microneedle patch loaded with methotrexate for improved treatment of psoriasis. ACS Appl Mater Interfaces. 2019;11(46):43588–98.
Dubuisson P, Picard C, Grisel M, et al. How does composition influence the texture of cosmetic emulsions? Colloids Surf A Physicochem Eng Asp. 2018;536:38–46.
EC. The European Parliament and the Council of the European Union. Regulation No. 1223/2009 of the European parliament and of the council of 30 November 2009 on cosmetic products. Official Journal of the European Union L 342/59. 2009. https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:342:0059:0209:en:PDF
Epstein H. Cosmeceutical vehicles. Clin Dermatol. 2009;27(5):453–60.
Faieta M, Corradini MG, Di Michele A, et al. Effect of encapsulation process on technological functionality and stability of *Spirulina platensis* extract. Food Biophys. 2019;15:50–63.
Fallacara A, Baldini E, Manfredini S, Vertuani S. Hyaluronic acid in the third millennium. Polymers. 2018;10(7):701. https://doi.org/10.3390/polym10070701.
Fiume MM, Heldreth B, Bergfeld WF, et al. Safety assessment of microbial polysaccharide gums as used in cosmetics. Int J Toxicol. 2016;35(1):5S–49S.
Foroughi-Heravani N, Hadavandkhani M, Arjmand B, et al. Principles of good clinical practice. In: Arjmand B, Payab M, Goodarzi P, editors. Biomedical product development: bench to bedside. Cham: Springer Nature; 2020. p. 99–106.
Frankel A, Sohn A, Patel RV, et al. Bilateral comparison study of pimecrolimus cream 1% and a ceramide-hyaluronic acid emollient foam in the treatment of patients with atopic dermatitis. J Drugs Dermatol. 2011;10(6):666–72.
Freitas F, Alves VD, Reis MAM. Bacterial polysaccharides: production and applications in cosmetic industry. In: Ramawat KG, Mérillon JM, editors. Polysaccharides. Cham: Springer International Publishing; 2014. p. 2017–43.
Gallegos AMA, Carrera SH, Parra R, et al. Bacterial cellulose: Bacterial cellulose. Bioresources. 2016;11(2):5641–55.
Gruber JV. Polysaccharide-based polymers in cosmetics. In: Goddard ED, Gruber JV, editors. Principles of polymer science and technology in cosmetics and personal care. Medicine & health science books, vol. 22. 1st ed. New York: Marcel Dekker Inc; 1999. p. 325–59.
Guetta O, Mazeau K, Auzely R, et al. Structure and properties of a bacterial polysaccharide named Fucogel. Biomacromolecules. 2003;4(5):1362–71.
Halachmi S, Amitai DB, Lapidoth M. Treatment of acne scars with hyaluronic acid: an improved approach. J Drugs Dermatol. 2013;12(7):121–3.

Halla N, Fernandes I, Heleno S, et al. Cosmetics preservation: a review on present strategies. Molecules. 2018;23(7):1571. https://doi.org/10.3390/molecules23071571.
Hebert AA, Pacha O. Treating atopic dermatitis: safety, efficacy, and patient acceptability of a ceramide hyaluronic acid emollient foam. Clin Cosmet Investig Dermatol. 2012;5:39–42.
Hosoi J, Koyama J, Ozawa T. New aspects of cosmetics and cosmetic science. In: Sakamoto K, Lochhead RY, Maibach HI, Yamashita Y, editors. Cosmetic science and technology: theoretical principles and applications. Amsterdam: Elsevier; 2017. p. 87–100.
Hussain-Gambles M. Halal personal hygiene and cosmetics. In: Al-Teinaz YR, Spear S, Abd El-Rahim IHA, editors. The halal food handbook. New York: Willey-Blackwell; 2020. p. 183–96.
Huynh A, Priefer R. Hyaluronic acid applications in ophthalmology, rheumatology, and dermatology. Carbohydr Res. 2020;489:107950. https://doi.org/10.1016/j.carres.2020.107950.
Jesumani V, Du H, Pei P, et al. Unravelling property of polysaccharides from Sargassum sp. as an anti-wrinkle and skin whitening property. Int J Biol Macromol. 2019;140:216–24.
Jindal M, Khattar J. Microbial polysaccharides in food industry. In: Grumezescu AM, Holban AM, editors. Biopolymers for food design. Cambridge: Academic; 2018. p. 95–123.
Kanlayavattanakul M, Lourith N. Biopolysaccharides for skin hydrating cosmetics. In: Ramawat KG, Mérillon JM, editors. Polysaccharides. Cham: Springer International Publishing; 2014. p. 1867–92.
Kim K, Kim K, Kim T et al. Cosmetic composition containing Levan having cell- proliferation, skin-moisturizing and irritation- alleviating effects Japan patent JP2003277225A. 2003. 2 October 2003
Kong M, Chen XG, Kweon DK, et al. Investigations on skin permeation of hyaluronic acid based nanoemulsion as transdermal carrier. Carbohydr Polym. 2011;86(2):837–43.
Kusumawati I, Indrayanto G. Natural antioxidants in cosmetics. Stud Nat Prod Chem. 2013;40:485–505.
Lai-Cheong JE, McGrath JA. Structure and function of skin, hair and nails. Medicine. 2013;41(6):317–20.
Lautenschläger H. Additives in cosmetic products. Kosmetische Praxis 2004;1:8–10.
Lawton S. Skin 1: the structure and functions of the skin. Nursing Times [online]. 2019;115(12):30–3.
Leijs MM, Esser A, Amann PM, Schettgen T, Gube M, Merk HF, Kraus T, Baron JM. Hyperpigmentation and higher incidence of cutaneous malignancies in moderate-high PCB- and dioxin exposed individuals. Environ Res. 2018;164:221–8.
Li J, Yuan X, Tang Y, Wang B, Deng Z, Huang Y, Liu F, Zhao Z, Zhang Y. Hydroxychloroquine is a novel therapeutic approach for rosacea. Int Immunopharmacol. 2020;79:106178.
Lima Fontes M, Meneguin AB, Tercjak A, et al. Effect of in situ modification of bacterial cellulose with carboxymethylcellulose on its nano/microstructure and methotrexate release properties. Carbohydr Polym. 2018;179:126–34.
Lintner K, Mas-Chamberlin C, Mondon F. Cosmeceuticals and active ingredients. Clin Dermatol. 2009;27:461–8.
Lochhead RY. The use of polymers in cosmetic products. In: Sakamoto K, Lochhead RY, Maibach H, Yamashita Y, editors. Cosmetic science and technology: theoretical principles and applications. Amsterdam: Elsevier; 2017. p. 171–221.
Long CC. Common skin disorders and their topical treatment. In: Walters KA, editor. Dermatological and transdermal formulations. New York: Informa Healthcare; 2002. p. 41–58.
Lourenço SC, Torres CAV, Nunes D, et al. Using a bacterial fucose-rich polysaccharide as encapsulation material of bioactive compounds. Int J Biol Macromol. 2017;104:1099–106.
Martins D, Estevinho B, Rocha F, et al. A dry and fully dispersible bacterial cellulose formulation as a stabilizer for oil-in-water emulsions. Carbohydr Polym. 2020;230:115657. https://doi.org/10.1016/j.carbpol.2019.115657.
Mathe N, Loffeld A. The management of eczema in children. Paediatr Child Health. 2019;29(2):52–8.
McCarthy RR, Ullah MW, Booth P, et al. The use of bacterial polysaccharides in bioprinting. Biotechnol Adv. 2019;37:107448.

Michelon M, Leopércio BC, Carvalho MS. Microfluidic production of aqueous suspensions of gellan-based microcapsules containing hydrophobic compounds. Chem Eng Sci. 2019;211:115314. https://doi.org/10.1016/j.ces.2019.115314.
Mitura S, Sionkowska A, Jaiswal A. Biopolymers for hydrogels in cosmetics: review. J Mater Sci Mater Med. 2020;31(6):50. https://doi.org/10.1007/s10856-020-06390-w.
Monteiro-Riviere NA. Structure and function of skin. In: Riviere JE, editor. Dermal absorption models in toxicology and pharmacology. Boca Raton: CRC, Taylor and Francis; 2006. p. 1–19.
Musa SH, Basri M, Fard Masoumi HR, et al. Enhancement of physicochemical properties of nanocolloidal carrier loaded with cyclosporine for topical treatment of psoriasis: in vitro diffusion and in vivo hydrating action. Int J Nanomedicine. 2017;12:2427–41.
Nohynek GJ, Antignac E, Re T, et al. Safety assessment of personal care products/cosmetics and their ingredients. Toxicol Appl Pharmacol. 2010;243(2):239–59.
Nwodo UU, Green E, Okoh AI. Bacterial exopolysaccharides: functionality and prospects. Int J Mol Sci. 2012;13(11):14002–15.
Pauwels M, Rogiers V. Safety evaluation of cosmetics in the EU. Toxicol Lett. 2004;151(1):7–17.
Pavicic T, Gauglitz GG, Lersch P, et al. Efficacy of cream-based novel formulations of hyaluronic acid of different molecular weights in anti-wrinkle treatment. J Drugs Dermatol. 2011;10(9):990–1000.
Poetschke J, Schwaiger H, Steckmeier S, et al. Anti-wrinkle creams with hyaluronic acid: how effective are they? MMW Fortschr Med. 2016;158(S4):1–6.
Ramalingam C, Priya J, Mundra S. Applications of microbial polysaccharides in food industry. Int J Pharmaceut Sci Rev Res. 2014;27(1):322–4.
Roca C, Alves VD, Freitas F, et al. Exopolysaccharides enriched in rare sugars: bacterial sources, production, and applications. Front Microbiol. 2015;6:288. https://doi.org/10.3389/fmicb.2015.00288.
Savary G, Grisel M, Picard C. Cosmetics and personal care products. In: Olatunji O, editor. Natural polymers. Cham: Springer International Publishing; 2016. p. 219–61.
Schlesinger TC, Rowland P. Efficacy and safety of a low-molecular weight hyaluronic acid topical gel in the treatment of facial seborrheic dermatitis. J Clin Aesthet Dermatol. 2014;7(5):15–8.
Shanmugam M, Abirami RG. Microbial polysaccharides – chemistry and applications. J Biol Active Prod Nature. 2019;9(1):73–8.
Silva SS, Fernandes EM, Pina S, et al. 2.11 polymers of biological origin. In: Ducheyne P, editor. Comprehensive biomaterials II. 2nd ed. Oxford: Elsevier; 2017. p. 228–52.
Siqueira EC, Rebouças JS, Pinheiro IO, et al. Levan-based nanostructured systems: an overview. Int J Pharm. 2020;580:119242. https://doi.org/10.1016/j.ijpharm.2020.119242.
Song B, Song R, Cheng M, et al. Preparation of Calcipotriol emulsion using bacterial exopolysaccharides as emulsifier for percutaneous treatment of psoriasis vulgaris. Int J Mol Sci. 2019;21(1):77. https://doi.org/10.3390/ijms21010077.
Turnbull SE. Cosmetics. In: Pacifici E, Bain S, editors. An overview of FDA regulated products. Cambridge: Academic; 2018. p. 217–29.
Ullah H, Santos HA, Khan T. Applications of bacterial cellulose in food, cosmetics and drug delivery. Cellulose. 2016;23(4):2291–314.
Vary JC. Selected disorders of skin appendages – acne, alopecia, hyperhidrosis. Med Clin N Am. 2015;99(6):1195–211.
Yamada M, Prow TW. Physical drug delivery enhancement for aged skin, UV damaged skin and skin cancer: translation and commercialization. Adv Drug Deliv Rev. 2020;153:2–17.
Yildiz H, Karatas N. Microbial exopolysaccharides: resources and bioactive properties. Process Biochem. 2018;72:41–6.
Zia KM, Tabasum S, Khan MF, et al. Recent trends on gellan gum blends with natural and synthetic polymers: a review. Int J Biol Macromol. 2018;109:1068–87.

Natural Polysaccharides for Skin Care

35

Mayuree Kanlayavattanakul and Nattaya Lourith

Contents

M. Kanlayavattanakul (✉) · N. Lourith
School of Cosmetic Science, Mae Fah Luang University, Chiang Rai, Thailand

Phytocosmetics and Cosmeceuticals Research Group, Mae Fah Luang University, Chiang Rai, Thailand
e-mail: mayuree@mfu.ac.th; nattayal@mfu.ac.th

J. M. Oliveira et al. (eds.), *Polysaccharides of Microbial Origin*,
https://doi.org/10.1007/978-3-030-42215-8_46

Abstract

Polysaccharides are proved to be one of the important cosmetic ingredients especially for skin care products. Among which, those of renewable, eco-friendly, and accountable for circular bio-economy productions are highly in demands of the cosmetic consumers, which hit the interests of the researchers. Of which, biotechnological production route on the basis of microbial produced polysaccharides is the appointed choices. In this chapter, microbial polysaccharides applicable for cosmetics are summarized exclusively for those of skin care applications. The specialty bacterial and fungal polysaccharides, those of emerging mushroom, producing and commercializing for cosmetics are enclosed. In addition, challenges and future prospects of microbial polysaccharides in cosmetic industries in the sectors of packaging and delivery system are included in the context as well as the promising sources and productions.

Keywords

Cosmetics · Skin care · Microbial polysaccharide · Cosmetic package · Active ingredient · Functional ingredient

1 Introduction

Classification of polysaccharides in cosmetics is on the basis of their actions in the products, i.e., functional and active polysaccharides. Functional polysaccharide is the compulsory ingredients to formulate the preparation with the desired preferences and textures. Thus, they act as gelling agent, viscosity adjuster, thickener and emulsifier, etc. Active polysaccharide is positively related with the claim efficacy of the skin care preparations, for instance, skin hydration, moisturizing, anti-wrinkle, or astringent agents. Furthermore, they can be categorized on the basis of their electrochemical charges in the structure that are anionic, cationic, nonionic and amphoteric polysaccharides. However, this categorization positively reflect onto those of functional polysaccharides rather than the active ones. In the view of the incorporated quantity of the polysaccharide in cosmetics, the high content of functional polysaccharide is required with the properties range from forming viscous solutions to exhibiting a pseudoplastic material nature, but low for active action (Kanlayavattanakul and Lourith 2015). Accordingly, the unit price for those of functional polysaccharides are lower than the active polysaccharides. Among which, naturally derived

polysaccharides are highly in demand than the functional ones. Although polysaccharides suppling for cosmetic and personal care industries are majorly derived from plants, recent biotechnology formation of polysaccharides with modified structures for expected properties can be achieved by using of microorganisms (Freitas et al. 2011, 2014) with the safety requirements that meet the standard of cosmetics in particular safety.

Polysaccharides derived from microorganisms, including bacteria, yeasts, fungi, and some algae, are emerging as an unexploited market. Polysaccharide biosynthesis and its accumulation generally take place after the growth phase of the microorganism. The polysaccharides produced by microorganisms can be classified into three main groups: (i) capsularpolysaccharides, which provide a carbon and energy sources for the cell; (ii) lipopolysaccharides that make up the cell wall; and (iii) exopolysaccharides (EPSs) that are exuded into the extracellular environment in the form of capsules or biofilm (Chen et al. 2010; Donot et al. 2012). In addition, microbial polysaccharides can be categorized into cellulose, xanthan, dextran, and alginate (Freitas et al. 2011, 2014). However, additional category of the microbial polysaccharides applicable in cosmetic industry are more versatile and feasibly suit with the dosage form and their expected claims as well. The two main industrially relevant microbial polysaccharides worldwide are cellulose and alginate with the revenue of over 22 billion USD annually and projected to reach 50 billion USD by 2025 (Anderson et al. 2018). Of which, microbial polysaccharides are regarded as the potential sources producing the specialty polysaccharides supplied for certain industries (Huertas and Matilla 2020) including skin care products. Amidst bacterial polysaccharides, exopolysaccharides are the main source of cosmetic industrial importance.

Exopolysaccharides with different sugar moieties and the structural charge produced by a wide range of microorganisms used in skin care cosmetics are exemplified in Table 1. Polysaccharides used in cosmetic proposes can be in a modified form and composited with different materials in an order to tailor-made the expected properties. The most familiar cosmetic awareness on microbial polysaccharides are their active actions as the safe and efficient moisturizer and

Table 1 Examples of microbial polysaccharides of different sources

Polysaccharide	Strain	Charge
Alginate	*Azotobacter vinelandii, Pseudomonas aeruginosa*	Anionic
Cellulose	*Gluconacetobacter* spp., *Acetobacterium* spp.	Nonionic
Chitin	*Aspergillus fumigatus, Saccharomyces cerevisiae*	Cationic
Chitosan	*Bacillus pumilus, Streptomyces griseus*	Cationic
Curdlan	*Agrobacterium* spp.	Nonionic
Gellan	*Sphingomonas paucimobilis*	Anionic
Hyaluronic acid	*Streptococcus* spp.	Anionic
Levan	*Zymomonas mobilis, Halomonas smyrnensis, Bacillus subtilis*	Nonionic
Pullulan	*Aureobasidium pullulans*	Nonionic
Xanthan	*Xanthomonas campestris*	Anionic

anti-wrinkle agent (Kanlayavattanakul and Lourith 2015, 2019; Lourith and Kanlayavattanakul 2016, 2019). Microbial polysaccharides are fond of more importance in cosmetic products accordingly. Those for skin care products are therefore exhaustively focused in this chapter. Of which, they are sometime known or called as specialty polysaccharides.

2 The Specialty Bacterial Polysaccharides for Skin Care Cosmetics

Bacterial polysaccharides have been exploited in a number of industrial uses. The particular application of a specific polysaccharide is a reflection of its unique physical properties. In cosmetic industry, different classes of polysaccharides are applied. Of which, cellulose, chitin and chitosan and hyaluronic acid are of importance in skin care products on the basis of their applications by functional and active actions (Lourith and Kanlayavattanakul 2018). The specialty bacterial polysaccharides, cellulose, chitin and chitosan, and hyaluronic acid will be therefore exclusively addressed in this section regarding on their widely used as skin hydrating agent besides their functional actions. In addition, xanthan gum, dextran, curdlan, gellan, levan, pullulan, and alginate are briefly addressed as per they are majorly implied as functional polysaccharides, although their skin hydrating capabilities are exhibited but in a moderate degree.

2.1 Cellulose

Cellulose, a homopolymer of (1-4)-β-linked glucose, is the most abundant biological polymer. Apart from being a major component of plant cell walls, cellulose is also synthesized by *Acetobacterium, Rhizobium, Salmonella* and *Saricana* spp. Bacterially derived cellulose is much purer than that extracted from plant tissue and as such it has been proposed for use in a number of medical applications including as an artificial skin, for topical drug delivery, and in wound dressings (Klemm et al. 2006; Rehm 2010; Shah et al. 2013), and of course for cosmetic applications.

Cosmetic benefits of this polysaccharide is due to the nature of its hygroscopic material with a flow behavior as a shear thinning fluid. The natural anionic nature of cellulose enables its interfacial activity. Not only the native form of cellulose that is largely used in cosmetics, derivatization into ethers (Fig. 1) with a different of degree of substitution (DS) that tailor making the physicochemical properties is accounted as the major form used.

In addition, physicochemical property adjustments can be also achieved in term of molar substation (MS) by propylene oxide. The resulting derivatives thereafter expand the applications for instance cellulose ether that prolonging its shelf-life and increase solubility in common vehicles used in skin care cosmetics. Derivatization of cellulose into ether forms change the anioinic nature of cellulose into nonionic

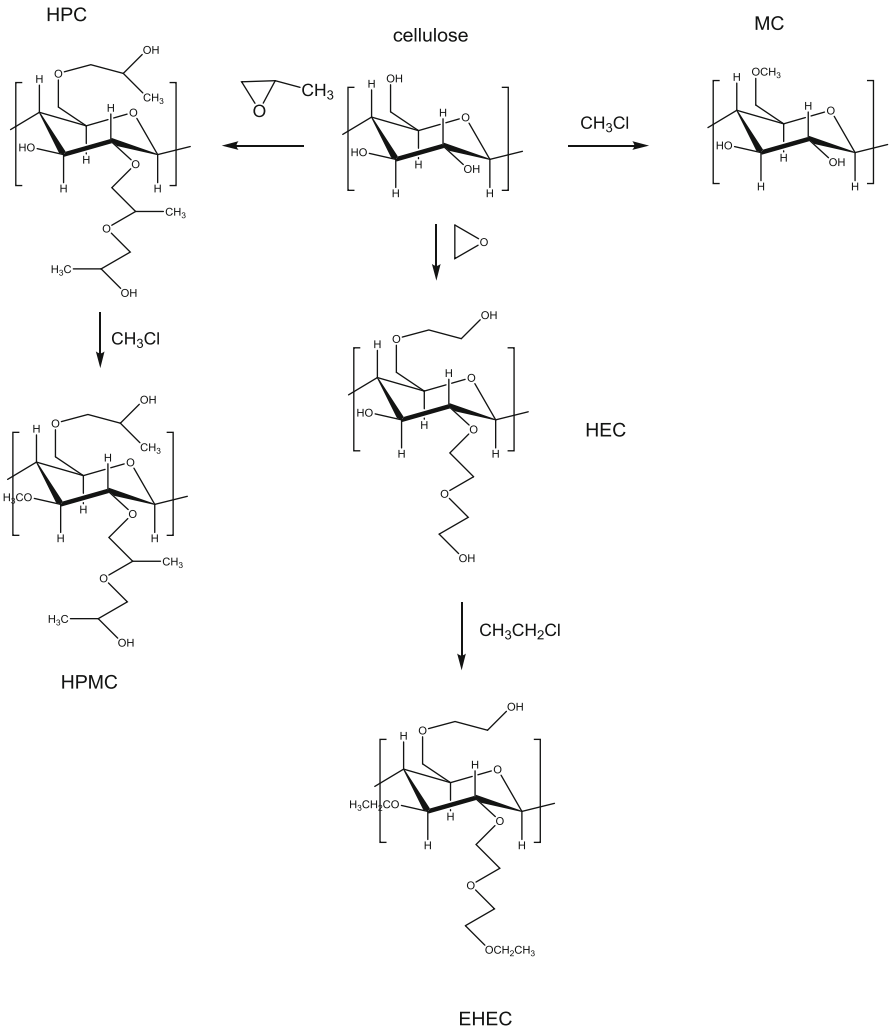


Fig. 1 Preparation of cellulose ethers

polymers with the maximum DS of 3 (3 hydroxyl group derivatizable). Cellulose ethers are generally recognized as safe (GRAS) in similar to the native form. Therefore, they gain highly acceptance to be incorporated as functional polysaccharides in cosmetics as summarized in Table 2.

2.1.1 Methylcellulose

Due to strong intermolecular hydrogen bonding between the hydroxyl groups on the cellulosic chains, unmodified cellulose is insoluble in water. However, methylation of cellulose introduces hydrophobic groups on the cellulosic chains producing methyl cellulose (MC), which can be easily dissolved in cold water. Furthermore,

Table 2 Cosmetic functions of cellulose ethers

Application	Celluloses	Function
Detergent	CMC, HPMC	Anti-redeposition, wetting ability, suspender, emulsifier
Cream, shampoo, lotion, ointment, gel	CMC, MC, HEC, HPMC	Thickener, binder, emulsifier, stabilizer, film former

MC can be modified into different form to fit with the desired properties for example hydroxypropyl methyl cellulose (HPMC) and so forth.

Carboxymethyl cellulose, popularly known as CMC, is a carboxymethyl ether of cellulose. The ionic polysaccharide is water soluble. It can be produced and commercialized in free or salt forms. Of particular, commercial CMC are those with DS of 0.4–1.5 that make them pH media unaffected between 5 and 9. CMC is widely available in a number of grades for specific applications in the industry. For instance, extra pure CMC grade is used in food products, pharmaceuticals, and toothpastes, while semi-purified and technical grades are used for detergents, etc.

2.1.2 Hydroxyethyl Cellulose

This cellulose ether is the most hydrophilic used cellulose ether with cloud point of more than 100 °C. Hydroxyethyl cellulose (HEC) tolerates extremes of pH and salts. Therefore, it is accounted and extensively used as surfactant with a several form of derivatizations of HEC with a modified properties such as ethylhydroxyethyl cellulose (EHEC).

2.1.3 Hydroxypropyl Cellulose

Hydroxypropyl cellulose (HPC) is the modified polysaccharide with cloud point arounds 45 °C is more lipophilic than HEC. Therefore, it is easily dissolved in cosmetic solvents and giving a higher thickening effect. In addition, its thermoplastic nature (film forming during melts) with liquid crystallinity makes it insensitive to pH fluctuations.

Once derivatized into ether forms, they turn to be nonionic polymer acting as thickening agents in the formulation with surface activity in addition to its task as film former. At a greater molecular weight, they become greater pseudoplasticity cellulose. Substitution with a higher methyl residue results in a harder gel. In contrary, if higher hydroxypropyl substitution took place (for example, HPMC), a softer gel will be offered. Therefore, MC & HPMC are DS unaffected viscosity cellulose ethers.

2.2 Chitin and Chitosan

Chitin is the second most abundant natural polysaccharide after cellulose. Chitin itself has low toxicity and is biodegradable polysaccharide with antibacterial properties, hydrophilicity, gel-forming properties, and affinity for proteins. Therefore, various applications according to its biological activities and wound healing effect are widely evidenced. Structural differences of chitin and its derivative, chitosan are shown in Fig. 2.

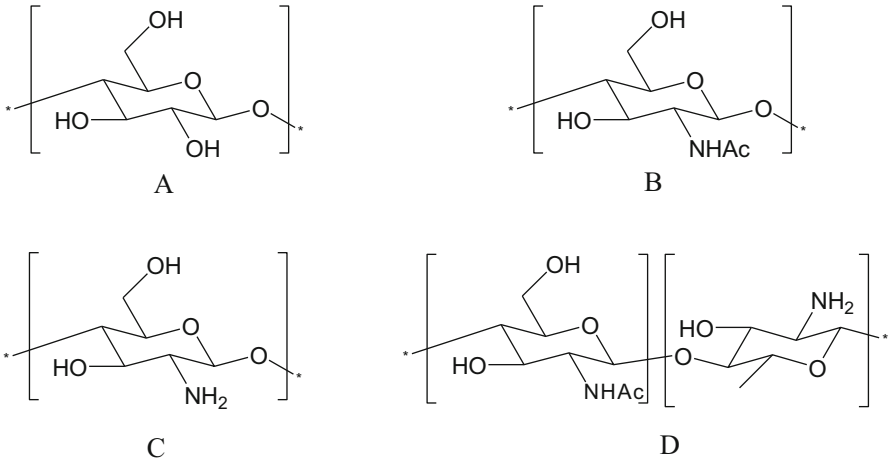


Fig. 2 Chemical structures of cellulose (**a**), chitin (**b**), chitosan (**c**) and partial deacetylated chitosan (**d**) repeating units

Chitin can be produced in the form of aminoglucan (poly-GlcNAc) by *Saccharomyces cerevisiae* (β-(1,6)-glucan), *Aspergillus fumigatus* (β-(1,3/1,4)-glucan), *Candida albicans*, *Fusarium oxysporum*, *Microsporum fluvum*, and *Epidermophyton stockdaleae*. On the means time, *Aspergillus niger*, *Humicolo lutea*, and *Fusarium moniliforme* are reported to be the producers of chitosan. Although two other strains that are commercially used to prepare chitosan are also reported, *Bacillus pumilus* chitosanse is more effective than *Streptomyces griseus* chitinase.

The antimicrobial activities, wound healing, and antioxidant properties including asthma and atopic dermatitis curing effects enable high quality of chitin application in cosmetics. It is able to maintain skin moisture, treat acne, skin toning, improve suppleness of hair, and reduce electrostatic of hair as well as its efficient surfactant and humectant functions. High molecular weight polysaccharides are especially utilized for skin care cosmetics. Of which, the polysaccharides with a molecular weight of 10^4–10^6 Da are used as broad-spectrum ingredient in hair care cosmetics. Significant trans-epidermal water loss (TEWL) reduction of skin treated with high molecular weight chitosan was reported. This, consequently brings softer and more flexible skin accordingly in addition to its irritation reduction efficacy. Improvement of sun protection and product's water resistance is attributed by the emulsion containing chitosan as well as adhering affinity of perfume containing chitosan onto skin that prolong wearing duration by a reduction of an evaporation rate. Moreover, its applications for deodorant and antiperspirant products are also available concerning to humectantcy of chitosan. The hydrophilicity of chitosan efficiently removes sebum and oils from hair, scalp, and skin (Lourith and Kanlayavattanakul 2018). Thus, it is appreciable to be used for greasy/oily skin treatment diminishing severity of acne and promote wear-ability of facial makeup applications.

2.3 Hyaluronic Acid

Skin is composed of two distinct areas; epidermis and dermis. Epidermis consists of many layers of dead skin that are supported by the dermis. Dermis is a three-dimensional network of collagen fibers and elastin fibers surrounded by gel-like material called the ground substance. Dermis accounts for most qualities considered on aesthetic property, i.e., the appearance of the skin. These include a moist, plump appearance and tautness of skin. The aging process is therefore considered skin that is less taut, less moist, less plump, and in some areas, sagging along lines that are flexed (also known as wrinkles) (Lourith and Kanlayavattanakul 2016, 2018, 2019).

The extracellular matrix (ECM) is a gel-like material filling the extracellular space in skin tissues that holds cells together and provides a porous pathway for the diffusion of nutrients and oxygen to individual cells. This structure is composed by fibrous proteins (collagen, elastin, fibronectin, and laminin) and heteropolysaccharides, namely, glycosaminoglycans (GAGs). These heteropolysaccharides are unbranched polysaccharide chains, composed of repeating disaccharide units. GAGs are highly negatively charged, due to the presence of sulfate or carboxyl groups on most of their sugar residues. Based on the disaccharide composition, linkage type, and on the presence of sulfate groups, GAGs have been divided into four main groups: (i) hyaluronic acid (HA), (ii) chondroitin sulfate (CS) and dermatan sulfate (DS), (iii) heparan sulfate and heparin (HS), and (iv) keratan sulfate (DeAngelis 2012). They are called GAGs because one of the two sugar residues in the repeating disaccharide is always an amino sugar (*N*-acetylglucosamine or *N*-acetylgalactosamine), which in most cases is sulfated. The second sugar is usually an uronic acid (glucuronic or iduronic), except for dermatan sulfate that contains galactose. GAGs are also known as mucopolysaccharides with biomedical benefits as summarized in Table 3.

Hyaluronic acid (HA), also named hyaluronan, is the mostly used GAGs in skin care products. HA is a polyanion that can self-associate and can also bind to water molecules (when not bound to other molecules), giving it a stiff, viscous quality similar to gelatin. HA is one of the major elements in ECM of vertebrate tissues. It is available in almost all body fluids and tissues. It is also involved in several important biological functions, such as regulation of cell adhesion and cell motility, manipulation of cell differentiation and proliferation, and providing mechanical properties to tissues. HA is responsible for providing the viscoelasticity of some biological fluids and controlling tissue hydration and water transport. In addition, HA has been

Table 3 Biomedical benefits of different GAGs

GAG	Biomedical benefits
HA	Surgical aid (ophthalmic; intra-abdominal anti-adhesive), viscoelastic supplementation (joint injection), moisturizer (eye drops, skin humectant), dermal filler, device coating, drug delivery, tissue engineering
CS	Surgical aid (ophthalmic, nutraceutical (joint health), tissue engineering)
DS	Anticoagulant
HS	Anticoagulant, device and stent coating, anti-proliferative, tissue engineering

found during embryonic development in the umbilical cord, suggesting that materials composed of HA may persuade favorable conditions for tissue regeneration and growth.

HA's characteristics, including its consistency, biocompatibility, and hydrophilicity, have made it an excellent moisturizer in cosmetic dermatology and skin care products. In cosmetic applications, HA is simply divided into high molecular weight (MW) HA (HMWHA; 10^5–10^7 Da) and how molecular weight HA (LMWHA; 2×10^4–4.5×10^5 Da). HMWHA with anti-angiogenic activity is also exhibited fibrinogen binding enhancement assisting in clot formation in wound healing and therefore inhibit scar formation in addition to its anti-inflammatory activity. LMWHA is a good collagen type I & VIII synthesis enhancer and also expresses activities toward matrix metalloproteinase (MMP)-3, MMP-9, MMP-13, and hyaluronan synthase (HAS)-2 that are responsible for firmness of skin (Lourith and Kanlayavattanakul 2016, 2018, 2019).

Biological functions of HA depend on chain length, molecular mass, and synthetic circumstances. Several microbes are differently used to generate hyaluronic acid, i.e., *Agrobacterium* sp., *Bacillus* sp., *Escherichia coli*, *Lactococcos lactis*, *Bacillus subtilis*, and *Streptpcoccus* sp. especially *S. zooepidermicus* (Mattila et al. 2018).

HA has been approved as a dermal filler, and were known to be the second most popular non-surgical procedure for women and the third most popular procedure for men. Many dermal fillers have been developed, which the widely commercializing ones, Restylane® and Juvéderm™ are made from *Streptococci* (Lourith and Kanlayavattanakul 2016, 2019).

2.4 Xanthan

Xanthan, a water soluble anionic polysaccharide, with the molecular weight of 2–50×10^6 Da has unique rheological properties demonstrating pseudo-plastic flow and viscoelasticity, retaining its physical properties over a broad temperature range (Freitas et al. 2011, 2014). Quantity of xanthan gum in cosmetics is positively affect onto sensory of the product especially integrity of shape, penetration force, wetness, ease of spreading, and gloss (Dubuisson et al. 2018). Xanthan gum is functionally used in cosmetics as stabilizer and thickening agent (Fiume et al. 2016). The main source of microbial production of xanthan gum for cosmetic products is *Xanthomonas campestris*, which can be further modified into xanthan gum cross polymer and xanthan gum hydroxypropyl trimonium chloride for variety of cosmetic function in regards with its good stability over a wide range of temperature, pH, and salt concentrations (Kanlayavattanakul and Lourith 2015).

2.5 Dextran

Dextran, a neutral polysaccharide, is low immunogenicity, has led to numerous clinical and pharmaceutical applications including cosmetics. Dextran is soluble in water, and

a dextran solution behave as Newtonian fluids and has a viscosity that changes as a function of concentration, temperature, and average molecular mass. The most common forms of dextran to be used in skin care preparation are native dextran and dextran sulfate that are produced from *Leuconostoc mesenteroides* with the molecular size of 10^6–10^8 Da (Kanlayavattanakul and Lourith 2015) to function as skin conditioning and bulking/binding agent. In addition, modifications of dextran into sodium dextran, carboxymethyl dextran, sodium carboxymethyl dextran, and dextran hydroxypropyl trimonium for cosmetics proposes are conducted to vary their function as suspending agent, film former, binder, and conditioning agent, respectively (Fiume et al. 2016). In addition to *Leuconostoc*, *Streptococcus*, *Weissella*, *Perdiococcus*, and *Lactobacillus* are able to produce dextran (Kanlayavattanakul and Lourith 2015).

2.6 Curdlan

Curdlan is a neutral linear 1,3-*β*-*D*-glucan with the molecular weight range from $5 \times 10^4 - 2 \times 10^6$ Da synthesized by several Gram negative bacteria, *Alcaligenes faecalis* including *Agrobacterium* spp., *Rhizobium* spp., *Bacillus* spp., and *Cellulomonas* spp. It is used for various industrial applications based on its uncharged structure. Although curdlan is insoluble in water, it could be dissolved in sodium hydroxide solutions and highly safe to be used not only for food products but also personal care preparations. It appeared to form triple helical structures under appropriate conditions. Thereafter, it serves as a gel forming agent in skin care products with a wide pH range application for either high or low setting gel (>80 or 55–60 °C) depends on its molecular weight (Kanlayavattanakul and Lourith 2015). For its biological activity applicable in cosmetics, curlan is one of the antioxidant polysaccharides (Giese et al. 2015) capable to enhance superoxide dismutase or SOD activity (Lin et al. 2008). Moreover, its stimulating capacity was exhibited in the wounded treatment examined in an animal model (Berdal et al. 2007). The biological activities are relating with its glucan backbone. Of which, glucan is regarded as the active polysaccharide with collagen synthesis stimulating activity in addition to inductions of reepithelization and collagen deposition processes associating its potency against cellular aging (Kwon et al. 2009).

2.7 Gellan

Gellan has been proved to be one of the interesting recent microbial polysaccharide introductions as xanthan competitor. This anionic polysaccharide is produced commercially from a strain of *Pseudomonas elodea* with the molecular weight approximately 5×10^6 Da in addition to its procurable strain, *Sphingomonas paucimobilis*. It mainly functions as a stabilizer and thickener, which is stable over a wide range of pH. The gelling properties of this polysaccharide are only fully revealed after chemical deacylation to remove the *O*-acetyl and *O*-glyceryl substituents. The native

polymer only formed soft, elastic gels but progressive removal of the acyl groups increased the brittleness of the gels produced in the presence of divalent cations. The extent of acetylation is thought to control the local crystallization of parts of the polysaccharide chains. Divalent ions linked the gellan molecules causing gelation. All possessed closely related chemical structures but none of the others formed gels although most yielded highly viscous solutions with considerable thermostability (Kanlayavattanakul and Lourith 2015).

2.8 Levan

Numerous Gram-positive bacteria, including *Bacillus* sp., and Gram-negative bacteria, including *Z. mobilis*, are able to produce levan. Fermenting saccharose with bacteria, such as *Zymomonas mobilis* or *Bacillus subtilis* or by enzymatic synthesis using saccharose as substrate produces the nonionic polysaccharide leven, with the molecular weight that less than 10^8 Da (Kanlayavattanakul and Lourith 2015). In addition to its film forming ability, levan is regarded as a thickening agent with a skin protecting property (Fiume et al. 2016). Antioxidant activity of levan was reported to be 81% of the standard vitamin C (Srikanth et al. 2015). Its safety was approved using human fibroblast cells at which its non-cytotoxicity dose was relatively high at 100μg/ml. Once it was tested on keratinocyte cell, cell proliferation effect was exhibited that was conformed to 3D-artifical skin test. Moreover, anti-inflammatory effect of levan was exhibited in 3D-artifical skin, at which IL-1α was suppressed. These information support the applications of the new promising polysaccharide, levan, in skin care cosmetics. Skin moisturizing efficacy of leven was evidenced in 10 female volunteers participating in a short terms efficacy test. Transepidermal water loss (TEWL) was suppressed by a single topically applied levan solution (1%) for 6 h monitoring by Vapometer®. In addition, skin hydrating efficacy of levan (0.2%) assessed by Corneometer® in the same group of the volunteers topically treated for 1 time with a monitoring time of 30 min. The efficacy was substantially with the benchmark hyaluronic acid at the same test concentration (Kim et al. 2005).

2.9 Alginate

Alginate or algin is microbial produced from two strains; *Pseudomonas* and *Azotobacter* in acid and salt forms, among which *P. aeruginosa* and *A. vinelandii* are the commercial available sources (Hay et al. 2013, 2014). Alginate, an anionic polysaccharide with 1.0–1.4 10^3 kDa, is used for applications such as thickening aqueous solutions, forming gel and water-soluble film, and stabilizer. Sodium alginate is a commonly applied form that is often used as a powder, either pure or mixed with other ingredients for topical products. Wound healing property of alginate was exhibited. The polysaccharide base stimulates reparative processes, prepares the wound for scarring, and displays protective and coating effects, shielding mucous

membranes and damaged skin against irritation from unfavorable environments. Calcium alginate promotes the proliferation of fibroblasts and inhibits the proliferation of microvascular endothelial cells and keratinocytes. Profound wound healing effects have also been reported for a gelatin–alginate sponge impregnated with antiseptics and antibiotics (Balakrishnan et al. 2006). In addition, alginate possess antimicrobial activity with anti-elastase and anti-MMP-2, and anti-inflammatory as well which the activities pronounces for silver-containing alginate (Wiegand et al. 2009). This polysaccharide is therefore appointed for several skin care cosmetics for variety of claims.

3 The Specialty Fungal Polysaccharides for Skin Care Cosmetics

Fungal polysaccharides comprise a large group of biopolymers which are either part of the cell wall or may form intracellular inclusions and serve as energy reserve, or are excreted extracellularly providing a mechanism for cell protection or attachment to other surfaces (El Enshasy and Hatti-Kaul 2013). Of which, pullulan is the most widely known fungal polysaccharides supplied for cosmetics.

3.1 Pullulan

Pullulan is a linear homopolysaccharide composed of glucose. *Aureobasidium pullulans* is described in the literature as producing high pullulan concentrations with the molecular weight of $5 - 900 \times 10^3$ Da (Kanlayavattanakul and Lourith 2015). However, the commercial pullulan is available with the weight of $8 \times 10^3 - 2 \times 10^6$ Da. The polysaccharide is highly soluble in water, and stable in a form of aqueous low viscosity solution over a wide pH range (Fiume et al. 2016) with the potency on oxidizing protection of the active cosmetic ingredients (Krochta and De Mulder-Johnson 1997). Besides an application of pullulan as the functional ingredients for cosmetics, its action as an active ingredient is continuously explored. Topical application of pullulan was exhibited to suppress UVB-induced skin damage in an animal model. Glutahione, depletion, MMP activation, and production of IL-1ß were attenuated as per the ability to decrease IL-10 and keratinocyte apoptosis. Thus, pullulan can be used as a protective agent against photoaging of skin (Kim et al. 2015). In regards with the fact of high cost pullulan, it is not commonly used as a single polysaccharide in skin care cosmetics. The most commonly used form is blending or compositing with another polysaccharides.

In addition to pullulan, many of fungal polysaccharides derived from edible mushrooms, such as the Maitake or Shiitake or Oyster mushroom. Although many fungi are known for their health-promoting properties and have been widely consumed in Asia for centuries, those with pharmaceutical properties applicable for cosmetics are exemplified in Table 4.

Table 4 Health beneficial mushroom

Scientific name	Common name
Agaricus subrufescens	Almond/Himematusutake
Cordyceps sinensis	Caterpillar fungus
Ganoderma lucidum	Reishitake
Grifola fondosa	Maitake
Hericium erinaceus	Lion'Mane
Inonotus obliquus	Chaga
Lentinula edodes	Shiitake
Pleurotus ostreatus	Oyster
Poria cocos	Tuckahoe
Trametes versicolor	Turkey tail

The biological activities of several fungal polysaccharides have been reported repeatedly in the last years and efforts have been made to elucidate their structure–function relationships. In the following section, the commercial available mushroom polysaccharides applicable for skin care cosmetics are summarized (Lourith and Kanlayavattanakul 2018).

3.2 *Lentinus edodes*

One of the most common and well-studied medicinal fungal polysaccharides is lentinan, a glucan elaborated by the edible mushroom *Lentinus* (or *Lentinula*) *edodes*, also known as Shiitake mushroom. It is composed of a main chain of *β*-(1,3)-*D*-glucose residues to which *β*-(1,6)-*D*-glucose side groups are attached (one branch to every third main chain unit), and an average molecular weight of about 5×10^5 Da. Lentinan is a high molecular weight glucan extracted from the cell wall of the fruiting body of *L. edodes* with mostly *β*-(1→3)-glucose linkages in the regularly branched back-bone and *β*-(1→6)-glucose side-chains.

3.3 *Ganoderma lucidum*

Ganoderma lucidum or Reishitake mushroom is another well-studied medicinal mushroom of the *Basiodiomycetes* family, which has been used in traditional East Asian medicine. It produces ganoderan, a typical *β*-(1,3) bioactive glucan branched at C-6 with *β*-(1,6) glucose units, with a varying molecular weight and degree of branching, especially when isolated from the water-soluble fraction of the fruit body, while the glucan isolated from filtrates of liquid-cultured mycelia which has a MW of $1.2–4.4 \times 10^6$ Da. Notably, as occurs with other mushroom biopolymers, the fruit bodies of *G. lucidum* also produce several more heteroglucans and proteoglucans with immunostimulating activity.

3.4 *Agaricus subrufescens*

Another edible and medicinal mushroom, *Agaricus subrufescens* or Almond or Himematsutake mushroom, is the source of several polysaccharides contained in its fruit body. These include a *β*-(1,6);*β*-(1,3) glucan, an acidic *β*-(1,6);*α*-(1,3) glucan, and an acidic *β*-(1,6);*α*-(1,4) glucan. By contrast to most mushroom glucans, the above glucans have a main chain of *β*-(1,6) glycopyranose, instead of the more common *β*-(1,3) linked main chain. The fruit body also contains water-soluble proteoglucan of 3.8×10^5 Da with a *α*-(1,4) glucan main chain and *β*-(1,6) glucopyranoside branches at a ratio of 4:1, as well as two immunostimulating heteroglucans containing glucose, galactose, and mannose, one consisting of glucose and ribose and a xyloglucan.

3.5 *Grifola frondosa*

Other immunostimulating biopolymers from *Basidiomycetes* include grifolan, a gel-forming β-(1,3)-*D*-glucan with *β*-(1,6) glucosidic bonds at every third glucopyranosyl residue, found in *Grifola frondosa* or Maitake mushroom with a *β*-(1,3)-*D*-glucan.

3.6 *Trametes versicolor*

The edible Turkey tail mushroom *Coriolous versicolor* or *Trametes versicolor* has been commercialized in Asia as an effective immunostimulative agent with the glucan structure similarly to polysaccharide from maitake.

3.7 *Pleurotus ostreatus*

The popular culinary Oyster mushroom, *Pleurotus ostreatus*, synthesizes bioactive *β*-glucans, such as pleuran, an insoluble *β*-(1,3/1,6)-*D*-glucan, which is another potential candidate for the development of nutraceuticals.

3.8 *Poria cocos*

Heteropolysaccharides isolated from *P. cocos* or Tuckahoe mushroom mainly contained glucose, galactose, and mannose, and exhibited anti-tumor activities both *in vitro* and *in vivo*.

Nonetheless, commercial production of mushroom polysaccharides for cosmetic industry is infeasible in several aspects. Especially, high production or purification costs, low or erratic polysaccharide yields, and unstable chemical characteristics (i.e., composition, molecular weight, and degree of branching). Such problems are encountered mainly during the production of these biopolysaccharides from mushroom fruit bodies; however, they can be ameliorated to some extend by the use of

fungal mycelium grown in submerged cultures under controlled process conditions (Lourith and Kanlayavattanakul 2018).

4 Challenges and Future Prospects of Microbial Polysaccharides for Cosmetics

The global demand on natural cosmetic ingredients derived from the renewable, eco-friendly, or sustainable sources of particular from the biotechnological production route is increasing year by year including for the sector of microbial polysaccharides that are biodegradable. This high demand is not only serve for the formulation of skin care cosmetics but including for cosmetic packaging and those of delivery system. Thus, challenges of microbial polysaccharides upon these issues are additionally addressed as well as the further uses and perspective production technology and source of microbial polysaccharides.

4.1 Biodegradable Material for Packaging

In addition to functional and active tasks of polysaccharides serve for skin care cosmetics, the polysaccharides are appointed to be used for cosmetic packaging proposes. Accordingly, general information on cosmetic packaging is briefly introduced. The beneficial applications of microbial polysaccharides for cosmetic packaging are highlighted (Lourith and Kanlayavattanakul 2018).

Cosmetic packaging is the materials that enclose/surround the product since the time of manufacturing to till final usage. Cosmetic packaging functions in two aspects; protection and presentation. Protection function is environmental and physical/mechanical protections. While presentative functions of cosmetic packaging are identification of the product, providing information of the product with a convenience during handling, and advertising and marketing of product in the same time. The most important concerning issue that is needed for cosmetic packaging is the compatibility of the packaging and the containing cosmetics. To achieve compatibility there are according concerning issues as followings;

1. Adsorption onto the surface and absorption into the package

When formulation components are removed by a package two different processes are involved: adsorption onto the surface and absorption into the package wall by diffusion. A component may also get desorbed from the outer surface of the package and pass into the atmosphere if it is volatile enough. Strong adsorption or absorption requires a strong chemical interaction between the component and the packaging material. In addition, a high level of absorption will occur when the packaging material is permeable to the component. When adsorption and absorption occur together, there is rarely a distinction made between the two and therefore the term "sorption" will be used to indicate both processes are taking place.

2. Leaching

Most leaching problems occur with plastics because of the presence of additives such as fillers, activators, and plasticizers. Leaching can cause discoloration, precipitation, change in pH, and contamination that can lead to increased toxicity or instability of the active ingredients.

3. Permeation

The transmission of gases, vapors, or liquids through packaging materials can have an adverse effect on the shelf life of the cosmetic products. Permeation of water vapor and oxygen through the polymeric wall into the cosmetics can present a problem if the dosage form is sensitive to hydrolysis and oxidation. Temperature and humidity are important factors influencing the permeability of oxygen and water through the package. An increase in temperature reflects an increase in the permeability of the gas. Permeation can therefore alter properties of polymeric package and may also lead to degradation. Some solvent systems have been found to be responsible for considerable changes in the mechanical properties of the package.

4. Physical or chemical alteration

The physical or chemical alteration of packaging materials by cosmetic products is called modification. Permeation, sorption, and leaching are physical alterations that can lead to degradation.

Polysaccharide is biodegradable polymer or so called biopolymer can be found on its application for packaging including for cosmetic product. Biopolymer is simply divided into (i) biopolymers that are originated from living organisms and (ii) bioplastics or bio-based polymers that are manufactured from a natural or renewable sources and can be biodegraded. Microbial polysaccharide is accounted as biopolymer regarding on this two categories. Of which, the desired characters of cosmetic packaging can be summarized as followings;

- Oxygen-scavenging
- Moisture-control
- Anti-microbial
- Natural & Eco-friendly

Nowadays, natural trend influences customer buying a product including skin care cosmetics. Use natural materials to imitate looks and add more trust and credibility to natural lovers. In addition, eco-friendly packing not only meets the emotional needs of consumers, but also reflects simple and eye-catching design, in particular the skin care cosmetic consumers of the niche market. The production biodegradable polysaccharides are expected to be increased to 2.44 million tons in 2020 (Gontard et al. 2018) to supply for the certain industries including cosmetics, of which the desired characters of skin care cosmetic package are illustrated as shown in Fig. 3.

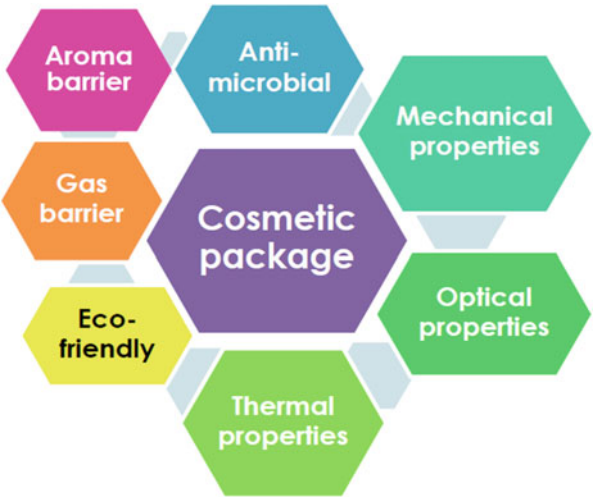


Fig. 3 Desired characters of cosmetic package

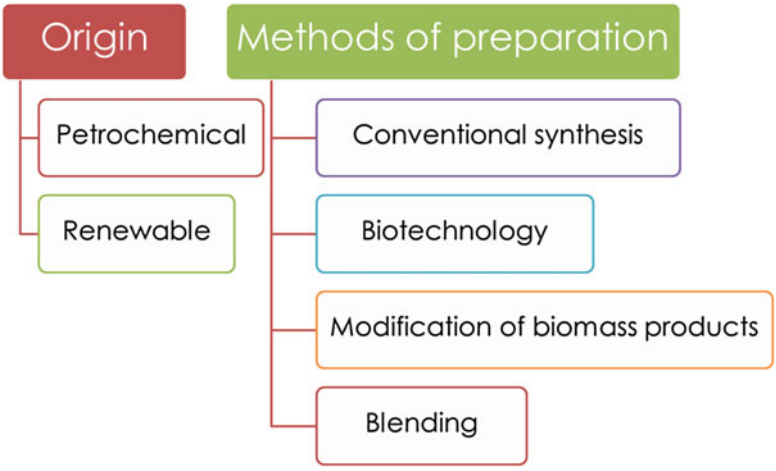


Fig. 4 Preparation of biodegradable or compostable polymer

Biodegradable material for cosmetic package is highly in demand. Biopolymeric materials with the desired characters for cosmetic package (Fig. 3) can be archived by several methods as shown in Fig. 4. Among which, cellulose is widely used as biodegradable cosmetic package nowadays.

Cellulose esters, such as cellulose acetate (CA), cellulose acetate propionate (CAP), and cellulose acetate butyrate (CAB), that are characterized by their stiffness, moderate heat resistance, high moisture vapor transmission, grease resistance, clarity

and appearance, and moderate impact resistance (Mekonnen et al. 2013; Rhim and Ng 2007). Benefits of the cellulose-based packaging materials is its wide heat sealing range (70–200 °C), static-free, and can be varied in the levels of thickness (23 and 30μm). More important, it is claimed to be gas-selective and permeable (shelf life boost).

In addition to an application based on a single polysaccharide. The mixture of polysaccharides in the forms of composites and blends with improvements of thermal and mechanical properties as well as biological properties in some cases are found more applicable to be used. Blending of pullulan with alginate by a ternary co-blended film technique was shown to improve water vapor permeability and oxygen barrier property of the films as per the moisture retention ability that positively achieve mechanical property (Pan et al. 2014). Pullulan-nanofibrillated cellulose composite had been developed and exhibited as the translucent film that is thermal stable and highly achieve on Young's and tensile strength to 5500% and 8000%, respectively (Trovatti et al. 2012). In spite of the excellent property of pullulan-derived packaging, its unit price might infeasible the application.

4.2 Delivery System

A variety of natural polysaccharides are finding increasing applications in cosmetics and personal care markets (Ammala 2013). Encapsulation of active ingredients using biodegradable polymeric carriers can facilitate increased active efficacy and bioavailability. They can also be chemically functionalized to give enhanced properties over conventional carrier materials. Many of the conventional nano delivery system (e.g., liposomes, micelles, and polymer-based nanodevices) have reached the late stages of development, and some of them were approved. Various kinds of vehicles, i.e., liposomes, polymersomes, microemulsions, vesicles, and hydrogel have been developed in cosmetic industries, in which the duty of vehicles can be protection and delivery of actives. In particular, well-defined structure of synthetic polymers is attractive in percutaneous treatments due to the ease of synthesis and the wide diversity of material selection (Cho et al. 2014). However, the polymers used in topical delivery should meet several stringent requirements, e.g., low toxicity or skin irritation, biocompatibility or biodegradability, appropriate molecular weights and amphiphilicity (Lochhead 2010). Application of microbial polysaccharide in terms of a delivery system for skin care cosmetics is summarized as followings (Lourith and Kanlayavattanakul 2018).

Cellulose is the polysaccharide used in delivery system especially in its microcrystalline form. In addition, chitosan has been reported on its ability to enhance permeation across the skin by altering the structure of keratin. It also increases the water content of the stratum corneum and cell membrane fluidity. Further, due to its positive charge under slightly acidic conditions, it can depolarize the negatively

charged cell membrane and in doing so, it decreases the membrane potential and drives the active component or drug through the skin. Encapsulation of active ingredients using chitosan in the forms of nanoparticles, microspheres and hydrogels are possible. Which, a controlled release has been demonstrated by increasing the viscosity of the polymer matrix. Alginate is reported for delivery applications as they can form gels with divalent metal ions such as calcium. The fact that relatively mild conditions can be used to incorporate actives within alginates makes them excellent candidates for delivery of proteins that can minimize any denaturation. The spherical alginate capsules can contain water-soluble or dispersible active agents as well as liposoluble additives and can include biological compounds, colored pigments, sunscreens, and perfume. Hyaluronic acid is used as a delivery system to transfer keratinocyte cells from tissue culture to skin wounds, in particular burns, with high rates of healing. The stability of hyaluronic acid-based nanoemulsions as trans-dermal carriers was reported. Electrostatic, steric and hydrophobic effects were found to play a key role in the stability of the emulsions. Encapsulation was capable of lipophilic additives with desirable stratum corneum permeability, efficient partitioning capacity, and was able to be diffused deeper into the dermis. The small size of the emulsion droplets (50–200 nm) was also noted as significant as this enabled greater surface-to-volume ratio which has greater contact points between the emulsion and the skin (Ammla 2013).

Gellan was evidenced as one of the appointed polysaccharide for delivery system applicable for skin care products. Nanohydrogel of gellan was proved to be the appointed delivery system for the cutaneous active agent. Of which, the system could enhance the retention of the active in the epidermis (Musazzi et al. 2018). Gellan-hyaluronic acid hydrogel was prepared and examined on its wound healing property in mice. It was shown to significantly increase epidermis thickness. The mechanism was by stimulating activities toward neoepidermis formation and neovascularization associating with its hydroscopic nature limiting inflammatory exudate promote cellular differentiation and angiogenesis and wound healing in terns (Cerqueira et al. 2014). Gellan had been developed into the form of gellan-transfersomes lading with the skin active ingredient, baicalin. Skin restoration activity was shown with the significant inhibitory effect against inflammatory mediators, TNFα and IL-1ß in an animal model (Manconi et al. 2018). In addition to the incorporation of organic active with gellan, nanoparticle biofilm incorporated with gellan and titanium dioxide was developed and characterized including biological activity. The developed nanoscale particle exhibited anti-microbial activity, *Staphylococcus aureus* and *Escherichia coli*, the prohibited microbe in cosmetic products. Its cellular activity stimulating cell density and adherence. Moreover, it's in vivo cell proliferation and migration promoting activities associated in wound healing process was evidenced in an animal model (Ismali et al. 2019).

Moreover, levan can be prepared in the form of nanoparticle and loaded with vitamin E derivative to be used for skin care product (Nakapong et al. 2013).

Calcium alginate nanocarrier was developed for hydrophobic active delivery, and the system was shown to be safe in keratinocyte with a rapidly enter the cell and dissociated. This delivery system was suggested as the potential carrier for cosmetic actives applicable with skin cell (Nguyen et al. 2016).

4.3 Source and Production

Microbial polysaccharides prepared from natural sources are of interests among the consumers and the researchers as well. Diversity of the natural sources surplus with an advancement on biotechnology feasibly serve the demands. In addition, ecological condition suitable with the growth environment of the specific microbial is one issue that can be set. In addition, new source of microbe producing polysaccharide applicable for cosmetic application is continuously revealed for instance *Deinococcus radiodurans*. Exopolysaccharide of 80–100 kDa was synthesized by *D. radiodurans* and it was exhibited to be constructed with xylose, galactose, fucose, glucose, arabinose, and fructose at the molar ratios of 10.6:6.1:4.2:3.8:2.6:1.0. The polysaccharide showed cellular antioxidant activity by suppression of intracellular radical formation induced by peroxyl radicals and ultraviolet exposure at the dose of 10μg. In addition, its protective effect against lethal doses of irradiation was evidenced in an animal model (Lin et al. 2020). However, production cost turns to be the disruptive issues for commercial development of microbial polysaccharides for certain industries. Of which, extraction and purification of the specific polysaccharides are obviously consume lots of cost during the production processes. Thus, biosynthetic control or metabolic engineering on the basis of gene and coding enzyme manipulations would be overwhelm this obstacles. Although the biosynthetic pathway of microorganisms have been continuously revealed, additional researches on the specific step in the pathway producing the desired polysaccharide are of challenge.

5 Conclusions

Microbial-derived polysaccharides are currently serving as the important cosmetic ingredients. They are applicable for different tasks not only for active and functional actions but include delivery systems and packaging as well. Their cosmetic benefits are enclosed therein. The biotechnological production of microbial polysaccharides is accountable as the natural and organic cosmetic ingredients that are highly appreciated among the consumers rather than those of the synthetic ones. The specialty microbial polysaccharides are therefore gaining the high interest in terms of the researchers' point of view on challenging for the fabricated production of these sort of actives serving in health and personal care products industry, among which skin care products are the major section as enclosed. The cosmetic formulators will be widen in the choices on the formulation developments accordingly. In

regard with the consumers' demand and economically feasibility of microbial polysaccharides, safety is the mandatory issue that must be highly concerned.

References

Ammala A. Biodegradable polymers as encapsulation materials for cosmetics and personal care markets. Int J Cosmet Sci. 2013;35:113–24.

Anderson LA, Islam MA, Prather KLJ. Synthetic biology strategies for improving microbial synthesis of "green" biopolymers. J Biol Chem. 2018;293:5053–61.

Balakrishnan B, Mohanty M, Fernandez AC, Mohanan PV, Jayakrishnan A. Evaluation of the effect of incorporation of dibutyl cyclic adenosine monophosphate in an insitu forming hydrogel wound dressing based on oxidized alginate and gelatin. Biomaterials. 2006;27:1355–61.

Berdal M, Apelbom HI, Eikerm JH, Lund A, Zykova S, Busund L-T, Seljelid R, Jenssen T. Aminated β-1,3-d-glucan improves wound healing in diabetic db/db mice. Wound Repair Regen. 2007;15:825–32.

Cerqueira MT, da Silva LP, Santos TC, Pirraco RP, Correlo VM, Reis RL, Marques AP. Gellan gum-hyaluronic acid spongy-like hydrogels and cells from adipose tissue synergize promoting neoskin vascularization. ACS Appl Mater Interfaces. 2014;6:19668–79.

Chen P, Cho SY, Jin HJ. Modification and applications of bacterial cellulose in polymer science. Macromol Res. 2010;18:309–20.

Cho HK, Cho JH, Jeong SH, Cho DC, Yeum JH, Cheong IW. Polymeric vehicles for topical delivery and related analytical methods. Arch Pharm Res. 2014;37:423–34.

DeAngelis PL. Glycosaminoglycan polysaccharide biosynthesis and production: today and tomorrow. Appl Microbiol Biotechnol. 2012;94:295–305.

Donot F, Fontana A, Baccou JC, Schorr-Galindo S. Microbial exopolysaccharides: main examples of synthesis, excretion, genetics and extraction. Carb Polym. 2012;87:951–62.

Dubuisson P, Picard C, Grisel M, Savary G. How does composition influence the texture of cosmetic emulsions? Colloid Surface A. 2018;536:38–46.

El Enshasy HA, Hatti-Kaul R. Mushroom immunomodulators: unique molecules with unlimited applications. Trend Biotechnol. 2013;31:668–77.

Fiume MM, Heldreth B, Bergfeld WF, Belsito DV, Hill RA, Klaassen CD, Liebler DC, Marks JJG, Shank RC, Slaga TJ, Snyder PW, Andersen FA. Safety assessment of microbial polysaccharide gums as used in cosmetics. Int J Toxicol. 2016;35:5S–49S.

Freitas F, Alves VD, Reis MAM. Advances in bacterial exopolysaccharides: from production to biotechnological applications. Trend Biotechnol. 2011;29:388–98.

Freitas F, Alves VD, Reis MA, Crespo JG, Coelhoso IM. Microbial polysaccharide-based membranes: current and future applications. J Appl Polym Sci. 2014;131:40–7.

Giese EC, Gascon J, Anzelmo G, Barbosa AM, da Cunha MAAA, Dekker RFH. Free-radical scavenging properties and antioxidant activities of botryosphaeran and some other β-D-glucans. Int J Biol Macromol. 2015;72:125–30.

Gontard N, Sonesson U, Birkved M, Majone M, Bolzonella D, Celli A, Angellier-Coussy H, Jang G-W, Verniquet A, Broeze J. A research challenges vision regarding management of agricultural waste in a circular bio-based economy. Crit Rev Environ Sci Technol. 2018;48:614–54.

Hay ID, Rehman ZU, Moradali MF, Wang Y, Rehm BHA. Microbial alginate production, modification and its applications. Microb Biotecnol. 2013;6:637–50.

Hay ID, Wang Y, Moradali MF, Rehman ZU, Rehm BHA. Genetics and regulation of bacterial alginate production. Environ Microbiol. 2014;16:2997–3011.

Huertas MJ, Matilla MA. Training bacteria to product environmentally friendly polymers of industrial and medical relevance. Microb Biotechnol. 2020;13:14–6.

Ismali NA, Amin KAM, Majid FAA, Razali MH. Gellan gum incorporating titanium dioxide nanoparticles biofilm as wound dressing: physicochemical, mechanical, antibacterial properties

and wound healing studies. Mater Sci Eng C. 2019;103:109770. https://doi.org/10.1016/j.msec.2019.109770.
Kanlayavattanakul M, Lourith N. Biopolysaccharides for skin hydrating cosmetics. In: Ramawat KG, Mérillon J-M, editors. Polysaccharides: bioactivity and biotechnology. Cham: Springer; 2015. p. 1867–92.
Kanlayavattanakul M, Lourith N. Cosmetics: active polymers. In: Mirshra M, editor. Encyclopedia of polymer. Boca Raton: CRC Press; 2019. p. 705–21.
Kim KH, Chung CB, Kim YH, Kim KS, Han CS, Kim CH. Cosmeceutical properties of levan produced by *Zymomonas mobilis*. J Cosmet Sci. 2005;56:395–406.
Kim KH, Park SJ, Lee YJ, Lee JE, Song CH, Choi SH, Ku SK, Kang SJ. Inhibition of UVB-induced skin damage by exopolymers from *Aureobasidium pullans* SM-2001 in hairless mice. Basic Clin Pharmacol Toxicol. 2015;116:73–86.
Klemm D, Schumann D, Kramer F, Heßler N, Hornung M, Schmauder H-P, Marsch S. Nanocelluloses as innovative polymers in research and application. Adv Polym Sci. 2006;205:49–96.
Krochta J, De Mulder-Johnson C. Scientific status summary – edible and biodegradable polymer films. Food Technol. 1997;51:61–74.
Kwon AH, Qiu Z, Hashimoto M, Yamamoto K, Kimura T. Effects of medicinal mushroom (*Sparassis crispa*) on wound healing in streptozotocin-induced diabetic rats. Am J Surg. 2009;197:503–9.
Lin YC, Vaseeharn B, Chen J-C. Identification of the extracellular copper–zinc superoxide dismutase (ecCuZnSOD) gene of the mud crab *Scylla serrata* and its expression following β-glucan and peptidoglycan injections. Mol Immonol. 2008;45:1346–55.
Lin SM, Baek CY, Jung J-H, Kim WS, Song H-Y, Lee JH, Ji HJ, Zhi Y, Kang BS, Bahn Y-S, Seo HS, Lim S. Antioxidant activities of an exopolysaccharide (DeinoPol) produced by the extreme radiation-resistant bacterium *Deinococcus radiodurans*. Sci Rep. 2020;10:55. https://doi.org/10.1038/s41598-019-56141-3.
Lochhead RY. A review of recent advances in the polymeric delivery of attributes in cosmetics and personal care products. In: Morgan S, Lochhead RY, editors. Polymeric delivery of therapeutics. Washington, DC: ACS; 2010.
Lourith N, Kanlayavattanakul M. Biopolymeric agents for skin wrinkle treatment. J Cosmet Laser Ther. 2016;18:301–10.
Lourith N, Kanlayavattanakul M. Polymer in cosmetics. Bangkok: Chulalongkorn University Press; 2018.
Lourith N, Kanlayavattanakul M. Tissue engineering: polymeric dermal filler. In: Mirshra M, editor. Encyclopedia of polymer. Boca Raton: CRC Press; 2019. p. 2676–82.
Manconi M, Manca ML, Caddeo C, Valenti D, Cencetti C, Diez-Sales O, Nacher A, Mir-Palomo S, Terencio MC, Demurtas D, Gomez-Fernandez JC, Aranda FJ, Fadda AM, Matricardi P. Nanodesign of new self-assembling core-shell gellan-transfersomes loading baicalin and *in vivo* evaluation of repair response in skin. Nanomed Nanotechnol. 2018;14:569–79.
Mattila PH, Marnila P, Pihlanto A. Wild and cultivated mushrooms. In: Yahia EM, editor. Fruit and vegetable phytochemicals: chemistry and human health. 2nd ed. Hoboken: Wiley; 2018. p. 1279–304.
Mekonnen T, Mussone P, Khalil H, Bressler D. Progress in bio-based plastics and plasticizing modifications. J Mater Chem A. 2013;1:13379–98.
Musazzi UM, Cencetti C, Franzé S, Zoratto N, Di Meo C, Procacci P, Matricardi P, Cilurzo F. Gellan nanohydrogels: novel nanodelivery systems for cutaneous administration of piroxicam. Mol Pharm. 2018;15:1028–36.
Nakapong S, Pichyangkura R, Ito K, Iizuka M, Pongsawasdi P. High expression level of levansucraase from *Bacillus licheniformis* RN-01 and synthesis of levan nanoparticles. Int J Biol Macromol. 2013;54:30–6.

Nguyen HTP, Allard-Vannier E, Gaillard C, Eddaoudi I, Miloudi L, Soucé M, Courpa I, Munnier E. On the interactions of alginate-base core-shell nanocarriers with keratinocytes *in vitro*. Colloids Surf B Biointerfaces. 2016;142:272–80.

Pan H, Jiang B, Chen J, Jin Z. Assessment of the physical, mechanical, and moisture-retention properties of pullulan-based ternary co-blended films. Carb Polym. 2014;112:94–101.

Rehm BH. Bacterial polymers: biosynthesis, modifications and applications. Nature Rev Microbiol. 2010;8:578–92.

Rhim J-W, Ng PKW. Natural polymer-based nanocomposite films for packaging application. Crit Rev Food Sci Nutr. 2007;47:411–33.

Shah N, Ul-Islam M, Khattak WA, Park JK. Overview of bacterial cellulose composites: a multiprupose advanced material. Carb Polym. 2013;98:1585–98.

Srikanth R, Siddartha G, Sundhar RCH, Harish BS, Janaki RM, Uppuluri KB. Antioxidant and anti-inflammatory levan produced from *Acetobacter xylinum* NCIM2526 and its statistical optimization. Carb Polym. 2015;123:8–16.

Trovatti E, Fernandes SCM, Rubatat L, Perez DDS, Freire CSR, Silverstre AJD, Neto CP. Pullulan-nanofibrillated cellulose composite films with improved thermal and mechanical properties. Compos Sci Technol. 2012;72:1556–61.

Wiegand C, Heinze T, Hipler UC. Comparative *in vitro* study on cytotoxicity, antimicrobial activity, and binding capacity for photophysiological factors in chronic wounds of alginate and silver-containing alginate. Wound Repair Regener. 2009;17:511–21.

Immunogenicity and Vaccines of Polysaccharides

36

Benjamin Obukowho Emikpe and Michael Ayodele Odeniyi

Contents

Abstract

There is an increased interest in the use of plant extracts as therapeutic agents exploring their ability to inhibit growth of pathogenic microorganisms; however, there has been a dearth of information on their application as adjuvants and vaccine delivery agents. Gums and mucilages from several plants have been evaluated for both bioadhesive and phytogenic properties. Gums were prepared from *Abelmoschus esculentus, Irvingia gabonensis*, and *Boswellia carteri* and were used as mucosal delivery agents for PPR vaccine in goats and sheep using the intranasal route of vaccine application. The application was comparable to the conventional subcutaneous route of PPR vaccination in terms of humoral immune response induction and duration of antibodies. Mixtures of gums were prepared

B. O. Emikpe (✉)
Departments of Veterinary Pathology, University of Ibadan, Ibadan, Nigeria

M. A. Odeniyi
Department of Pharmaceutics and Industrial Pharmacy, University of Ibadan, Ibadan, Nigeria
e-mail: ma.odeniyi@ui.edu.ng

J. M. Oliveira et al. (eds.), *Polysaccharides of Microbial Origin*,
https://doi.org/10.1007/978-3-030-42215-8_47

from *Cedrela odorata, Khaya senegalensis,* and *Boswellia carteri* as oral and ocular delivery agents for Newcastle Disease and Infectious Bursal Disease vaccines in broilers. The gums act as adjuvants to enhance immune response to the poultry diseases. Gums and mucilages of plant origin have great potential as both delivery agents and adjuvants in vaccines for the prevention and treatment of poultry and animal diseases.

Keywords

Phytogenic gums · Vaccines · Poultry · Adjuvants

1 Gums/Polymers from Phytogenic Origin Used for Vaccine Delivery

1.1 Introduction

The use of plant extracts as therapeutic agents is enjoying increasing attention in the scientific research area. This interest has been geared toward the capacity for these plant extracts to inhibit the growth of pathogenic microorganisms and to be used as veterinary vaccine delivery agents. Reports by Emikpe et al. (2016) and Oyebanji et al. (2017a, b) have proven that gums from *Khaya senegalensis* and *Cedrela odorata* which are bioadhesives have shown the ability to interact with biologic tissues for veterinary applications such as vaccine delivery in Newcastle disease of poultry as well as in infectious disease vaccine of poultry (Adeniran et al. 2019, 2020a, b). Recent studies have also showed that gums prepared from *Abelmoschus esculentus, Irvingia gabonensis,* and *Boswellia carteri* are capable of serving as delivery agents for PPR vaccine in goats and sheep (Ezeasor et al. 2020a, b; Mumin et al. 2020) using the intranasal route of vaccine application. This application was comparable to the conventional subcutaneous route of PPR vaccination in terms of humoral immune response induction and duration of antibodies. The responses in the two animals, sheep and goats, are similar which gives a wider application of this plant polysaccharide as vaccine delivery agents in small ruminants. The probable explanation for the enhancement of immune response seen in studies done clearly revealed the adjuvant property of the gum.

1.2 Plant Gums

Gums, by definition, are substances made up of a mixture of chemical compounds that are able to conglomerate into gels that increase the viscosity of solutions as well as cause stability in foams and emulsions (Granzotto et al. 2017). Gums are commonly associated with water-soluble and modified polysaccharides and do not necessarily designate a class of chemical compounds. As plant gums are deemed to be natural, they have been reported to be obtained from different parts of plants including plant cell walls, seeds, trees or shrub exudates, and seaweed extracts (Granzotto et al. 2017).

Gums obtained from plants have been identified to have several uses. Usage of gums from plants has been traced back to 2600 B.C. through the Fourth Dynasty of Egyptian time where they were used as binders and adhesives for mineral pigments on cartonnage and in the practices of mummification (Granzotto et al. 2017). Binding media and adhesives used in artworks and cultural artifacts have also been identified to be products made from plant gums. These benefits indicate strongly the significance of plant gums in the world. Currently, gums have been widely used, for example, as binders for watercolor and gouache paints. In reference to the distinguishing properties of plant gums, certain plant gums including that of gum Arabic (from *Acacia senegal* and *seyal*) and locust bean gum (LBG) (*Ceratonia siliqua*), *Khaya senegalensis, Cedrela odorata, Abelmoschus esculentus, Irvingia gabonensis,* and *Boswellia carteri* have been investigated for use in the food, cosmetics, textiles, and biomedical/pharmaceutical industries in recent studies (Ofoefule and Chukwu 2001; Stephen and Churms 2006; Kalu et al. 2007).

Structurally, plant gum polysaccharides have been found to be complex in nature reflected in having high-molecular mass (up to several million Dalton), highly branched structures with a large variety of monosaccharides and glycosidic linkages, high polydispersity, and heterogeneity, while some have covalently attached protein moieties (Granzotto et al. 2017). In as much as polysaccharides are studied by different analytical techniques, limited information is provided in the literature about the makeup in their chemical structure. The structural analyses of plant gum polysaccharides focus on the monosaccharide composition and sequences, molecular weight and distribution, linkage positions between the glycosidic linkages and branches, ring size (furanosidic/pyranosidic rings), anomeric configuration (α/β), substitutions, and identification/localization of potentially attached proteins. With this knowledge on their structure, their use as mucoadhesive delivery agents for vaccine is limited in literature. Recent studies are on veterinary vaccines such as Newcastle disease (Emikpe et al. 2016; Oyebanji et al. 2017a, b), infectious disease vaccine (Adeniran et al. 2019, 2020a, b) of poultry, and PPR vaccine for control of morbillivirus of sheep and goats (Ezeasor et al. 2020a, b; Mumin et al. 2020).

2 Mucosal Adjuvants

Adjuvants are of major concern in recent scientific discourse. Most often, living antigens are used as adjuvants, but in recent times, emphasis is being laid on adjuvants that are phytogenic in nature. With this current shift and choice for phytogenic adjuvants for mucosal delivery, it is important to investigate their bioadhesive strength. There has been varied approaches and techniques to evaluate the mucoadhesive properties of gums either ex vivo or in vivo. Among these techniques are the Wilhelmy plate technique, electromagnetic force transducer for the tensile stress, while adhesion tests are based on measurement of the force required to separate two polymer-coated glass slides with a film of mucus sandwiched between, to know the shear stress as observed in the design of a flow chamber method (Calero et al. 2010). Other approaches are the rheological method (Prabhu et al. 2010) and the viscometric method (Thirawong et al. 2008) which evaluate in vivo behavior of mucoadhesive

polymers and quantify mucin-polymer bioadhesive strength, respectively. In some recent studies, especially in limited resource environments, a tablet dissolution machine was adaptively used to evaluate the interaction between the gum polymer and mucosal tissue surfaces (Emikpe et al. 2016; Mumin et al. 2020). This often simulates the interplay of factors which plays out between the mucosal surface and the mucoadhesive polymer under an in vivo condition.

2.1 Living Antigens

Living antigens which have been used as adjuvants along mucosal surfaces, especially the gastrointestinal mucosal surface, had yielded positive results. This was evident by easy uptake of such microorganisms which seems to have a lectin-like interaction with specific carbohydrate receptors on M cells for easy uptake. Examples of such candidates are *Salmonella typhi th21a*, *Vibro cholera*, and Poliovirus strains. These have also been modified to express heterogeneous antigens for wider protection against a wide range of pathogens. Other adjuvant candidates for mucosal vaccines include liposomes, microparticles and nanoparticles, cytokines, ISCOMs, monophosphoryl lipid A, CpG, and detoxified ADP-ribosylating toxins (Hu et al. 2001).

2.2 Adjuvants of Phytogenic Origin

It is worth mentioning that the use of adjuvants in veterinary vaccines preparations seems to have broader candidates. However, the growing toxicity concerns of most adjuvant/delivery systems, the development of specialized vaccines, newer less invasive routes of applications, and the need for broader spectrum of responses, amid growing concerns of zoonotic infectious diseases have necessitated the review of available options for readily available, relatively stable adjuvants which are adaptable for less invasive routes, and broader response such as from phytogenic origin is inevitable. In this regard, plant products that have been generally explored are large carbohydrates, gums, and mucilages.

Gamma inulins from *Compositae* family are identified as large carbohydrates which have proven to have strong stimulatory effects on cells of the immune and reticulo-endothelial system. Studies have shown they stimulate good cytotoxic and humoral responses without the observed associated adverse reaction seen with older adjuvants such as FCA (Petrovsky and Aguilar 2004). The combination of large phytogenic carbohydrates with some other traditional adjuvants such as Alum products often yields excellent and safe results with a more vigorous Th2 response. This result is easily attributable to Alum and is boosted by the addition of such phytogenic large carbohydrate. Candidate antigens tested in animal models include respiratory syncytia virus, diphtheria, tetanus toxoid, Hepatitis B surface antigens, Haemophilus influenza, and influenza hemagglutinin antigens.

In another dimension, gums and mucilages which are natural polysaccharide products obtained from tree exudates have existed over 4,000 years. They are often produced by plants belonging *to Leguminosae, Sterculiaceae, Bixaceae,*

Compositae, Combretaceae, and *Gigarginaceae* families, e.g., gum acacia, gum tragacanth, and gum karaya. Economically, they have been used as binding, stabilizing, thickening, emulsifying agents and matrix components in pharmaceutical and food industries. They have also gained uses in cosmetics, textiles, adhesives, lithography, paints, and paper industries (Bhosale et al. 2014).

2.3 Gums and Mucilages: Salient Features

Gums as they exist are formed when plants undergo pathological changes such as injuries as a result of unfavorable environmental conditions, such as drought which results into dissolution of cell walls. This process is termed as gummosis while mucilages are formed within the cell (intracellular formation) due to physiological metabolism of plants. Mucilages may serve as food reserves or water-holding structures of the plant (Bhosale et al. 2014).

Certain gums (Acacia, Guar, and Tragacanth gums) have been observed to dissolve in water naturally while certain mucilages (Phoenix & Cassia Tora mucilages) form masses which are slimy in nature (Bhosale et al. 2014). In obtaining gums, incised trees barks or a tree trunk serves as the major source while mucilages can be harvested from other plant parts such as leaves (especially in the case of *Aloe barbadensis* mucilage).

Despite the differences in their morphology and botanical source, gums and mucilages have been reported to have some similarities. Both products have been reported to be made up of hydrocolloids, translucent amorphous substances and polymers of monosaccharide units, or mixed monosaccharide constituents, such as starch instead of cellulose or hydrocellulose (Jania et al. 2004). Both products chemically yield mixtures of sugars and uronic acids upon hydrolysis. These hydrophilic molecules constituents could combine with water to form viscous solutions or gels depending on the nature of the containing compounds which ultimately reflects in the properties of different gums. Structurally, gums made up of linear polysaccharides are more viscous because they occupy more space than those containing highly branched compounds of the same molecular weight. The branched compounds form gels more easily and are more stable because of the impossibility of extensive interaction along the chains (Jania et al. 2004).

2.4 Characterization of Gums and Mucilages from Excipient Analysis

Gums and mucilages are often run through a panel of tests to detect primary structure, purity of the pure compound, and presence of any impurities, i.e., impurity profiling, physicochemical properties, and toxicity (Ansari 2006). Thereafter, a scientific dossier is developed for such gum or mucilage. Often for tablet/drug preparations, after incorporation into drug preparations, compatibility tests are performed using spectrophotometry, Fourier transfer Infra-Red (FTIR) spectroscopy, or Differential Scanning Calorimetry (Raymond et al. 2004).

Purity: Purity tests for alkaloids, amino acids, steroids, terpenes, saponins, oils and fats, phenol, and tannins are often carried out to standardize the constituents of the gums.

Toxicity: This is most often determined by following a fixed-dose method as recommended by OECD guidelines, No. 25. Determination of LD50 is often recommended by conducting a subacute toxicity study. An initial in vitro cytotoxicity test can also be performed while elimination of microbial load, or contamination is highly recommended.

2.4.1 Classification of Gums

Gums have been classified based on varied criteria. They have been classified based on their ionic charge, origin, pH, and molecular shape or size. These varied classifications of gums have been described by Jania et al. (2004) as follows:

- Based on the ionic charge, gums have been classified into anionic, cationic, and nonionic.
  - Anionic charged gums: tragacanth, Arabic, karaya, gellan, agar, pectin, algin, and carageenans
  - Cationic charged gums: chitosan
  - Nonionic charged gums: guar gum, locust bean gum, tamarind gum, arabinans, xanthan gum, amylase, and cellulose
- Based on the origin, gums can be of the marine, animal, or plant origin:
  - Marine (sea weeds gum): alginates, agar, and carageenans
  - Animal origin: chitin and chitosan, chondroitin sulfate, and hyaluronic acid
  - Plant origin:
    Seed gums – locust bean, guar, starch, cellulose, and amylase
    Tree exudates – gum arabic, tragacanth, ghatti, and karaya
    Tubers – potato starch. iv) extracts-pectin. d) microbial origin (fungi and bacteria): glycan, pullulan, dextran, xanthan, and gellan
- Based on the source, gums could be natural, modified, and synthetic in nature:
  - Natural, e.g., acacia, tragacanth, and xanthan
  - Modified or semi-synthetic, e.g., carboxymethylcellulose and microcrystalline cellulose
  - Synthetic, e.g., carboxypolymethylene and colloidal silicon dioxide
- Based on the morphology or the shape of the gum, it can be linear or branched in nature:
  - Linear: amylase, pectin, and cellulose
  - Branched:
    Short branched-guar gum, locust bean gum
    Long branched-amylopectin, karaya gum, gum tragacanth, and gum Arabic
- Based on pH:
  - Acidic, e.g., acacia, tragacanth, and albizia gums.
  - Neutral, e.g., asparagus gum and plantago seed gums.
  - Basic, no basic gum occurs in nature; natural gums are either acidic or neutral.

2.5 Gums as Delayed Transit and Continuous Release Systems

Delayed transit and continuous release systems are drugs or delivery systems designed to prolong their residence time as well as release duration within the gastrointestinal tract (GIT). Often because of the hydro, chemical, and pH dynamics of the GIT (saliva, stomach, small intestinal juices, and large bowel system), the dosage form is fabricated to be able to withstand these dynamics in each region of the GIT and efficiently release the drug component to the targeted site. Systems included in this category are mucoadhesives and size-based systems.

Most drugs or pharmaceutical preparations explored as delayed release are sensitive to stomach or intestinal juice, targeted at a specific site and, as such, need special formulation with excipients that could sequestrate agents such that gradual release at needed sites is achieved. Mainly, there are two types of delayed release systems, i.e., intestinal and colonic release systems.

For orally administered drugs, addition of slow release excipients allows for optimal time of antigenic exposure and uptake by phagocytic cells and antigen-presenting cells which are situated along specialized mucosa structures along the gastrointestinal tract. However, for noninvasively administered vaccines, such as through the oral route, an excipient or vehicular base which could easily simulate what obtains with delayed drug release within the GIT is needed.

This is against the backdrop that existing delivery systems for most common orally administered vaccines are water or Alum compounds which rapidly transit the GIT leaving behind little time for exposure and uptake. Hence, subsequent immune response is often nonvigorous, short lived, and nonprotective. Therefore, a repeated dose at close intervals is often needed for protection to be assured. Gums and mucilages from many plants have shown prospects as delayed or continuous release candidates for drugs. A few examples are given in Table 1.

2.6 Attempts at Use of Plant Polysaccharide for Vaccine Delivery

Vaccines are a prepared material that induces an immunologically mediated resistance to a disease but not necessarily an infection. Vaccines are generally composed of killed or attenuated organisms or subunits of organisms or DNA-encoding antigenic proteins of pathogens (Saroja et al. 2011).

In order to induce an effective protective immunity, vaccines require boosting with agents called “adjuvants.” Adjuvants are believed to act by forming complexes with the agent to be delivered from whom immunogens are slowly released. Adjuvants potentiate the immunostimulatory property of the antigen while being non-immunogenic, nontoxic, and biodegradable by themselves (Saroja et al. 2011).

Conventional immunization which involves prime dose and booster doses sometimes fails while the administration of vaccines via gums allows for the incorporation of doses of antigens so that booster doses are no longer necessary as antigens are released slowly in a controlled manner. Again, the spatial and temporal presentation of antigens to the immune system could be controlled when administered via gums,

Table 1 Examples of phytogenic gums and mucilages used in drug delivery systems

Common name	Botanical name	Family	Pharmaceutical applications
Acacia	*Acacia Senegal*	*Leguminosae*	Osmotic drug delivery
Bhara gum	*Terminalia bellericaroxb*	*Combretaceae*	Microencapsulation
Chitosan	–	–	Colon-specific drug delivery, microspheres, and nanoparticles
Cordia gum	*Cordia oblique willed*	*Boraginaecae*	Oral sustained release matrix tablets
Guar gum	*Cyamompsis Tetraganolobus*	*Leguminoseae*	Colon-targeted drug delivery, microspheres
Gellan gum	*Pseudomonas elodea*	–	Ophthalmic drug delivery, sustaining agent, beads, and hydrogels
Karaya gum	*Sterculiaurens*	*Sterculiaceae*	Mucoadhesive and buccoadhesive
Locust bean gum	*Cerataniasiliqua*	*Leguminoseae*	Controlled delivery
Mucuna gum	*Mucunaflagillepes*	*Papillionaceae*	Microspheres
Okra gum	*Hibiscus esculentus*	*Malvaceae*	Hydrophilic matrix for controlled release drug delivery
Sodium alginate	*Macrocytis pyrifera*	*Lessoniaceae*	Bioadhesive microspheres, nanoparticles
Xanthan gum	*Xanthomonas lempestris*	–	Pellets, controlled drug delivery system

Source: (Reproduced from Bhosale et al. (2014) under the creative commons attribution – non-commercial (CC BY 4.0) license IJPPR)

thereby promoting their targeting of the immune cells. These benefits have encouraged the use of gums in the delivery of vaccines (Saroja et al. 2011).

The use of mucoadhesive gums and biopolymers (Odeniyi et al. 2013, 2015) allows for the mucosal administration of vaccines, which offers protection against microorganisms which gain access to body via mucosal membranes. Patient compliance, ease of administration, reduction in possibility of needle-borne injections, and stimulation of both systemic and mucosal immunity are some of the advantages of this route of administration.

This route has been exploited by Emikpe et al. (2016), thereby combining both the adjuvant and adhesive properties of the investigated gums; furthermore, some of the gum especially *Boswellia carteri* has been used to deliver Newcastle disease vaccine in microbeaded form (Fig. 1) for oral delivery in Indigenous and backyard poultry. Similarly, the *Boswellia carteri*-PPR vaccine combination administered through the intranasal route also exhibited a similar antibody titer with a subcutaneous route and hence could be an innocuous, safe, and immunogenic noninvasive alternative method than the invasive subcutaneous route commonly used for PPR vaccination (Mumin et al. 2020). Similar result was also obtained in another study using *Irvingia gabonensis*-PPR vaccine in goats (Ezeasor et al. 2020b).

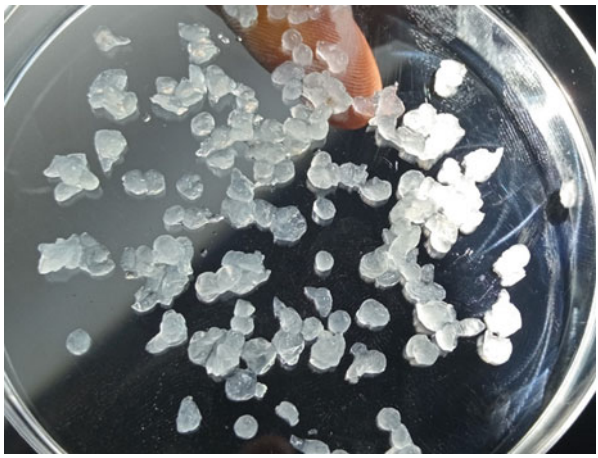


Fig. 1 Microbeads formulated from *Boswellia carteri* loaded with Newcastle disease ND vaccine

Another study evaluated the clinicopathological effects of the infectious bursal disease in broilers that were vaccinated orally and ocularly with a combination of infectious bursal disease vaccine (IBDV) and plant gums (*Khaya senegalensis* and *Cedrela odorata*) (Adeniran et al. 2020a, b). The prospects for phytogenic bioadhesives in vaccine delivery were explored by using mucilage from *Cedrela odorata* and *Khaya senegalensis* in established combination ratios of 1:1 (Emikpe et al. 2016). Their combined potential was harnessed for the optimal enhancement of the immune response to IBD virus and consequent protection against IBD virus (Adeniran et al. 2020a, b). Conclusively, this series of studies has clearly shown the importance of mucilage as adjuvant and immunomodulator; however, the mechanism for this needs further clarifications.

3 Conclusions

This chapter clearly showed that plant extracts and gum have potential as adjuvants and vaccine delivery agents. Though gums and mucilages from several plants have been evaluated for bioadhesive and phytogenic properties, especially those from *Abelmoschus esculentus, Irvingia gabonensis*, and *Boswellia carteri,* their evaluation for vaccine delivery should be the focus of research. The mucosal application of gum vaccine mixture, though comparable to the conventional subcutaneous route in some morbilliviral vaccines of small ruminant, should be explored for the eradication of PPR, a pneumo-enteritis viral infection in small ruminants. Mixtures of gum vaccine could be useful as oral and ocular delivery agents for Newcastle Disease and Infectious Bursal Disease vaccines in poultry especially Indigenous chicken in sub-Saharan Africa where poultry production is in subsistence form. This chapter revealed that gums and mucilages of plant origin, which are cheap and available in Africa, have great potential as both delivery agents and adjuvants of vaccines for the prevention and treatment of animal and poultry diseases in sub-Saharan Africa.

References

Adeniran GA, Ohore GO, Jarikre TA, Olawumi OO, Oyebanji V, Emikpe BO. Humoral and mucosal responses in challenged chickens vaccinated with infectious bursal disease vaccine using gums from *Cedrella odorata* and *Khaya senegalensis* as delivery agents. Immunoassay Immunohistochem. 2019;40(6):630–41. https://doi.org/10.1080/15321819.2019.1669640.

Adeniran GA, Jarikre TA, Ola OO, Ohore OG, Oyebanji VO, Emikpe BO. Clinicopathological assessment of infectious bursal disease vaccine using phytogenic mucoadhesive agents in challenged broilers. Acta Vet Eurasia. 2020a;46:75–80.

Adeniran GA, Emikpe BO, Jarikre TA, Ola OO, Odeniyi MA. Clinical and pathological responses of broilers to ocular vaccination using plant gum delivery and challenge with infectious bursal disease virus. Comp Clin Pathol. 2020b;29:721–7.

Ansari SH. Essential of pharmacognosy. New Delhi: Birla Publications Pvt. Ltd.; 2006. p. 2006.

Bhosale RR, Osmani RAM, Moin A. Natural gums and mucilages: a review on multifaceted excipients in pharmaceutical science and research. Int J Pharmacognosy Phytochem Res. 2014;6(4):901–12.

Calero N, Munoz J, Ramirez P, Guerrero A. Flow behaviour, linear viscoelasticity and surface properties of chitosan aqueous solutions. Food Hydrocoll. 2010;24:659–66.

Emikpe BO, Oyebanji VO, Odeniyi MA, Salaam AM, Oladele OA, Jarikre TA, Akinboade OA. *Ex-vivo* evaluation of the mucoadhesive properties of *Cedrela odorata* and *Khaya senegalensis* gums with possible applications for veterinary vaccine delivery. Springerplus. 2016;5:1289.

Ezeasor CK, Emikpe BO, Odeniyi MA, Shoyinka SVO. The mucoadhesive strengths of *Abelmoschus esculentus* and *Irvingia gabonensis* gums towards applications in veterinary vaccine delivery: ex-vivo and preliminary in-vivo evaluation. J Immunoassay Immunohistochem. 2020a;41(1):60–70. https://doi.org/10.1080/15321819.2019.1680388.

Ezeasor CK, Shoyinka SVO, Emikpe BO, Bodjo C. Intranasal Peste des petits ruminants virus vaccination of goats using *Irvingia gabonensis* gum as delivery system: hematological and humoral immune responses. 2020b; https://doi.org/10.1080/15321819.2020.1821215.

Granzotto C, Arslanoglu J, Rolando C, Tokarski C. Plant gum identification in historic artworks. Sci Rep. 2017;7:44538. https://doi.org/10.1038/srep44538.

Hu KF, Lövgren-Bengtsson K, Morein B. Immunostimulating complexes (ISCOMs) for nasal vaccination. Adv Drug Deliv Rev. 2001;51:149–59.

Jania KG, Shahb DP, Prajapatia VD, Jainb VC. Gums and mucilages: versatile excipients for pharmaceutical formulations. Asian J Pharm Sci. 2004;4(5):309–23.

Kalu VD, Odeniyi MA, Jaiyeoba KT. Matrix properties of a new plant gum in controlled drug delivery. Arch Pharm Res. 2007;30(7):884–9.

Mumin FI, Emikpe BO, Odeniyi MA. Evaluation of mucoadhesive property and the effect of *Boswellia carteri* gum on intranasal vaccination against small ruminant morbillivirus infection (PPR). J Immunoass Immunochem. 2020;41(3):311–21.

Odeniyi MA, Babalola AO, Ayorinde JO. Evaluation of Cedrela gum as a binder and bioadhesive component in ibuprofen tablet formulations. Braz J Pharm Sci. 2013;49(1):95–105.

Odeniyi MA, Khan HN, Peh KK. Release and mucoadhesion properties of diclofenac matrix tablets from natural and synthetic polymer blends. Acta Pol Pharm Drug Res. 2015;72(3):559–67.

Ofoefule SI, Chukwu A. Application of *Abelmoschus esculents* gum as a mini-matrix for furosemide and diclofenac sodium tablets. Indian J Pharm Sci. 2001;63:532–5.

Oyebanji VO, Emikpe BO, Oladele OA, Osowole OI, Salaam A, Odeniyi MA, Kasali O, Akinboade AO. Evaluation of immune response in challenged chickens vaccinated with Newcastle disease vaccine using gums from *Cedrela odorata* and *Khaya senegalensis* as delivery agents. J Immunoass Immunochem. 2017a;38(4):378–88. https://doi.org/10.1080/15321819.2016.1273237.

Oyebanji VO, Emikpe BO, Oladele OA, Osowole OI, Salaam A, Odeniyi MA, Kasali O, Akimboade AO. Clinicopathological evaluation of Newcastle disease virus vaccination using

gums from *Cedrela odorata* and *Khaya senegalensis* as delivery agents in challenged chickens. Int J Veterinary Sci Med. 2017b;5(2):135–42.

Petrovsky N, Aguilar JC. Vaccine adjuvants: current state and future trends. Immunol Cell Biol. 2004;82:488–96.

Prabhu N, Divya TR, Yamuna GK, Ayisha SS, Joseph PID. Synthesis of silver phyto nanoparticles and their antibacterial efficacy. Digest Journal of Nanomaterials and Biostructures 2010;5 (1):185–189.

Raymond CR, Paul JS, Paul JW, editors. Handbook of pharmaceutical excipients, vol. 2004. 5th ed. Washington, DC: The Pharmaceutical Press and The American Pharmaceutical Association; 2004. p. 89–92.

Saroja C, Lakshmi P, Bhaskaran S. Recent trends in vaccine delivery systems: a review. Int J Pharm Invest. 2011;1(2):64–74. http://www.jpionline.org/index.php/ijpi/article/view/39

Stephen AM, Churms SC. Introduction. In: Stephen AM, Phillips GO, Williams PA, editors. Food polysaccharides and their application. New York: Taylor and Francis, CRC Press; 2006. p. 1–24.

Thirawong N, Kennedy RA, Sriamornsask P. Viscometric study of pectin- mucin interaction and its mucoadhesive bond strength. Carbohydr Polym. 2008;71:170–9.

Bacterial Polysaccharides Versatile Medical Uses

37

Misu Moscovici and Cristina Balas

Contents

Abstract

During the last years, a lot of research results have been published concerning to new approaches to extend the use of microbial and particularly bacterial polysaccharides, in pharmaceutical and medical practice. Most of them were focused on new developments of already well-known, authorized on the health market, commercial polysaccharides (dextran, hyaluronic acid, xanthan, gellan, bacterial cellulose, levan), but regarding new derivatives and associations, having in view the progress in nanomedicine and preparative techniques. An overview of the state of the art in the main directions of research, starting from their present recognized applications, is presented, trying to notice challenges and perspectives. Efficient targeting and for controlled release drug carriers, especially of antitumor hydrophobic, low water soluble drugs, topical therapeutic agents, especially for wound healing, suitable scaffolds in tissue engineering, surgery, have been given major attention. Attempts of new producing strains and products were also mentioned. Among the challenges, the necessity of ensuring a compet-

M. Moscovici (✉) · C. Balas
National Institute for Chemical Pharmaceutical Research and Development, Bucharest, Romania

J. M. Oliveira et al. (eds.), *Polysaccharides of Microbial Origin*,
https://doi.org/10.1007/978-3-030-42215-8_48

itive ratio price/performance and full completion of the steps, especially in nonclinical (preclinical) development of an innovative/improved medicine or medical device, are highlighted.

Keywords

Bacterial polysaccharides · Pharmaceutical · Medical · Applications · New developments

Introduction During recent years, increasing attention has been further given to microbial polysaccharides in health improvement. This chapter tries to highlight the progress of knowledge in the field regarding such bacterial polymers. The information is presented according to their main categories of applications based on valuable properties in the field of health.

Bacterial polysaccharides have been used for decades in the medical field. Their biocompatible and biodegradable properties, as well as different rheology, other physicochemical properties associated to structure and molecular weight, have promoted them as valuable pharmaceutical ingredients, starting from blood plasma substitutes to thickeners, emulsifiers, stabilizers, as well as medical devices. For the already known and therapeutically approved bacterial polysaccharides, numerous studies presented modified derivatives acquiring new medical applications responding to current trends in therapy. To this field of research, the most important contributions are represented by their hydrogel forming property, as tunable matrices due to the hydrophilic structure and the numerous reactive hydroxyl groups. Meanwhile, new such biopolymers, isolated from different producing bacteria, with new properties, including useful biological activities, have been discovered.

1 New Developments of Well-Known, Commercial Bacterial Polysaccharides

There are few bacterial polysaccharides which have up to date commercial applications in therapeutics or health improvement, but they have been the subject of intense research efforts with promising results during the last years. By processing a large number of reactive groups, a huge number of derivatives with potential therapeutic applications have been prepared and studied.

1.1 Dextran

This neutral glucose homo polymer containing α-(1→6) and α-(1→4) glucopyranosyl linkages could be considered the first remarkable example for a microbial polysaccharide used in medicine, as plasma volume expander for controlling wounds shock in

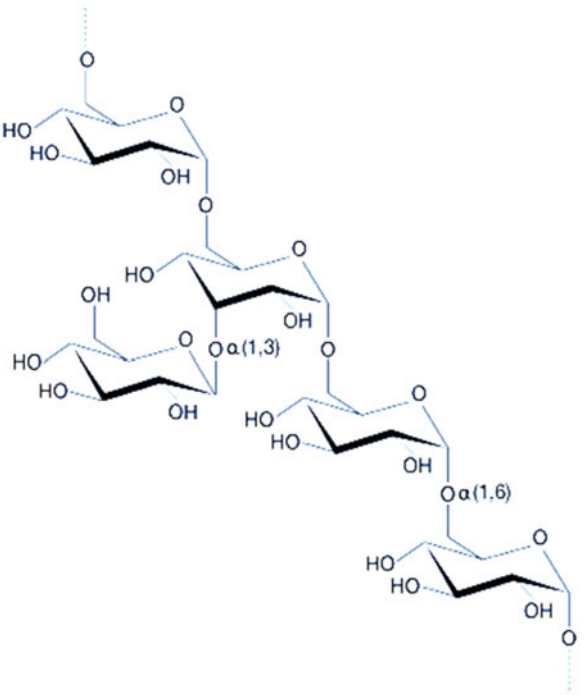


Fig. 1 Chemical structure of dextran (www.dextran.com/about-dextran/dextran-chemistry/dextran-structure)

surgery since 1953, officially acknowledged by pharmaceutical authorities (United States Pharmacopeia [USP 43]; National Formulary [NF 38]; European Phamacopoeia [EP 9] 2017). Other pharmaceutical applications are in eye-drops, due to its lubricating action (to relieve dry/irritated eyes, e.g., Visine), or as carrier and stabilizer in vaccines (www.dextran.com).

Almost linear, the degree of branching is approx. 5% in the native polymer, and the branches are mostly 1–2 glucose units long (Fig. 1).

It is produced by *Leuconostoc mesenteroides* on sucrose-containing media.

To medical applications of dextran seem to be dedicated the most publications of the late years. Some main considered ones are presented in Table 1.

1.1.1 Dextran Derivatives as Drug Carriers

It is probably the most studied field of new applications of polysaccharides. As therapeutic direction, anticancer drug delivery systems are very well represented, having in view the importance and gravity, as well as the fact that free drugs against such diseases are hydrophobic, with low absorption, short resilience (rapid excretion), and exhibit severe adverse reactions. A better targeting and sustained release, permitting their lower dosage and superior effectiveness, is an ongoing objective of the research studies.

Table 1 New developments of dextran medical applications

Application	Formulation (drug/polymer system)	Potential therapy	Reference
Drug carrier	Docetaxel/folate-dextran-polylactide-co- glycolide DT/FA-Dex-PLGA	Breast cancer	Alibolandi et al. (2016)
	Doxorubicin/carboxymethyl dextran-black hole quencer 3 Dox/CMD ex-BHQ3	Head and neck cancer	Son et al. (2018)
	PEG-ylated interferon-α-2a/Dex-tyramine PEG-IFN-α2a/Dex-Tyr	Hepatitis C	Bae et al. (2015)
	Vaccine protein antigen+adjuvant (murabutide)/acetalated dextran Antigen+adjuvant/Ace-Dex	Vaccines	Chen et al. (2018)
	Rivastigmine/dextran-polyacrylamide/ dextran-poly (vinylalcohol) (transdermal) RVS/Dex-PAA/Dex-PVA	Alzheimer	Patil et al. (2019)
	Camptothecin/2-methacryl ester hydroxyl-ethyl disulfide-dextran-poly [poly (ethyleneglycol)methyl ether methacrylate] (disulfide bond conjugated prodrug) CPT/Dex-POEGMA(DCO)	Human cervical cancer Breast cancer	Bai et al. (2018)
	Doxorubicin+campthotecin/dextran polyaldehyde-lipoic acid ester Dox+CPT/DexPA-LA	Breast cancer	Curcio et al. (2020)
In systems for topical therapeutics	Dexamethasone, indomethacin/dextran-aniline trimer-hexamethylene diisocyanate DMT,IND/Dex-AT-HDI	Inflammations	Qu et al. (2019)
	Curcumin/polyurethane-dextran Cur/PU-Dex	Infected wounds	Sagitha et al. (2019)
	Quaternary ammonium salts/dextran-siloran-polyurethane QAS/Dex-SI-PU	Infected wounds	Gharibi et al. (2019)
	Quaternized chitosan-polyaniline-dextran polyaldehyde QCh-PAN-DexPA	Infected tissue regeneration	Zhao et al. (2015)
	Dextran-β-tricalciumphosphate Dex-βTCP	Bone regeneration	Ghaffari et al. (2020)
	Polylactic acid-dextran PLA-Dex	Cardiac tissue engineering	Zhang et al. (2017)
	Poly(3-hydroxybutyrate-co-3-hydroxy-valerate)-dextran PHBV-Dex	Bone repair	Zou et al. (2016)

(continued)

Table 1 (continued)

Application	Formulation (drug/polymer system)	Potential therapy	Reference
Gene and cell delivery	hSET1 antisense/thiolated carboxymethyl dextran-chitosan hSET1anti-TCMD-Ch	Colon cancer	Kiani et al. (2016)
	miRNA(miR-145)-thiolated carboxymethyl dextran-aptamer AS1411/polyelectrolyte complex-deacetylated chitosan miR-145+AS1411/TCD-PEC-Ch	Breast cancer	Tekie et al. (2018)
	Dextran-dimethyl/diethyl/ aminoacrylate ethyl methacrylate-pEGFP-C1plasmid + (DNA)/siRNA (dextran based DNA, dextran based siRNA coacervates) p-EGFP-C1(DNA)/DexPDAA/Dex-PDEAEMA-siRNA Dex –DC, Dex-RC	Ovarian cancer	Wang et al. (2020)
	Arginylglycylaspartic acid (RGDpeptide)/ dextran RGD-Dex	Epithelial tissue engineering	Riahi et al. (2017)
	Chitosan-dextran-β-glycero-phosphate Ch-Dex-βGP	Cardiac tissue repair	Ke et al. (2020)
Other medical applications	**Dextran antimicrobial** Gentamicin/dextran (new nature isolated *Leuconostoc mesenteroides* strains)	Multidrug-resistant urinary infections	Salman et al. (2018)
	Dextran from a nature isolated *Leuconostoc pseudomesenteroides* strain	Infections with *S. aureus* and *E. coli*	Ye et al. (2019)
	Immunomodulatory Dextran from milk isolated *Leuconostoc mesenteroides* strains	Inflammations	Zarour et al. (2017)

One of the main approaches is the encapsulation of the drug in amphiphilic polymer particles, obtained by grafting of hydrophobic segments onto the hydrophilic polymeric backbone, forming self-associated thermodynamically stable nanogel structures, with an inner hydrophobic core. An interesting example is represented by polymersomes (a synthetic alternative approach of natural liposomes), consisting of bi-layer vesicles of amphiphilic block-copolymers with an aqueous core. Such drug carriers were prepared by conjugation of dextran with poly(lactic-co-glycolic acid) (PLGA). A folate group was attached to the conjugated Dex-PLGA polymer as cell-receptor ligand. The poor water-soluble anticancer drug docetaxel was encapsulated in the bilayer. The antitumor effect was determined on human and murine breast cancer cells MCF-7 and AT1, respectively, overexpressing folate receptors. *In vitro*

tests, confirmed by *in vivo* treatment of tumor-bearing mice, showed a higher cellular uptake and cytotoxicity of the loaded conjugate (FA-Dex-PLGA-DTX), compared to free drug formulation and nonfolate conjugate, corresponding to tumor-targeting capability, as well as a sustained drug-release reducing tumor growth and increasing survival rates of animals (Alibolandi et al. 2016).

The low oxygen concentration under the oxygen demand (hypoxia) of tumor microenvironment, accompanied by a reduction potential, particularly by over-expression of nitro- and azoreductases, was exploited by a hypoxia-responsive carboxymethyldextran (CMD), chemically modified (amide conjugate) with BHQ3 (black hole quencer 3), containing an azo bond (-NH=NH-) as hypoxia-sensitive moiety. The hydrophobic, poorly soluble anticancer doxorubicin was entrapped with a high loading capacity (71%) into the amphiphilic nanoparticles (DOX@CMD-BHQ3). The drug release was determined under hypoxic conditions (nitrogen, NADPH), noting a rapid release due to the bioreduction of azo bond, compared to normoxic (Fig. 2). The *in vitro* cytotoxicity and intracellular release were investigated in mouse head and neck carcinoma SCCT cells. Both were increased under hypoxic conditions.

In vivo systemic administration into SCCT tumor-bearing mice showed high selective tumor accumulation, compared to the other tissues (Son et al. 2018).

Protein incorporation in dextran derivatives was another direction of application for drug-delivery systems. The efficacy of protein drugs could be diminished by their proteinase-sensitivity leading to a biological short half-live and renal elimination. Covalent attachment to polyethylene glycol (PEGylation) is a way to avoid such inconveniences. An example is the PEGylated interferon-α2a (PEG-IFN- α2a), with a prolonged half-life than the free drug, which is used in hepatitis C therapy. However, frequent injections are necessary, increasing patient discomfort and the risk of adverse reactions. Considering the excellent biocompatibility and nonimmunogenicity and its ability to form aqueous two-phase system (ATPS) with PEG, a microstructured dextran derivative system was prepared aiming to a sustained release of PEG-IFN-α2a. Thus, a dextran-tyramine conjugate crosslinked by catalytic oxidative coupling of tyramine moieties adding horseradish peroxidase and H_2O_2, incorporated PEG-IFN-α2a as droplets in a continuous gel phase (Fig. 3). The drug release could occur by the gradually degradation of the gel, due to slow hydrolysis of carbamate (-O-CO-N-) bonds between dextran and tyramine. The dextran backbone could be further degraded by α-1-glucosidases present in various human organs and tissues. The system exhibited a drug sustained release for 3 months.

The *in vitro* antiviral activity was determined on Huv-7 (human hepatoma) cells carrying hepatitis C virus (HCV) replicon. The retained activity after encapsulation depended on the microgel particles size, a 3 μm size proved to retain approx. 95% after 2 weeks and was chosen for *in vivo* experiments. *In vivo* tests were performed on humanized (human immune system) mice and showed a prolonged blood circulation of PEG-IFN-α2a after one administration of drug-loaded hydrogel (2 weeks), compared to free PEG-IFN-α2a, the plasma half-live increased from 1.5 to 15.6 days. After 8 weeks of treatment, the therapeutic effect of one administration of drug-loaded hydrogel was the same of weekly injections, proving the sustained release and corresponding efficacy (Bae et al. 2015).

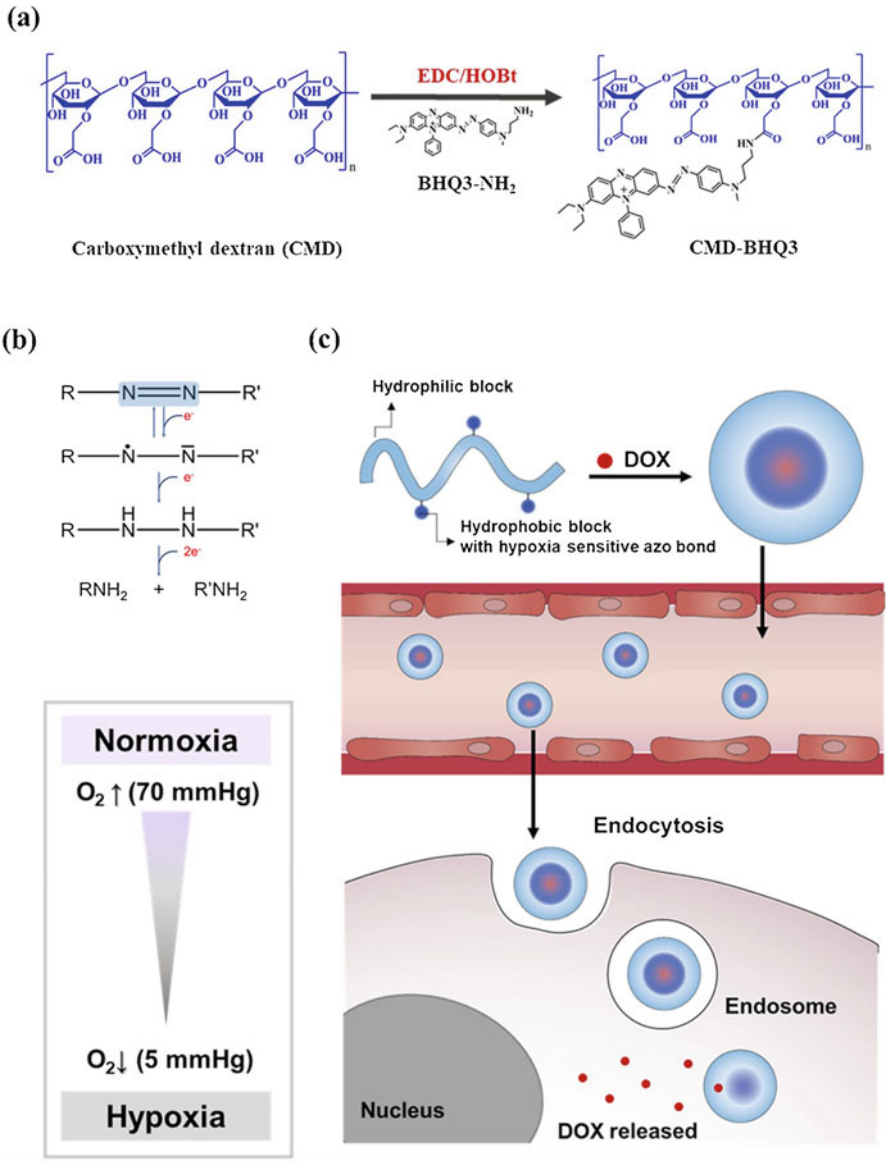


Fig. 2 Synthetic scheme of the doxorubicin-loaded hypoxia sensitive amphiphilic conjugate, tumor accumulation, and drug release (Elsevier permission, from Son et al. 2018)

Dextran-derivative microparticles with tunable degradation properties were investigated on possible controlled antigen and adjuvant release as vaccine ingredients, important for optimal immune response and safety. Acetalated dextran (Ace-Dex) with various degrees was prepared by reaction with 2-ethoxypropene.

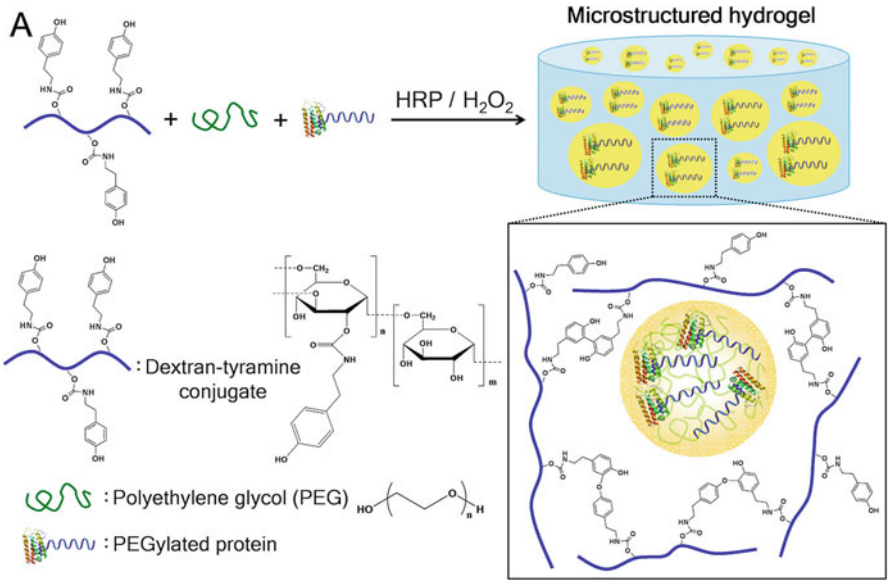


Fig. 3 Schematic preparation of microstructured hydrogels for sustained release of PEGylated proteins (Elsevier permission, from Bae et al. 2015)

Microparticles were obtained by electro-spraying. The loading substances (murabutide), a vaccine adjuvant, and ovalbumin, as model protein antigen, were encapsulated in the hydrogel during the same electrospray process. The degradation behavior of the polymer was studied at a physiological environment pH (7.4) and at pH 6.5, resembling the acidic conditions within lysosomes of phagocytic cells, the vaccine target (pH 6.5). The polymer showed an acidic sensitivity, depending on the proportion of cyclic acetals, more stable, and on the electrospray procedure, favoring polymer stability.

Protein release was faster for lower cyclic acetals, so the polymer could be a platform of immunization process control. The intracellular internalization and transport of antigen were revealed in murine bone marrow-derived dendritic cells (BMDCs). After an *in vivo* study of 42 h on mice, different kinetics responses were noted for encapsulated adjuvant and antigen, but permitting, by polymer cyclic acetal degree, a control of adjuvant and antigen release, enhancing the safety and effectiveness of both vaccine subunits (Chen et al. 2018).

An electro-responsive transdermal delivery system was prepared consisting of a hydrogel reservoir containing polyacrylamide-grafted dextran linked with glutaraldehyde, with encapsulated rivastigmine, a cholinesterase inhibitor in Alzheimer disease treatment, and rate controlling membranes composed of cross-linked dextran-poly (vinyl) alcohol films. The drug permeation release, which was determined *in vitro* on rats' skin excised samples, reached only 66% in the absence of electric current, decreasing with the crosslinking density of the hydrogel (higher content of glutaraldehyde), but increased with direct current application of 2–8 mA at every 30 min

intervals, to 97.5%. The electric responsiveness of the hydrogel is attributed to carbonyl groups resulted from partial hydrolysis of the amide ones (Patil et al. 2019).

The advantage of transdermal delivery is the direct systemic transfer of the drug, avoiding the higher doses requested by oral administration, with their associated side effects. Regarding the above presented transdermal system, its distinctive feature could be considered the drug delivery on demand, not only for rivastigmine. Concerning this drug, a commercial therapeutic transdermal medicine exists (Exelon 9.5 mg/24h transdermal patch of Novartis), formulated with poly (alkyl) methacrylates and polyester (www.medicines.org.uk/eme/product/7764).

The effect of combining two anticancer low water-soluble and bioavailable drugs (doxorubicin and camptothecin) by an oxidized dextran-lipoic acid prodrug formation and encapsulation in the self-assembled vesicles of the prodrug, respectively, was investigated. Dextran was oxidized to polyaldehyde and its non-oxidized hydroxyl groups were esterified by carboxylic group of lipoic acid, a natural antioxidant produced by the human body, which contains a disulfide bond. Then, doxorubicin was covalently linked to the dextran polyaldehyde group by a Schiff-base formation (imine bond CH=N- between the amine group of the drug and the aldehyde group of the polymer). The second anticancer drug (camptothecin) was entrapped in the prodrug obtained vesicles. The system was two stimuli release responsive: pH 5.0, specific to the cancer cell environment (favoring the hydrolysis of doxorubicin imino-bond) and redox, the presence of added glutathione enhancing the hydrolysis and reducing the disulfide bonds of lipoic acid residues.

Thus, the destabilization of the vesicle structure allowed the selective intracellular release of the drugs. Comparative results on breast cancer MCF-7 cells and healthy MCF-10A cells showed a better internalization of the loaded drugs than free drugs in the cancer cells and enhanced toxicity on cancer cells, compared to the combination of free drugs. The selectivity was also higher, proved by increased healthy cells viability (Curcio et al. 2020).

1.1.2 Dextran Derivatives in Systems for Topical Therapeutics

An electro-responsive delivery system was prepared from dextran cross-linked with electroactive aniline trimer (obtained by condensation of two aniline molecules and one of p-phenilenediamine, resulting N= intermolecular bonds) and hexamethylene diisocyanate, forming polyuretane groups (NH-CO-O-) with dextran hydroxyl and free aminogroups of aniline trimer (Dex-AT/HDI). The hydrogel exhibited good conductivity and corresponding electro-responsive properties. Different such hydrogels (with aniline trimer content of 0–11%, corresponding to pore size 5–15 μm) were loaded with anti-inflammatory dexamethasone and indomethacin. *In vitro* drug release at pH 7.4 was as expected, increasing with pore size and reaching for dexamethasone 77% after 180 min. Electric-driver release was determined applying different voltages (3,1,0 V) to the 5μm hydrogel, leading to significant improvement of cumulative dexamethasone release, reaching 90% after 120 min.

In vitro cell compatibility on L 929 fibroblast cells showed a good cell viability, even cell proliferation for 10 μm hydrogel, confirmed by a desirable biocompatibility

during *in vivo* (rats) tests. Thus, such hydrogels were considered as smart drug carriers for localized sustained release (Qu et al. 2019).

pH responsive curcumin-loaded polyurethane-dextran nanofibrous membranes were considered as potential wound dressing materials. Membrane composites of polyurethane/dextran with different concentration of dextran (5–25%) aiming to increase hydrophilicity, sorption capacity, hemostatic potential, mechanical reinforcement, and biodegradability were prepared by electrospinning. Encapsulating curcumin, for a synergistic antibacterial activity, such composites showed the best results of blood clotting, wettability, biocompatibility (max. viability of 3T3 mouse fibroblasts), and biodegradability for 25% dextran. The drug releasing performance was noticed at pH 5.8, compared with pH 1.2 and pH 7.4 (explained by poor solubility at acidic and neutral pH, but a complete protonation of dextran hydroxyl groups and interaction with β-diketone of curcumin). The *in vitro* antibacterial activity against *S. aureu*s was exhibited by more than 20% dextran containing membranes (Sagitha et al. 2019).

The hemolytic activity of antimicrobial quaternary ammonium salts was reduced by grafting dextran on a siloran-quaternary ammonium functionalized polyurethane dressing membrane, permitting to obtain a nonhemolytic dressing membrane with a preserved very good antimicrobial activity against meticillin-resistant *S. aureus, Ps. aeruginosa*, and *Candida albicans* (Gharibi et al. 2019).

Dextran polymers were also applied as tissue scaffolds, contributing to build new functional 3D tissues, despite the high water solubility of the polymer.

Electroactive antibacterial injectable hydrogels were prepared from chitosan grafted with polyaniline, crosslinked with oxidized dextran (Schiff-base). The presence of polyaniline in the copolymer, as electroconductive component, favored the proliferation of C2C12 mouse skeletal muscle cells and enhanced the antibacterial activity, assayed against *E. coli* and *S. aureus*. *In vivo* subcutaneous injection (rat) of the hydrogel encapsulating bacteria showed a gel formation and an antibacterial activity confirming the *in vitro* study results (Zhao et al. 2015).

A composite of dextran and crystalline β-tricalcium phosphate (β-TCP) nanoparticles was prepared by incorporation of TCP in the hydrogel during crosslinking with epichlorhydrin. The highest porosity was obtained with 5% wt TCP. *In vitro* biomineralization by formation of hydroxyapatite in simulated body fluid (SBF) and cellular proliferation, assayed with 3T3 fibroblasts, were observed, thus considering a promising scaffold in bone regeneration (Ghaffari et al. 2020).

A similar dextran functionalization to methacrylate derivative was used to UV photo-crosslinking *in situ* of poly (lactic acid) (PLA) grafted with dextran through maleic anhydride, obtaining nanofibers by co-axial electrospinning. Variable dextran compositions of PLA-dextran nanofibers scaffold produced different cell attachment, proliferation, and differentiation of mouse V1 embryonic stem cells to cardiac muscle ones (Zhang et al. 2017).

An interesting application was covalently surface grafting dextran onto polyhydroxyalkanoate: [poly(3-hydroxybutyrate-co-3-hydroxyvalerate)] (PHBV) electrospun fibers scaffold methacrylic acid functionalized. This hydrophilic modification significantly enhanced the proliferation of bone marrow-derived mesenchymal stem cells (BMSCs) (Zou et al. 2016).

1.1.3 Dextran Derivatives in Gene and Cell Delivery

Gene therapy, introducing new genes into the cells, represents a new hopeful way in therapy, especially in cancer, when their introduction into a tumor cell or its surrounding tissue aims to cause cell death or its growth rate reduction.

Nanoparticles are considered possible efficient carriers.

Polyelectrolyte complexes of carboxymethyl dextran, thiolated carboxymethyl dextran, by conjugation with cysteine (amide formation) and chitosan nanoparticles, were prepared; loading the antisense of hSET1 enzyme, overexpressed in malignant cells, could determine their regression, without affecting normal ones. After a stability study in serum, simulated gastric and intestinal fluids, a mucoadhesive study, the specific biological activity was assayed by cell toxicity on SW480 colon cancer cells, cellular uptake, and expression of hSET1 (gene silencing). The best results were obtained with high degree thiolated carboxymethyl dextran (Kiani et al. 2016).

A similar role to thiolated carboxymethyl dextran and chitosan polyelectrolyte complex (TCD/Ch) nanoparticles was given to carry miR-145, a tumor suppressive miRNA, whose presence is abnormally reduced in some cancers. That gene silencing agent was accompanied by antinucleolin aptamer AS1411, a guanine-rich oligodeoxy nucleotide aptamer, potential inducer of apoptosis, which binds to nucleolin, overexpressed on the surface of certain cancer cells, a nucleolar protein involved in antiapoptosis. The components of the polysaccharide complex were synthesized as above (conjugation of carboxymethyldextran with L-cysteine and depolymerization of fully de-acethylated chitosan). MiR-145 was firstly conjugated with TCD and the aptamer was finally added to the polyelectrolytic complex. The system was redox-responsive, the disulfide bonds being susceptible to be broken by reductive action of the high cytoplasmatic concentration of glutathione. Contrary to naked miR-145, the system proved to be gene protective against RNase. *In vitro* studies on human breast cancer MCF-7 cells showed that miR-145 concentration significantly increased in the treated cells (RT-PCR), the cell proliferation was reduced, and the apoptosis was considerably increased. This double targeted gene delivery system was considered helpful in breast cancer therapy (Tekie et al. 2018).

A dextran-based system aimed to solve the problem of cationic polymers toxicity, an obstacle in their efficient use to transport negative charged genes. Thus, a dextran graft copolymer was obtained introducing cationic monomers (dimethyl/diethyl/amino acrylate/methacrylate) in a dextran solution, a polymerization initiator, and a disulfide bond containing monomer (dialyl disulfide) as crosslinker. Dextran-based coacervates were prepared adding pEGFP-C1 plasmid (DNA) and a small interfering RNA (siRNA), respectively. The coacervates were formed by electrostatic interaction between amino cationic groups and the anionic genes. *In vitro* experiments were performed on human embryonic kidney cells 293T and ovarian cancer cells OVCAR-3. After endocytosis, the gene release was due to disulfide bond cleavage by glutathione reduction, as above. The efficient transfection was proved by protein expression (green fluorescent protein, GFP) of pEGFP-C1 into 293T cells and detection of fluorescent labeled si-RNA in the cancer cells (Wang et al. 2020).

Cell delivery could be considered a first step of tissue engineering. A method to graft the RGD peptide (arginylglycylaspartic acid) to dextran through a divinyl

sulfone linker to obtain a cell delivery system to achieve a porous, three dimensional matrix, was developed. A dextran conjugate with divinyl sulfone was firstly obtained, by covalent binding of one vinyl group to dextran C2 glucopyranosyl unit hydroxyl (-SO_2-CH_2-CH_2-O-). The free vinyl sulfone reacted with a free amino group of RGD peptide by a Michael type addition (-NH-CH_2-SO_2-). The peptide dextran derivative was crosslinked with sodium trimetaphosphate. RGD, known as cell adhesion agent, supported the attachment, proliferation, and migration into the hydrogel scaffold of the human ombilical vein endothelial cells (HUVEC). Optimal conditions, especially regarding RGD content, were determined (Riahi et al. 2017).

A thermosensitive hydrogel, capable of controlled gelation at 37°C, and therefore considered injectable, was prepared from chitosan, dextran, and β-glycero-phosphate. The biodegradability tests at pH 7.4 showed a 70–80% degradation after 28 days, more pronounced in enzymatic conditions (lysozyme). With an excellent biocompatibility (3T3 and HUVEC cells), the hydrogel exhibited a linear cumulative cell delivery during 7 days (HUVEC), effective proliferation cell viability after injection by dual syringe applicator, and genetic differentiation (umbilical cord mesenchymal stem cells UCMSC). The hydrogel was considered a promising vehicle for injectable cell therapy of cardiac repair after myocardial infarction (Ke et al. 2020).

1.1.4 Dextran in Other Medical Applications

Dextran antimicrobial Dextran-type biopolymers produced by nature isolated *Leuconostoc mesenteriodes* strains exhibited antibacterial activities as their own property: one of them, single or blended with gentamycin, was active against a biofilm of multidrug-resistant microorganisms on urinary catheters (Salman et al. 2018) and another, produced by a *Leuconostoc pseudomesenteroides* isolated from mango juice, presented against *S. aureus* and *E. coli* MIC of 3 mg/ml and 2 mg/ml, respectively (Ye et al. 2019).

Immunomodulatory Dextrans produced by *Lc. mesenteroides* strains isolated from human milk, *Lc. mesenteriodes* and *Lactobacillus sakei* isolated from meat products, exhibited immunomodulatory activity changing the ratio TNF-α/IL-10 in human macrophages costimulated with *E. coli* lipopolysaccharides, corresponding to an anti-inflammatory effect (Zarour et al. 2017).

1.2 Hyaluronic Acid

It was discovered in 1934 and its chemical structure was established in the 1950s as anionic linear glucosaminoglycan composed of disaccharide units of (1→4)-β-D-glucuronic acid and (1→3)-β-N-acetyl-D-glucosamine. Physiologically present in almost all mammalian tissues, including human body, hyaluronic acid entered easily between the officially approved pharmaceutical ingredients: as sodium salt (hyaluronan), it has a monograph in the European Pharmacopoeia (EP9, 2017) (Fig. 4).

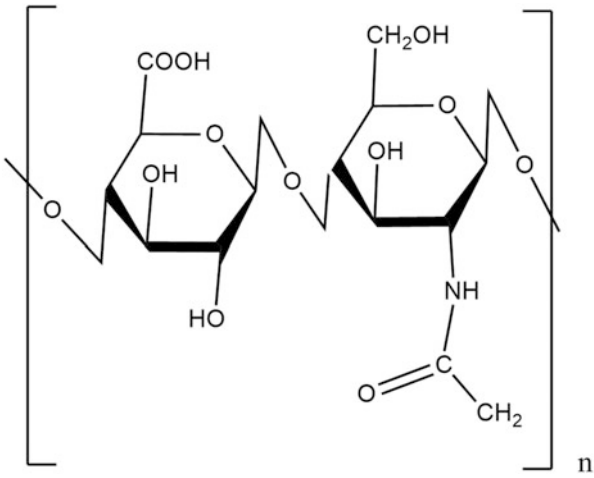


Fig. 4 Chemical structure of hyaluronic acid

Its first and remained medical application was considered a vitreous substitution/replacement during eye surgery in the late 1950s. Originally extracted from animal tissues, especially rooster combs, it is now produced by recombinant bacteria. The bacterial biopolymer was firstly approved only for topical applications: chronic, difficult wound healing, for example, Hyiodine, Sorelex (Contipro), and cosmetics. Lately, medical devices with bacterial hyaluronan were approved for eye surgery and intra-articular injections in osteoarthrities, for example, BioLon (Altacor-BTG), approved EU in 1995 (EC), USA-FDA 1998, EUFLEXXA (Ferring Pharmaceuticals, Savient Pharmaceuticals BTG), EC2004, FDA 2011, DUROLANE (Bioventus), EC 2001, FDA 2017. A sustained release injectable formulation of somatropin (recombinant human growth hormone), SP-hGH-Declage (L G Life Sciences), using sodium hyaluronate microparticles, has been approved by Korean FDA since 2008 and is now in extended clinical trials as LGO3002 (Eutropin Plus inj.) (Hwang et al. 2018). The first formulation approved by FDA for treatment of vesicoureteral reflux was a stabilized dextran/hyaluronic acid composite (NASHA/Dx) and gained European approval for the treatment of stress urinary incontinence (Huerta-Ángeles et al. 2018; Elzayat and Corcos 2008).

Another cited commercial hydrogel wound dressing containing hyaluronic acid promoting autolytic debridement was Restore Hydrogel produced by Hollister Inc. (Aswathy et al. 2020). New published results are presented in Table 2.

1.2.1 Hyaluronic Acid in Wound Healing

Hydrogels are considered the most promising material for wound dressing, providing a moist environment, the removal of exudates, infection prevention, and suitable environment for tissue regeneration. The healing process includes hemostasis, reducing inflammation, cell proliferation, and tissue remodeling (Aswathy et al. 2020). The microbial hydrogels should compete with synthetic ones.

Table 2 New developments of hyaluronic acid medical applications

Application	Formulation	Potential therapy	Reference
Wound healing	Hyaluronan alginate	Wound closure	Catanzano et al. (2015)
	Gentamycin, vancomycin/ hyaluronan-D,L-lactide HA-PLA (Defensive Antibacterial Coating (DAC)), antiseptic	Coating for implantable biomaterials (orthopedics, traumatology, dentistry, maxillo-facial surgery)	Gaetano et al. (2018)
	Antiseptic+β-lactam antibiotic/hyaluronic acid-cellulose in ionic liquid GUMBOS/HA-CEL	Patches for (infected) wound care	Lopez et al. (2020)
Drug and gene therapy	SN38 Active metabolite of irinotecan(CPT-11)/-hyaluronic acid bioconjugate prodrug ONCOFID-S	Ovarian cancer	Montagner et al. (2015)
	miRNA/hyaluronan/ cholanic acid/Zn-dipicolylamine miRNA/hyaluronic acid/ chitosan miRNA/hyaluronic acid/ protamine sulfate miRNA/hyaluronic acid/ dipalmitoyl phosphatidylcholine-PLGA-pluronic F127	Gene silencing in colon, breast, prostate cancer	Fernandez-Piñeiro et al. (2017)
Tissue engineering	Hyaluronic acid methacrylated-hyaluronic acid nanoparticles (paste-like) *in vivo* photo crosslinked	Bone implants	Beck et al. (2015)
	Cytomodulin-2-*in vivo* crosslinked hyaluronic acid Cx-HA-CM (injectable)	Cartilage-like tissues	Park et al. (2019)
	Thiolated adamantane-hyaluronic acid-methacrylated β-cyclodextrin-hyaluronic acid Dual crosslinked (second *in vivo*: Michael addition) (injectable)	Soft tissue reconstruction (nucleus pulposus, myocardial after infarct)	Rodell et al. (2015)

The presence of hyaluronan through cross-linking with alginate promoted wound closure *in vivo* (rats) (Catanzano et al. 2015).

A hyaluronic-based antibacterial hydrogel coating for implantable biomaterials in orthopedics, traumatology, dentistry, maxillofacial surgery was developed through a

EU-FP7 project. It consisted of hyaluronan grafted with hydrophobic poly-D, L-lactide (HLA-g-PLA). The hydrogel formed a uniform, resistant coating, entrapping antibiotics, as antiadhesive antimicrobial film on the prosthetic materials. Antibiotic (gentamicin/vancomycin)-loaded hydrogel reduced by 75–80% biofilm formation with *S. epidermidis* and *S. aureus*. Drug release started after the implant, providing a concentration higher than MIC, ensuring the effectiveness. The product was administered as prefilled syringe of sterile powder, filled at surgery with sterile water and antibiotic. After preclinical studies (rabbits), with 72–99% bacterial load reduction, without side effects, the product, developed by the Italian company Novagenit, with the commercial name DAC (Defensive Antibacterial Coating) was clinically studied on two groups of patients (380 and 256) with good results (Gaetano et al. 2018).

The wound healing process is considered containing three main events, every one involving hyaluronic acid (HA): a matrix rich in HA laid down in a cell-depleted space; stimulated mesenchymal cell migration from adjacent tissues which infiltrate the HA matrix; cells within the HA matrix secrete hyaluronidase which degrades the HA, sulfated glucosaminoglycans, and collagen which replace the HA and the tissue is remodeled (Gupta et al. 2019).

Composites of hyaluronic acid and cellulose were prepared dissolving the biopolymers in an ionic liquid (green technology, full solvent recovery), aiming at exploiting favorable biomedical properties of both biopolymers in patches for wound care (Fig. 5). Antibacterial ionic synergic combinations of antiseptic, beta-lactam antibiotic, composed of chlorhexidine-oxacillin/cephalothin, known as GUMBOS, active against multidrug-resistant bacteria, were loaded. *In vitro* burst drug release, *S. aureus* inhibition zones, and high swelling capacities (to efficiently absorbing exudates) were presented as promising for wound healing (Lopez et al. 2020).

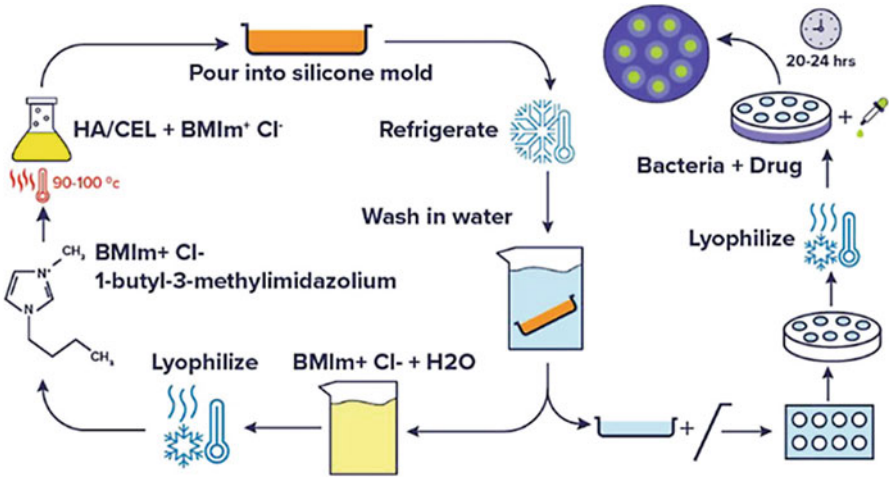


Fig. 5 Scheme of preparing hyaluronic acid-cellulose composites in an ionic liquid (green technology) as patches for loading antibacterial agents in wound care (ACS permission for Lopez et al. 2020; further permission related to the material excerpted should be directed to the ACS).

1.2.2 Hyaluronic Acid in Drug and Gene Therapy

A bioconjugate of SN-38, the active metabolite of anticancer pro-drug irinotecan (CPT-11) and hyaluronan, named ONCOFID-S, was obtained and studied as anticancer agent *in vitro* and *in vivo* by local-intraperitoneal administration in ovarian cancer. A selective internalization in tumor cells was proved *in vitro* through the binding to the CD44 receptor of three ovarian tumor cell lines: 1-GROV-1, OVCAR-3, and SKOV-3. The *in vivo* experiments on tumor bearing mice with the irinotecan conjugate showed a strong antitumor activity vs. controls and even an increased therapeutic effect and survival, compared to irinotecan, for 1-GROV-1 bearing animals (Montagner et al. 2015).

Four systems containing hyaluronic acid were cited as miRNA nanocarriers for targeted delivery to gene silencing in cancer therapy which passed *in vivo* studies: hyaluronan/cholanic acid/Zn dipicolylamine, hyaluronic acid/chitosan, hyaluronic acid/protamine sulfate, hyaluronic acid/dipalmitoyl phosphatidylcholine-PLGA-pluronic F127 for different cancer types: colon, breast, and prostate. Administered intravenously, they ensured a selective delivery, permitting to reduce doses and unwanted effects (Fernandez-Piñeiro et al. 2017).

1.2.3 Hyaluronic Derivatives in Tissue Engineering

The capacity of hyaluronic acid-based colloidal gels to fully recover after compression was exploited aiming at applications including cartilage regeneration.

Cytomodulin-2 (CM), a chondrogenic differentiation peptide factor, was chemically immobilized in hyaluronic acid (HA), crosslinked (Cx) by click reaction with tetrazine and transcyclooctene, separately and mixed using a double barrel syringe: Cx-HA+CM→Cx-HA-CM (Fig. 6). This injectable formulation was tested *in vitro* and *in vivo* (mice) for enhancing chondrogenic differentiation of human periodontal ligament stem cells (hPLSC). The chemical attachment of CM resulted in long time persistence (28 days after injection), favoring a significant expression and induction of the formation of cartilage-like tissues. This injectable system was considered suitable for repair of damaged articular cartilage (Park et al. 2019).

A supramolecular injectable assembly of two guest-host, HA derivatives (HA-adamantane and HA-β-cyclodextrin), was developed at the aim to obtain firstly an ex vivo crosslinking with shear thinning delivery as liquid with high retention at the target site, followed by a secondary covalent crosslinking in situ, time-controlled, clinically required, to enforce and stabilize the hydrogel network. The covalent bonds were created by Michael addition reaction with slow kinetics between thiol and methacrylate groups previously provided at the HA before modifications. *In vivo* experiments on rats with myocardial infarct showed stabilization, progressive remodeling, and trend toward increased contractile function. The results were thought potentially useful for soft tissue reconstruction, for example, nucleus pulposus replacement, treatment of myocardial infarct (Rodell et al. 2015).

Comprehensive reviews regarding a very wide range of possible chemical modifications of HA and potential product applications including tissue engineering were published (Khunmanee et al. 2017; Highley et al. 2016).

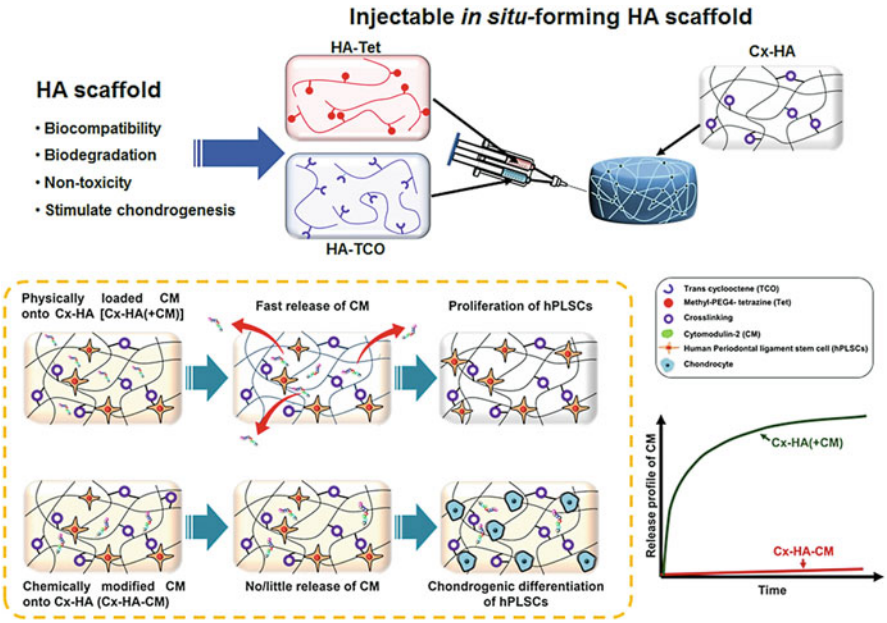


Fig. 6 Scheme showing injection of a formulation of human periodontal ligament stem cells (hPLSC)-loaded hyaluronic acid-tetrazine-cytomodulin (HA-Tet-CM) and hPLSC-loaded hyaluronic acid-transcyclooctene-cytomodulin (HA-TCO-CM) for chondrogenic differentiation in an *in vivo* formed crosslinked-hyaluronic acid-cytomodulin (Cx-HA-CM) (permission confirmed by Copyright Clearance Center, from Park et al. 2019)

1.3 Xanthan

Discovered in 1950, it is an anionic branched heteropolysaccharide with a backbone of (1→4)β-D-glucose disaccharide units with alternate branches consisting of three other hexose units: glucuronic acid between two mannose units, the whole monomer being a pentasaccharide. The terminal mannose can have a pyruvate group attached to C_5–C_6 and the mannose linked to the backbone glucose may have an acetyl group to C_6. The ratio pyruvate/acetate depends on fermentation conditions (Fig. 7).

Xanthan is produced in aerobic fermentation of *Xanthomonas campestris*. With high molecular weights (more than 1000 kDa) and viscosities, it was firstly used for enhanced oil recovery and later on in food industry, as a thickener and suspension stabilizer. Approved as food additive in the USA (1969), by FAO in 1974, in Europe in 1982, it was subsequently included in the USA and European Pharmacopeias (USP 43-NF38, EP9, 2017), as pharmaceutical ingredient.

1.3.1 Xanthan as Drug Carrier

Polyelectrolyte complexes of chitosan and xanthan were used for coating rifampicin-loaded liposomes (chitosomes). The microparticle vesicles aimed to lung delivery by

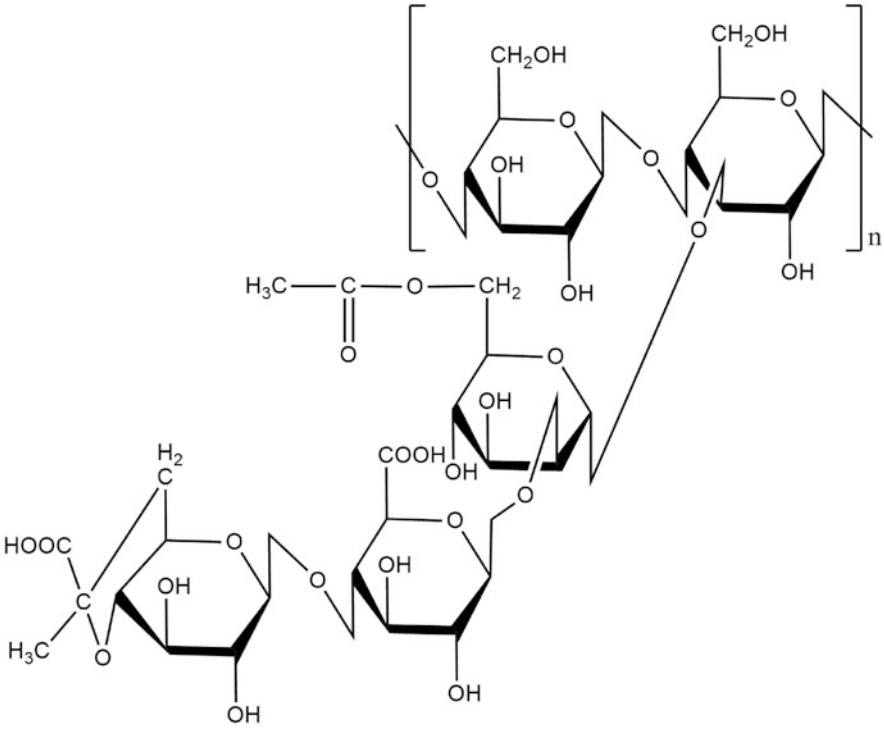


Fig. 7 Chemical structure of xanthan

nebulization. A chitosan-xanthan ratio of 1:0.5 was found the best to improve the drug total mass output and deposition (Manca et al. 2012).

To improve its low mechanical stability and excessive swelling, which limited xanthan application in drug delivery and tissue engineering, the polysaccharide was blended with silk fibroin to prepare a robust and with controlled porosity drug delivery scaffold. The chloramphenicol antibiotic was loaded as model drug and *in vitro* drug release (phosphate buffer pH 7.4, 37°C) kinetics was studied and optimized by response surface methodology (RSM) and artificial neural network modeling (ANN), using cumulative percentage release of antibiotic. The predictive model with blend combination, porosity, and swelling as variables was verified. The ANN was found as better predicting than RSM (Shera et al. 2018).

1.3.2 Xanthan in Tissue Engineering and Wound Healing

Magnetically responsive nanoparticles of xanthan-chitosan polyelectrolyte complex hydrogels for tissue engineering were prepared, incorporating iron oxide magnetic nanoparticles. Deacetylated chitosan was added to a xanthan solution containing D-(+)-glucuronic acid δ-lactone as acidifying agent with dispersed Fe_3O_4 nanoparticles, and an electrostatic complex was formed followed by hydrogen-bonding

interactions. Cell adhesion and proliferation of NIH3T3 fibroblasts were favored under magnetic field acting on the incorporated magnetic nanoparticles. Such tunable magnetic hydrogels were considered suitable for skin, cartilage, muscle, and connective tissue engineering (Rao et al. 2018).

Xanthan/konjac glucomannan blend hydrogels were obtained. Konjac glucomannan is a polysaccharide extracted from the tubers of *Amorphophallus konjac* plant. Xanthan presents in aqueous solutions at low temperature an ordered double helical strand structure which at about 40–50°C forms a three dimensional network gel-like. This property and a mechanical strength effect on konjac glucomannan were exploited in the blend, obtained by autoclaving the polymeric solutions at 121°C and decreasing the temperature to 37°C, when the sol-gel transition forms a gel-like morphology, permitting a direct application at the wound site, acquiring its shape and size. The biocompatibility, cell adhesion and infiltration, as well as cell migration in a wound healing scratch assay were proved on cultures of human dermal fibroblast (NHDF) cells (Alves et al. 2020).

1.4 Gellan

Discovered in 1978, gellan is a linear anionic heteropolysaccharide, with a tetrasaccharide repeating unit of 1,3-β-D-glucose, 1,4-β-D-glucuronic acid, 1,4-β-D-glucose, and 1,4-α-L-rhamnose. The native form contains glyceryl and acetyl groups (Fig. 8).

Gellan is produced by fermentation of *Sphingomonas paucimobilis*, also referred to as *Sphingomonas elodea*. It is produced and marketed under different trade names: Gelrite™, Kelcogell™, Phytagel, Gelzan, Gelrite, and Applied gel.

Acetyl groups can be easily removed by alkaline hydrolysis and deacylated gellans are mostly used in tissue engineering and pharmaceuticals. The average

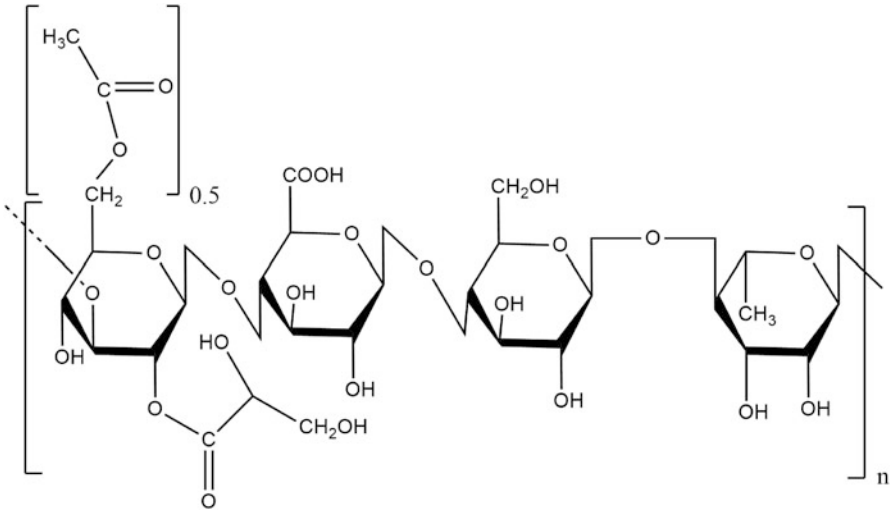


Fig. 8 Chemical structure of gellan

molecular weight is about 500 kDa. Dissolved in water, heated and mixed with cations (mono or divalent), gellan forms a gel by decreasing temperature. The gels are thermoreversible. The acetylated form yields soft and elastic gels, the fully deacetylated, hard, and brittle gels. With a large area of applications in food industry, it has been approved by FDA as a stabilizer and thickener in food since 1990 and in Europe as food additive (E418) since 2012. A low acyl form is used in solid dosage pharmaceutical formulations of immediate or sustained release (Osmalek et al. 2014). In ophthalmic preparations, low acetyl gellan is exploited for gel forming behavior *in situ*, in the presence of cations (tear fluids) ensuring a prolonged contact time and sustained release, for example, antiglaucoma timolol maleate gel forming solution, marketed as Timoptic-XE (www.rxlist.com/timoptic-xe-drug) or Blocadren Depot (www.ndrugs.com). Such applications determined the inclusion of low and high acyl forms of gellan in US Pharmacopeia (USP 43-NF38, 2020).

1.4.1 Gellan Developments as Drug Carriers

Dozens of delivery systems containing gellan and derivatives have been developed based on its gelling stimuli responsive properties, mainly temperature and ionic strength (Palumbo et al. 2020).

A drug delivery pH-responsive nanosystem was obtained consisting of sericin/rice bran albumin embedded gellan. Gellan was conjugated with sericin protein by CO-NH amide groups. The anticancer doxorubicin was loaded by encapsulation. The drug release was faster and greater at acidic pH (4.0), possibly due to the polymer ester bonds hydrolysis. The sustained release favored a decrease of the survival rate of the tumor MCF-7 cells, compared to single drug. A strong tumor cell internalization of the nanoparticles was proved (Arjama et al. 2018).

A thiol derivative of gellan was also prepared via amide bond formation by conjugation with 2-(2-aminoethyldisulfanyl)nicotinic acid, obtaining different degrees of thiolation. Keeping biocompatible, vaginal films casted from the obtained biopolymer exhibited a several fold increased dynamic viscosity in porcine vaginal mucus and 3-fold improved adhesion on mucosal surface compared to gellan films. It ensured a sustained release of metronidazol, thus proving to be a promising novel excipient for coating vaginal films (Jalil et al. 2019).

1.4.2 Gellan in Wound Healing and Tissue Engineering

Gellan-methacrylate was combined with up to 1% laponite XLG, a synthetic layered silicate clay, water insoluble, but swellable, forming colloidal dispersions. The mixture appeared as a nanocomposite hydrogel, which was UV photocrosslinked. It was considered a novel possible wound dressing material, having also in view the drug sustained release of a model antimicrobial, ofloxacin (Pacelli et al. 2016).

Gellan was added to gelatin, forming a blank hydrogel (heating and cooling) which incorporated tannic acid as antibacterial. Thus, the tannic acid-loaded hydrogel proved to be active *in vitro* against *E. coli, S. aureus*, and methicillin multidrug-resistant *S. aureus*. Tested in vivo on mice, that electrostatic complexation hydrogel showed a good injectability and synergistic gel formation and promoted cell adhesion and migration, leading to complete healing by skin regeneration after 12 days,

without scars, compared to slower and unsatisfactory results of a control, treated with single tannic acid (Zheng et al. 2018).

A comprehensive and well-ordered review on medical applications of gellan-based systems was published by Palumbo et al. (2020), highlighting the exploited important properties of that biopolymer, as gelling ability, thermal and ion sensitivity mucoadhesion.

Cell therapy by tissue engineering and scaffolds formation for regenerative medicine seems to be the major perspective application of gellans.

As collagen-derived protein, hydrazide-modified gelatin and oxidized to aldehyde gellan were covalently crosslinked to a 3D matrix mimicking the extracellular one. Cardiomyocytes derived from human-induced pluripotent stem cells (hiPSC) were successfully cultivated in that matrix and they retained their normal beating behavior (contraction-relaxation), the gelatin-gellan hydrogel proving to be suitable for 3D cardiac tissue modeling and study of such diseases (Koivisto et al. 2019).

A low-acyl gellan-hydroxyapatite spongy-like composite hydrogel obtained by Manda et al. (2018) was aimed to mimic the extracellular matrix of bone formation. $CaCl_2$ was used as crosslinker. When human adipose-derived stem cells hASC were seeded into the polymeric 3D structure, cell adhesions and spreading, as well as the precipitation of apatite similar to bone-apatite in modified simulated body fluid were noticed.

Santhanam et al. (2019) prepared injectable fibrillary hydrogels composed of deacylated gellan and semiflexible polyelectrolyte copolymer of poly[methacrylamide-co-methacrylic acid], both thiolated and crosslinked by disulfide bonds. Two products proved good and superior properties as vitreous biomimetic-biocompatible replacers on rabbit experiment, compared to chemically accepted silicone oil. They were stable at least for 30 days, maintaining optical transparency, physiological intraocular pressure, intact retinal layers.

Vilela et al. (2018) obtained a low acyl methacrylated gellan and used it as chondrogenic polymer for cartilage repair. *In vitro* chondrogenesis was studied on hydrogel encapsulated human nasal cartilage cells (NC) and human adipose stem cells (hASC). The *in vivo* study was performed on a rabbit model, by chondral lesion induction and repair with autologous rabbit ASC encapsulated in the hydrogel. After 8 weeks, a full thickness regeneration of size lesions and good integration with native cartilage were noticed. The biopolymer-derivative was considered a support for cartilage repairing surgery.

1.5 Bacterial Cellulose

Firstly observed by ancient Chinese producing a fermented beverage from acetic bacteria and firstly described by J. M. Brown in 1886 (Azeredo et al. 2019; Abeer et al. 2014), bacterial cellulose (BC) presents the same chemical structure as plant cellulose, as linear homopolysaccharide composed of β-1,4-glucose units (Fig. 9).

It is produced by fermentation of numerous bacterial species, but *Gluconacetobacter* or *Komagataeibacter xylinus* is the most studied and considered for

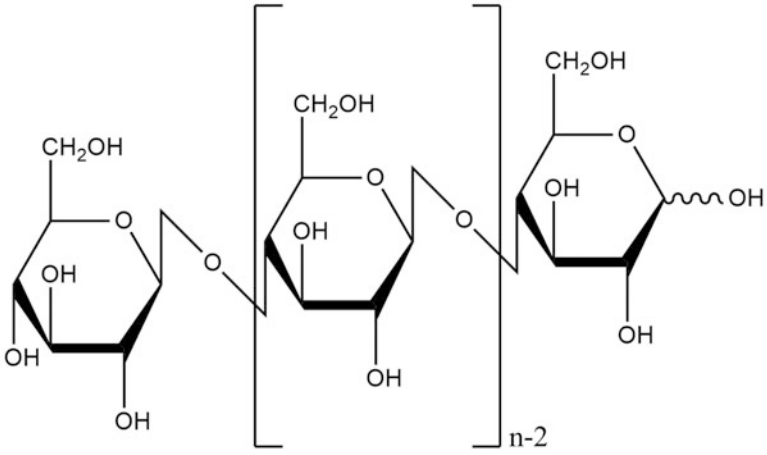


Fig. 9 Chemical structure of bacterial cellulose

commercial production, and static batch cultivation is preferred for the surface pellicle product morphology, even limited by the too large requested surface.

Apart from the advantage of the possibility to control the product characteristics (shape, network) and yield by bioprocess parameters, BC shows high purity, high degree of polymerization (2000–10000 glucose units), high crystallinity (80–95%), high fiber stability (similar to steel), very high water holding capacity (more than 99%), forming stable hydrogels (Moscovici et al. 2017), but due to process limitations, its applications are currently limited to high value, of justified performance products (Freitas et al. 2014).

Approved by FDA and the European Food Safety Authority (EFSA) as a dietary fiber in foods, it was also approved as component of other products for various clinical indications and as primary and unique component of some wound dressings. Numerous such products were cited as existing on the market, including vessel implants, artificial skin, tendon repair (Abeer et al. 2014; Ludwicka et al. 2016), as well as US and EU patents regarding similar products.

Despite the existence of commercial products and challenges in efficient production, the bacterial cellulose medical applications are continuously developed, as shown by the increased number of publications (Gorgieva 2020).

1.5.1 Bacterial Cellulose Developments in Wound Healing with Antimicrobials Delivery and Tissue Engineering

A dressing saturated with gentamycin antibiotic was obtained and tested for bone film infections. After *in vitro* experiments on a microbial biofilm on a simulated bone environment and evaluation of cytotoxicity of the dressing product in osteoblast (U2-OS) cultures, *ex vivo* experiments were performed on rat femur and jaw. Efficient inhibition was reached against *S. aureus* and *Ps. aeruginosa* biofilms and

an antibiotic prolonged release, compared to gentamycin-loaded collagen sponge (Junka et al. 2019).

Silver nanoparticles were deposited in the BC matrix by Ag^+ ions reduction of a silver nitrate solution impregnated in the BC membrane, using UV light irradiation. A strong inhibition of *E. coli* was reached at a concentration of 10^{-2} M Ag/BC sample (Pal et al. 2017).

A "mussel" mimetic transdermal patch was prepared on a moiety of dopamine (cathecol containing) modified by amidation-carboxymethyl BC/reduced graphene oxide (by CM-BC hydroxyl groups) composite, doped with Ag^+ nanoparticles. *In vitro* tests regarded cytotoxicity on 3T3 fibroblast cells and wound healing of scratched cell layers of 3T3 and A549 human lung epithelial cell lines and antimicrobial activity against *S. aureus, Lysinibacillus fusiformis, E. coli*, and *Ps. aeruginosa*. The composite enhanced proliferation of 3T3 cells, especially by the presence of Ag nanoparticles compared to a control; efficient wound healing for both cell types was noticed, and the antibacterial activity was significant, even compared to a ciprofloxacin containing control (Khamrai et al. 2019).

Roman et al. (2019) published an evaluation of BC-based tissue engineering scaffolds, starting from the specific criteria requested to the tissue scaffolds for implantation: biocompatible (nontoxic, nonimmunogenic), biodegradable, biomimetic, and ideally, bioactive, stimulating cell differentiation and tissue regeneration. Thus, BC was considered accordingly. With the advantage of a relative physical and chemical stability, permitting heat, chemical, and radiation sterilization, with a prolonged structural integrity of implants, its resistance to *in vivo* biodegradation in the absence of cellulolytic enzymes in mammalian tissues was considered the main limitation of this biomaterial. To overcome it, some approaches were cited, starting from incorporation of commercial enzymes for a gradual biodegradation, to metabolic engineering and irradiation. Thus, Yadav et al. (2015) obtained a genetically modified *Gluconacetobacter xylinus* which produced modified bacterial cellulose lysozyme susceptible as chondrogenic scaffold.

A genetically modified BC producer *Komagataeibacter hansenii* culture produced BC membranes with increased pore size and relaxed fiber structure, superior to membranes produced by a wild-type strain, as scaffold of chondrogenic model ATDC5 cells *in vitro* (Jacek et al. 2018).

1.5.2 Bacterial Cellulose in Drug Delivery

Transdermal drug delivery of antimicrobials from BC membranes has been firstly applied in wound dressings, including commercial ones, but it has been extended to other drugs to penetrate the skin, due to the significant advantage of direct transport with controlled release to the bloodstream through skin penetration and to the target site, avoiding the gastrointestinal tract, where the drug substance may undergo degradation or cause adverse side effects.

A transdermal potential system from BC was investigated by a study on crocin, an antioxidant carotenoid extracted from safron (*Crocus sativa L.*) with several medicinal properties, including antitumorigenic and selective antitumor activity. BC

membranes were loaded by drug adsorption and the diffusion experiments through mouse epidermal skin showed a slow release profile (Abba et al. 2019).

A multifunctional theranostic platform was designed consisting of magnetic hydrogel nanoparticles containing immobilized folic acid, encapsulated anticancer doxorubicin, and hematoporphyrin monomethyl ether which loaded bacterial cellulose membranes. So, folic acid was a target molecule, enhancing the accumulation of nanocomposite, the magnetic nanoparticles absorb a 633 nm laser radiation and are photosensitizer for the oxidation damage of tumor cells by the singlet oxygen generated from the hematoporphyrin derivative under light exposure. Thus, the tumor cells could suffer a dual attack, drug and singlet oxygen O· (photodynamic treatment).

The in vivo study of the composite immobilized on a medical adhesive tape on the skin of MCF-7 breast cancer cells tumor bearing mice exposed to laser irradiation and magnetic field showed a synergistic effect of approx. 80% inhibition after 14 days (Zhang et al. 2019).

1.6 Levan

With production insight since 1930, it is a neutral, water-soluble homopolysaccharide composed of fructose units linked in a backbone of β-2,6, with β-2,1 side branches, produced by a few plants (*Agropyron cristatum*, *Dactylis glomerata*, *Poa secunda*) and numerous microorganisms: fungi (e.g., *Aspergillus sydowii, A. versicolor*) and a wide range of bacteria, including species of *Erwinia, Streptococcus, Pseudomonas, Zymomonas, Lactobacillus, Bacillus, Aerobacter*, with different MW (Fig. 10). Levan

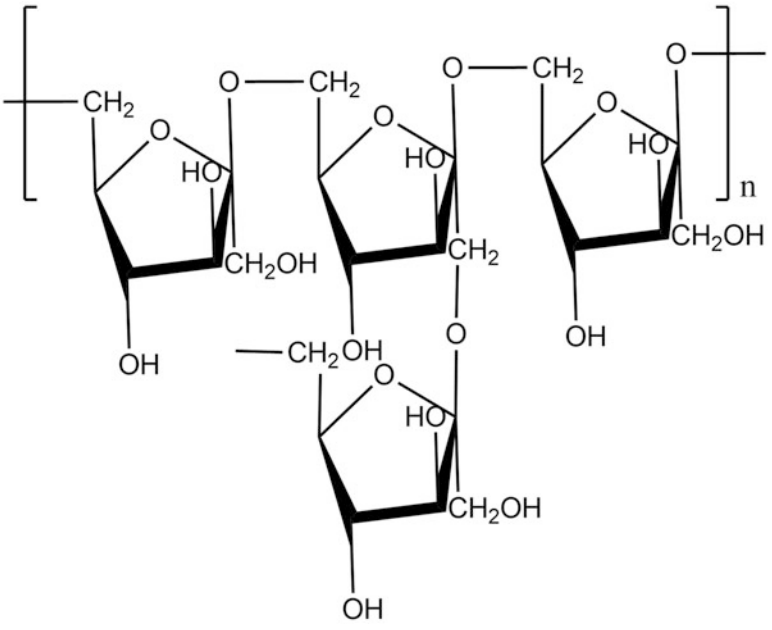


Fig. 10 Chemical structure of levan

is usually synthesized from sucrose by the enzyme levansucrase (EC 2.4.1.10) (Freitas et al. 2014; Srikanth et al. 2015). It has been approved as food additive in the USA, Europe, Japan.

As different from other microbial polysaccharides, levan exhibits biological activities by itself.

1.6.1 Biological Activities of Levan for Health

Antioxidant Products with antioxidant activity are very important, combating oxidative stresses, as a cause of numerous serious diseases. Those of natural origin are preferred. Hydrogen donating ability for free radical scavenging and heavy metal ions chelation by their functional groups are considered the main features of exopolysaccharides as antioxidants (Andrew and Jayaraman 2020).

A high MW (20 Mda) levan produced by a *B. subtilis* strain showed a very strong antioxidant activity *in vitro*, much superior to butylated hydroxyanisole (BHA) (Bouallegue et al. 2020).

Levan produced by *B. licheniformis* as cholesterol hydrophobized derivative incorporated fullerene C_{60} nanotubes, forming a noncovalent supramolecular hybrid exhibiting a high dose dependent antioxidant activity (Kop et al. 2020).

A levan produced by *Leuconostoc mesenteroides* showed a strong antioxidant activity, but also an immunomodulatory role, inducing a dose-dependent anti-inflammatory cytokine IL-4 production in HT-29 (human colon adenocarcinoma) cells (Taylan et al. 2019).

Antitumor A dose-related antiproliferative effect of levan produced by a halophilic bacterium *Halomonas smyrnensis* on human MCF-7 breast cancer cells was noticed and associated with cell apoptosis and oxidative stress, which was considered dependent on cell type and redox status (Queiroz et al. 2017).

Similar results were obtained with the oxidized aldehyde derivative of the same levan on other human cancer cell lines: A549 (lung), Hep2/C3A (liver), AGS (gastric) (Sarilmiser and Oner 2014).

Prebiotic Mild acid hydrolysis of levan produced by a strain of *Erwinia* sp. led to hydrolysate (MW approx. 3kDa) which stimulated *Bifidobacterium* and *Eubacterium rectale-Clostridium coccoides* growth in a gut model system stronger than inulin (Liu et al. 2020).

Two levan producing *Bacillus subtilis* strains isolated from honey were evaluated as probiotic-prebiotic systems (synbiotics) *in vivo* in a mouse model. Positive results were noticed regarding gut microbiota, immune system improvement, and protection from *Salmonella typhimurium* liver infection; levans probiotics showed antioxidant activity (Hamdi et al. 2017).

1.6.2 Levan in Tissue Engineering

Blends of poly (ε-caprolactone), gelatin, and hydrolysate of *Halomonas* produced levan were prepared in suitable compositions for 3D-bioprinting. The presence and

increased concentration of levan hydrolysate in the scaffolds increased the proliferation of human osteoblast cells (Duymaz et al. 2019).

Free-standing layer-by-layer adhesive films prepared from chitosan, alginate, with levan sulfate addition, non- or crosslinked with genipin, were obtained and tested on mouse C2C12 myoblast cell line culture. The presence of sulfated levan and crosslinking conferred a higher mechanical resistance to the membranes and enhanced cell compatibility and proliferation, for a possible application in cardiac tissue engineering (Gomes et al. 2018).

1.6.3 Levan as Drug Carrier

A carboxymethyl derivative of *Halomonas* levan hydrolysate was incorporated as crosslinker in poly-N-isopropyl acrylamide. 5-Aminosalycylic acid, an anti-inflammatory drug for bowel diseases (ulcerative colitis, Crohn's disease), was encapsulated in the obtained hydrogels, which were tested for biocompatibility on mouse fibroblast L929 cells. The increased levan concentration favored the biocompatibility and a controlled release (8 h) of the drug at 37°C and pH 7.4 (Osman et al. 2017).

Another levan produced by a strain of *Acinetobacter nectaris* and its carboxymethyl derivative were studied as anticancer 5-fluorouracil carriers. The drug was loaded by encapsulation. Drug loading reached a maximum (approx. 60%) at basic pH (12) and the drug release was higher for levan at pH 7 (more than 70%) than for carboxymethyl derivative, which retained the drug by an amide formed with a NH group of the drug (Tabernero et al. 2017).

2 New Discovered Bacterial Polysaccharides with Potential Medical Applications

Some of the new polysaccharides showed biological activities of interest regarding widespread serious diseases.

An exopolysaccharide (EPS) produced by a *Bacillus subtilis* strain isolated from mangrove marine sediment, with a composition of mannuronic, glucuronic acids, glucose, galactose, mannose (1.6:1.5: 1.0:2.3:1.4), showed antioxidant activity and improved hyperglycemia, dyslipidemia (molecular markers), and cardiovascular disease risk (histopathological) in streptozocin-induced diabetic rats (Ghoneim et al. 2016).

A strain of *Bacillus mycoides* isolated from a gas station was found as producer of an exopolysaccharide, composed of galactose, mannose, glucose, and glucuronic acid, which exhibited selective antitumor activity against cancer cells HepG2 and Caco-2 with IC_{50} of 138 $\mu g.ml^{-1}$ and 151 $mg.ml^{-1}$, respectively (Farag et al. 2020).

60 *Lactobacilli* were isolated from 55 herbal plants and 42 dairy samples, among them 21 producing significant concentrations of EPS. The produced EPS exhibited antioxidant, different anticollagenase, antielastase activities. No significant toxicity on normal primary human fibroblasts was observed, but some cell proliferation.

Positive results were noticed in a scratch test for wound healing. The EPS from *L. casei* showed positive effect regarding down-regulation and inhibition of matrix-

metal proteinases gene-expression, skin-aging related. D-glucuronic acid was found in some of the EPS compositions and was suggested as a key molecule in the skin antiaging process. The EPS products were considered promising for skin antiaging agents to tissue engineering scaffolds (Shirzad et al. 2018).

An exopolysaccharide produced by a new isolated *Bacillus* strain from mangrove marine sediment composed of galacturonic and glucuronic acid (1:1) was studied for 90 days in aluminum chloride-induced Alzheimer disease rats. Reduction of oxidative stress parameters (malondialdehyde, hydrogen peroxide, nitric oxide), stimulation of antioxidant ones (catalase, superoxide dismutase), and especially, inhibition of cholinesterase, a key activity in the treatment of neurodegenerative diseases, were considered potent anti-Alzheimer effects. A subchronic study revealed the EPS as nontoxic (Asker et al. 2015).

3 Conclusions and Perspectives

Bacterial polysaccharides already play an established role in medicine and health improvement.

During the last years, numerous smart medical developments of known bacterial polysaccharides have been published, as well as bioprospecting for new strains producing valuable polymers of this class continued.

From the medical-pharmaceutical authorization point of view, their current developing applications could be divided into two major classes: drugs and medical devices.

In the drug class, a very large area belongs to new drug carriers, especially as ingredients of smart targeted nanopharmaceuticals. Here, there are still limitations/barriers to overcome and corresponding challenges. So far, no polysaccharide nanoparticle formulations have obtained regulatory approval, only drug nanocrystals, liposome and lipid nanoparticles, PEG-conjugated nanodrugs, other polymers: protein-based nanopharmaceuticals (e.g., albumin), synthetic polymers (Farjadian et al. 2018) have been approved. As a general remark, physiological polymers or with a very known past are preferred.

Apart from financial, ethical issues, common to all new pharmaceuticals, we consider the regulatory issues the most important. In this view, larger *in vivo* studies, in different complexity targets, confirming *in vitro* results, should prove the efficacy and safety. As other innovative medicines, the nanomedicines should present a complete Common Technical Document according the guidelines of the International Council for Harmonization (ICH), with the very important safety chapter (M4-5) containing pharmacology, pharmacokinetics, and toxicology preclinical reports.

Generally, nanoparticle formulated drugs, by their advantages (reducing doses and side effects, possibly cheaper, due to their more precise delivery to the target) and a higher accumulation in the targeted cells and tissues, could represent a major direction in the future therapy, including personalized medicine. Concerning to bacterial polysaccharides, reliable, reproducible products, with stable properties, *in vivo* confirmed and quantitatively detectable in physiological fluids and tissues, are necessary conditions for their authorized entry in therapy.

A different image, with already existing approved bacterial polysaccharide content, even as nanomaterials, presents the medical devices. Though mostly as wound dressings, new developments of bacterial polysaccharides have a good chance to enter the market, easier as physiological polymers, like hyaluronic acid.

Some requirements are common with medicines, regarding the chemical purity determined according to the Pharmacopeias and other physicochemical properties depending on destination (viscosity, molecular weight and its distribution, mechanical properties).

Generally, and particular for Europe and USA, there are three medical devices categories, depending on the type of device (surface or implant), duration of tissue contact, and mainly on type of tissue in contact with the device (intact skin, bone, damaged tissue, blood, etc.). Cytotoxicity, sensitization, and irritation assessments are recommended for all medical devices (Huerta-Ángeles et al. 2018).

Open wound dressings and implants in contact with damage tissues are generally considered in second or third category and safety conditions are more severe. Preclinical reports should demonstrate that the product is nonpyrogenic, mutagenic, toxigenic, hemolytic, and immunogenic (norm ISO 10933). It is important to select adequate cell lines for biocompatibility/cytotoxicity test. If the device is in contact with blood, quantitative detection and systemic *in vivo* toxicity are necessary.

As intensive research is going on and a lot of results are relatively new, certainly novel medical applications of bacterial polysaccharides, efficient and safe, will reach the market and satisfy people's needs.

References

Abba M, Ibrahim Z, Chong CS, Zawawi NA, Kadir MRA, Yusof AHM, Razak SIA. Transdermal delivery of crocin using bacterial cellulose membrane. Fibers Polym. 2019;20:2025–31. https://doi.org/10.1007/s12221-019-9076-8.

Abeer MM, Amin MCIM, Martin C. A review of bacterial cellulose-based drug delivery systems: their biochemistry, current approaches and future prospects. J Pharm Pharmacol. 2014;66:1047–61.

Alibolandi M, Abnous K, Hadizadeh F, Taghdisi SM, Alabdollah F, Mohammadi M, Nassirli H, Ramezani M. Dextran-polylactide-co-glycolide polymersomes decorated with folate-antennae for targeted delivery of docetaxel to breast adenocarcinoma *in vitro* and *in vivo*. J Control Release. 2016;241:45–56. https://doi.org/10.1016/j.jconrel.2016.09.012.

Alves A, Miguel SP, Araujo ARTS, de Jesús Valle MJ, Navarro AS, Correia IJ, Ribeiro MP, Coutinho P. Xanthan gum–konjac glucomannan blend hydrogel for wound healing. Polymers. 2020;12:99. https://doi.org/10.3390/polym12010099.

Andrew M, Jayaraman G. Structural features of microbial exopolysaccharides in relation to their antioxidant activity. Carbohydr Res. 2020;487:107881. https://doi.org/10.1016/j.carres.2019.107881.

Arjama M, Sivaraj M, Rajan M, Jeyaraj M. Sericin/RBA embedded gellan gum based smart nanosystem for pH responsive drug delivery. I J Biol Macromol. 2018;120 Part. B:1561–1571. https://doi.org/10.1016/j.ijbiomac.2018.09.146.

Asker MMS, Ibrahim AY, Mahmoud MG, Mohamed SS. Production and characterization of exopolysaccharide from novel Bacillus sp. M3 and evaluation on development sub-chronic aluminum toxicity induced Alzheimer's disease in male rats. Am J Biochem Biotechnol. 2015;11(2):92–103. https://doi.org/10.3844/ajbbsp.2015.92.103.

Aswathy SH, Narendrakumar U, Manjubala I. Commercial hydrogels for biomedical applications. Heliyon. 2020;6(e03719):1–13. https://doi.org/10.1016/j.heliyon.2020.e03719.

Azeredo HMC, Barud H, Farinas CS, Vasconcellos VM, Claro AM. Bacterial cellulose as a raw material for food and food packaging applications. Front Sust Food Syst. 2019;3:7. https://doi.org/10.3389/fsufs.2019.00007.

Bae KH, Lee F, Xu K, Keng CT, Tan SY, Tan YJ, Chen Q, Kurisawa M. Microstructured dextran hydrogels for burst-free sustained release of PEGylated protein drugs. Biomaterials. 2015;63:146–157. https://doi.org/10.1016/j.biomaterials.2015.06.008.

Bai S, Gao YE, Ma X, Shi X, Hou M, Xue P, Kang Y, Xu Z. Reduction stimuli responsive unimolecular polymeric prodrug based on amphiphilic dextran-framework for antitumor drug delivery. Carbohydr Polym. 2018;182:235–44. https://doi.org/10.1016/j.carbpol.2017.11.028.

Beck EC, Lohman B, Tabakh D, Kieweg SL, Gehrke SH, Berkland CJ, Detamore MS. Enabling surgical placement of hydrogels through achieving paste-like rheological behavior in hydrogel precursor solutions. Annals of Biomedical Engineering. 2015;43(10):2569–2576. https://doi.org/10.1007/s10439-015-1277-8.

Bouallegue A, Casillo A, Chaari F, La Gatta A, Lanzetta R, Corsaro MM, Bachoual R, Ellouz-Chaabouni S. Levan from a new isolated Bacillus subtilis AF17: purification, structural analysis, and antioxidant activities. Int J Biol Macromol. 2020;144:316–324. https://doi.org/10.1016/j.ijbiomac.2019.12.108.

Catanzano O, D'Esposito V, Acierno S, Ambrosio MR, De Caro C, Avagliano C, Russo P, Russo R, Miro A, Ungaro F, Calignano A, Formisano P, Quaglia F. Alginate-hyaluronan composite hydrogels accelerate wound healing process. Carbohydr Polym. 2015;131:407–414. https://doi.org/10.1016/j.carbpol.2015.05.081.

Chen N, Johnson MM, Collier MA, Gallovic MD, Bachelder EM, Ainslie KM. Tunable degradation of acetalated dextran microparticles enables controlled vaccine adjuvant and antigen delivery to modulate adaptive immune responses. J Control Release. 2018;273:147–159. https://doi.org/10.1016/jconrel.2018.01.027.

Curcio M, Cirillo G, Paolì A, Naimo GD, Mauro L, Amantea D, Leggio A, Fiore PN, Iemma F. Self-assembling Dextran prodrug for redox- and pH-responsive co-delivery of therapeutics in cancer cells. Colloids Surf B: Biointerfaces. 2020;185:110537. https://doi.org/10.1016/j.colsurfb.2019.110537.

Duymaz BT, Erdiler FB, Alan T, Aydogdu MO, Inan AT, Ekren N, Uzun M, Sahin YM, Bulus E, Oktar FN, Selvi SS, Oner ET, Kilic O, Bostan MS, Eroglu MS, Gunduz O. 3D bio-printing of levan/polycaprolactone/gelatin blends for bone tissue engineering:Characterization of the cellular behavior. Eur Polym J. 2019;119:426–37. https://doi.org/10.1016/j.eurpolymj.2019.08.015.

Elzayat EA, Corcos J. Chapter 31: Urethral injectables in the management of stress urinary incontinence. In: Rez S, Rodriguez LV, editors. Female Urology. 3rd ed. Elsevier; 2008. p. 348–61.

Farag MMS, Moghannem SAM, Shehabeldine AM, Azab MS. Antitumor effect of exo-polysaccharide produced by Bacillus mycoides. Microb Pathog. 2020;140:103947. https://doi.org/10.1016/j.micpath.2019.103947.

Farjadian F, Ghasemi A, Gohari O, Roointan A, Karimi M, Hamblin MR. Nanopharmaceuticals and nanomedicines currently on the market: challenges and opportunities. Nanomedicine, Future Medicine Ltd. 2018; https://doi.org/10.2217/nnm-2018-0120.

Fernandez-Piñeiro I, Badiola I, Sanchez A. Nanocarriers for microRNA delivery in cancer medicine. Biotechnol Adv. 2017;35:350–60. https://doi.org/10.1016/j.biotechadv.2017.03.002.

Freitas F, Alves VD, Reis MAM. Bacterial polysaccharides: Production and applications in cosmetic industry. In: Ramavat KG, Merillon JM, editors. Polysaccharides – Bioactivity and Biotechnology. Springer International; 2014;66:2017–43.

Giammona G, Pitarresi G, Palumbo FS, Maraldi S, Scarponi S, Romanò CL. Hyaluronic-Based Antibacterial Hydrogel Coating for Implantable Biomaterials in Orthopedics and Trauma: From Basic Research to Clinical Applications. Hydrogels. Intechopen; 2018;9:179–201. https://doi.org/10.5772/intechopen.73203.

Ghaffari R, Salimi-Kenari H, Fahimipour F, Rabiee SM, Adeli H, Dashtimoghadam E. Fabrication and characterization of dextran/nanocrystalline β-tricalcium phosphate nanocomposite hydrogel scaffolds. Int J Biol Macromol. 2020;148:434–48. https://doi.org/10.1016/j.ijbiomac.2020.01.112.

Gharibi R, Kazemi S, Yeganeh H, Tafakori V. Utilizing dextran to improve hemocompatibility of antimicrobial wound dressings with embedded quaternary ammonium salts. Int J Biol Macromol. 2019;131:1044–56. https://doi.org/10.1016/j.ijbiomac.2019.03.185.

Ghoneim MAM, Hassan AI, Mahmoud MG, Asker MS. Effect of polysaccharide from Bacillus subtilis sp. on cardiovascular diseases and atherogenic indices in diabetic rats. BMC Complement Altern Med. 2016;16:112. https://doi.org/10.1186/s12906-016-1093-1.

Gomes TD, Caridade SG, Sousa MP, Azevedo S, Kandur MY, Oner ET, Alves NM, Mano JF. Adhesive free-standing multilayer films containing sulfated levan for biomedical applications. Acta Biomater. 2018;69:183–95. https://doi.org/10.1016/j.actbio.2018.01.027.

Gorgieva S. Bacterial cellulose as a versatile platform for research and development of biomedical materials. Processes. 2020;8:624. https://doi.org/10.3390/pr.8050624.

Gupta RC, Lall R, Srivastava A, Sinha A. Hyaluronic acid: molecular mechanisms and therapeutic trajectory. Front Vet Sci. 2019;6:192. https://doi.org/10.3389/fvets.2019.00192.

Hamdi AA, Elattal NA, Amin MA, Ali AE, Mansour NM, Awad GEA, Farrag ARH, Esawy MA. In vivo assessment of possible probiotic properties of Bacillus subtilis and prebiotic properties of levan. Biocatal Agric Biotechnol. 2017;13:190–197. https://doi.org/10.1016/j.bcab.2017.12.001.

Highley CB, Prestwich GD, Burdick JA. Recent advances in hyaluronic acid hydrogels for biomedical applications. Curr Opin Biotechnol. 2016;40:35–40. https://doi.org/10.1016/j.copbio.2016.02.008.

Huerta-Ángeles G, Nešporová K, Ambrožová G, Kubala L, Velebný V. An effective translation: the development of hyaluronan-based medical products from the physicochemical, and preclinical aspects. Front Bioeng and Biotechnol. 2018;6:62. https://doi.org/10.3389/fbioe.2018.00062.

Hwang JS, Lee HS, Lee K-H, Yoo H-W, Lee D-Y, Suh B-K, Ko CW, Chung WY, Jin D-K, Shin CH, Han H-S, Han S, Kim H-S. Once-weekly administration of sustained-release growth hormone in Korean prepubertal children with idiopathic short stature: a randomized, controlled Phase II study. Horm Res Paediat. 2018;90:54–63. https://doi.org/10.1159/000489262.

Jacek P, Szustak M, Kubiak K, Gendaszewska-Darmach E, Ludwicka K, Bielecki S. Scaffolds for chondrogenic cells cultivation prepared from bacterial cellulose with relaxed fibers structure induced genetically. Nanomaterials. 2018;8:1066. https://doi.org/10.3390/nano8121066.

Jalil A, Asim MH, Le N-MN, Laffleur F, Matuszczak B, Tribus M, Benkop-Schnurch A. S-protected gellan gum: decisive approach towards mucoadhesive antimicrobial vaginal films. Int J Biol Macromol. 2019;130:148–157. https://doi.org/10.1016/j.ijbiomac.2019.02.092.

Junka A, Bartosewicz M, Dziadas M, Szymczyk P, Dydak K, Zywicka A, Owczarek A, Bil-Lula I, Czajkowska J, Fijalkowski K. Application of bacterial cellulose experimental dressings saturated with gentamycin for management of bone biofilm in vitro and ex-vivo. J Biomed Mat Res Part B. 2019;108:30–37. https://doi.org/10.1002/jbm.b.34362.

Ke X, Li M, Wang X, Liang J, Wang X, Wu S, Long M, Hu C. An injectable chitosan/dextran/β-glycerophosphate hydrogel as cell delivery carrier for therapy of myocardial infarction. Carbohydr Polym. 2020;229:115516. https://doi.org/10.1016/j.carbpol.2019.115516.

Khamrai M, Banerjee SL, Paul S, Ghosh AK, Sarkar P, Kundu PP. A mussel mimetic, bioadhesive, antimicrobial patch based on dopamine-modified bacterial cellulose/rGO/AgNPs: a green approach toward wound healing applications. ACS Sustain Chem Eng. 2019;7:12083–97. https://doi.org/10.1021/acssuschemeng.9b01163.

Khunmanee S, Jeong Y, Park H. Crosslinking method of hyaluronic-based hydrogel for biomedical applications. Intelligent Scaffolds for Modulating and Promoting Tissue Regeneration. J Tissue Eng. 2017;8:2041731417726464. https://doi.org/10.1177/2041731417726464.

Kiani M, Tekie FSM, Dinarvand M, Soleimani M, Dinarvand R, Atyabi F. Thiolated carboxymethyl dextran as a nanocarrier for colon delivery of hSET1 antisense: In vitro stability and efficiency study. Mater Sci Eng C Mater Biol Appl. 2016;62:771–8. https://doi.org/10.1016/j.msec.2016.02.009.

Koivisto JT, Gering C, Karvinen J, Cherian RM, Belay B, Hyttinen J, Aalto-Setala K, Kelomaki M, Parraga J. Mechanically biomimetic gelatin-gellan gum hydrogels for 3D culture of beating

human cardiomyocytes. ACS Appl Mater Interfaces. 2019;11:20589–602. https://doi.org/10.1021/acsami.8b22343.

Kop TJ, Jakovljevic DM, Zivkovic LS, Zekic A, Beskoski VP, Milic DR, Gojgic-Cvijovic GD, Bjelakovic MS. Polysaccharide-fullerene supramolecular hybrids: Synthesis, characterization and antioxidant activity. Eur Polym J. 2020;123:109461. https://doi.org/10.1016/j.eurpolymj.2019.109461.

Liu C, Kolida S, Charalampopoulos RA, Rastall RA. An evaluation of the prebiotic potential of microbial levans from Erwinia sp. 10119. J Funct Foods. 2020;64:103668. https://doi.org/10.1016/j.jff.2019.103668.

Lopez KM, Ravula S, Pérez LR, Ayala CE, Losso JN, Janes ME, Warner IM. Hyaluronic acid−cellulose composites as patches for minimizing bacterial infections. ACS Omega. 2020;5:4125–32. https://doi.org/10.1021/acsomega.9b03852.

Ludwicka K, Jedrzejczak-Krzepkowska M, Kubiak K, Kolodziejczyk M, Pankiewicz T, Bielecki S (2016) Chapter 9: Medical and cosmetic applications of bacterial nanocellulose. In: Gama M, Dourado F, Bielecki S (eds) Bacterial Nanocellulose-From Biotechnology to Bio-Economy, Elsevier, pp. 145–165. https://doi.org/10.1016/B978-0444-63458-0.00009-3.

Manca ML, Manconi M, Valenti D, Lai F, Loy G, Matricardi P, Fadda AM. Liposomes coated with chitosan-xanthan gum (chitosomes) as potential carriers for pulmonary delivery of rifampicin. J Pharm Sci. 2012;101:566–75. https://doi.org/10.1002/jps.22775.

Manda MG, daSilva LP, Cerqueira MT, Pereira DR, Oliveira MB, Mano JF, Marques AP, Oliveira JM, Correlo VM, Reis RL. Gellan gum-hydroxyapatite composite spongy-like hydrogels for bone tissue engineering. J Biomed Mater Res A. 2018;106:479–90. https://doi.org/10.1002/jbm.a.36248.

Montagner IM, Merlo A, Carpanese D, Zuccolotto G, Renier D, Campisi C, Pasut G, Zanovello P, Rosato A. Drug conjugation to hyaluronan widens therapeutic indications for ovarian cancer. Oncoscience. 2015;2(4):373–81. https://doi.org/10.18632/oncoscience.150.

Moscovici M, Hlevca C, Casarica A, Pavaloiu R-D. Nanocellulose and nanogels as modern drug delivery sustems. In: Jawaid M, Mohammad F, editors. Nanocellulose and Nanohydrogel Matrices – Biotechnological and Biomedical Applications. Wiley-VCH; 2017. p. 209–69.

Osmalek T, Freelich A, Tasarek S. Application of gellan gum in pharmacy and medicine. Int J Pharm. 2014;466(1-2):328–40. https://doi.org/10.1016/j.ijpharm.2014.03.038.

Osman A, Oner ET, Eroglu MS. Novel levan and pNIPA temperature sensitive hydrogels for 5-ASA controlled release. Carbohydr Polym. 2017;165:61–70. https://doi.org/10.1016/j.carbpol.2017.01.097.

Pacelli S, Paolicelli P, Moretti G, Petralito S, Di Giacomo S, Vitalone A, Casadei MA. Gellan gum methacrylate and laponite as an innovative nanocomposite hydrogel for biomedical applications. Eur. Polym. J. 2016; 77:114–123. https://doi.org/10.1016/j.eurpolymj.2016.02.007.

Pal S, Nisi R, Stoppa M, Licciulli A. Silver functionalized bacterial cellulose as antibacterial membrane for wound-healing applications. ACS Omega. 2017;2:3632–9. https://doi.org/10.1021/acsomega.7b00442.

Palumbo FS, Federico S, Pitaressi G, Fiorica C, Giamonna G. Gellan gum-based delivery systems of therapeutic agents and cells. Carbohydr Polym. 2020;229:115430. https://doi.org/10.1016/j.carbpol.2019.115430.

Park SH, Seo JY, Park JY, Ji YB, Kim K, Choi HS, Choi S, Kim JH, Min BH, Kim MS. An injectable, click-crosslinked, cytomodulin-modified hyaluronic acid hydrogel for cartilage tissue engineering. NPG Asia Mater. 2019;11(30):1–16. https://doi.org/10.1038/s41427-019-0130-1.

Patil SB, Inamdar SZ, Reddy KR, Raghu AV, Soni SK, Kulkarni RV. Novel biocompatible poly (acrylamide)-grafted-dextran hydrogels: Synthesis, characterization and biomedical applications. J Microbiol Methods. 2019;159:200–10. https://doi.org/10.1016/j.mimet.2019.03.009.

Qu J, Liang Y, Shi M, Guo B, Gao Y, Yin Z. Biocompatible conductive hydrogels based on dextran and aniline trimer as electro-responsive drug delivery system for localized drug release. Int J Biol Macromol. 2019;140:255–64. https://doi.org/10.1016/j.ijbiomac.2019.08.120.

Queiroz EAIF, Fortes ZB, da Cunha MAA, Sarilmiser HK, Dekker AMB, Oner ET, Dekker RFH, Khaper N. Levan promotes antiproliferative and pro-apoptotic effects in MCF-7 breast cancer

cells mediated by oxidative stress. Int J Biol Macromol. 2017;102:565–70. https://doi.org/10.1016/j.ijbiomac.2017.04.035.

Rao KM, Kumar A, Han SS. Polysaccharide-based magnetically responsive polyelectrolyte hydrogels for tissue engineering applications. J Mater Sci Technol. 2018;34:1371–7. https://doi.org/10.1016/j.jmst.2017.10.003.

Riahi N, Liberelle B, Henry O, De Crescenzo G. Impact of RGD amount in dextran-based hydrogels for cell delivery. Carbohydr Polym. 2017;161:219–27. https://doi.org/10.1016/j.carbpol.2017.01.002.

Rodell CB, Mc Arthur Jr JW, Dorsey SM, Wade RJ, Wang LL, Woo YJ, Burdick JA. Shear thinning supramolecular hydrogels with secondary autonomous covalent crosslinking to modulate viscoelastic properties in vivo. Adv Funct Mater. 2015;25:636–44. https://doi.org/10.1002/adfm.201403550.

Roman M, Haring AP, Bertucio TJ. The growing merits and dwindling limitations of bacterial cellulose-based tissue engineering scaffolds. Cur Op Chem Eng. 2019;24:98–106. https://doi.org/10.1016/j.coche.2019.03.006.

Sagitha P, Reshmi CR, Sundaran SP, Binoy A, Mishra N, Sujith A. In-vitro evaluation on drug release kinetics and antibacterial activity of dextran modified polyurethane fibrous membrane. Int J Biol Macromol. 2019;126:717–30. https://doi.org/10.1016/j.ijbiomac.2018.12.155.

Salman JAS, Salman MAS, Shather MF, Salim MZ. [54] Preparation of biopolymer (dextran) and gentamycin blend against multi-drug resistant bacterial infections associated with catheters. Abstracts/Arab J Urol. 2018;16:S26-S27. https://doi.org/10.1016/j.aju.2018.10.007.

Santhanam S, Shui Y-B, Struckhoff J, Karakoçak BB, Hamilton PD, Harocopos GJ, Ravi N. Bioinspired fibrillary hydrogel with controlled swelling behavior: applicability as an artificial vitreous. ACS Appl Bio Mater. 2019;2:70–80. https://doi.org/10.1021/acsabm.8b00376.

Sarilmiser HK, Oner ET. Investigation of anticancer activity of linear and aldehyde activated levan from Halomonas smyrnensis AAD6^{T}. Biochem Eng J. 2014;92:28–34. https://doi.org/10.1016/jbej.2014.06.020.

Shera SS, Sahu S, Banik RM. Preparation of drug eluting natural composite scaffold using response surface methodology and artificial neural network approach. Tissue Eng Regen Med. 2018;15(2):131–143. https://doi.org/10.1007/s13770-017-0100-z.

Shirzad M, Hamedi J, Motevaseli E, Modarressi MH. Anti-elastase and anti-collagenase potential of Lactobacilli exopolysaccharides on human fibroblast. Artif Cells Nanomed Biotechnol. 2018;46:1051–61. https://doi.org/10.1080/21691401.2018.1443274.

Singh S, Gupta A, Sharma D, Gupta B. Dextran based herbal nanobiocomposite membranes for scar free wound healing. Int J Biol Macromol. 2018;113:227–39. https://doi.org/10.1016/j.ijbiomac.2018.02.097.

Son S, Rao NV, Ko H, Shin S, Jeon J, Han HS, Nguyen VQ, Thambi T, Suh YD, Park JH. Carboxymethyl dextran-based hypoxia-responsive nanoparticles for doxorubicin delivery. Int J Biol Macromol. 2018;110:399–405. https://doi.org/10.1016/j.ijbiomac.2017.11.048.

Srikanth R, Siddartha G, CHSSS Reddy, Harish BS, Ramaiah MJ, Uppuluri KB. Antioxidant and anti-inflammatory levan from *Acetobacter xylinum* NCIM2526 and its statistical optimization. Carbohydr Polym. 2015;123:8–16. https://doi.org/10.1016/jcarbpol.2014.12.079.

Tabernero A, Gonzales-Garcinuno A, Sanchez-Alvarez JM, Galan MA, del Valle EMM. development of a nanoparticle system based on a fructose polymer: Stability and drug release studies. Carbohydr Polym. 2017;160:26–33. https://doi.org/10.1016/j.carbpol.2016.12.025.

Taylan O, Yilmaz MT, Dertli E. Partial characterization of a levan type exopolysaccharide (BPS) produced by Leuconostoc mesenteroides showing immunostimulatory and antioxidant activities. Int J Biol Macromol. 2019;136:436–44. https://doi.org/10.1016/jijbiomac.2019.06.078.

Tekie F, Soleimani M, Zakerian A, Dinarvand M, Amini M, Dinarvand R, Arefian E, Atyabi F. Glutathione responsive chitosan-thiolated dextran conjugated miR-145 nanoparticles targeted with AS1411 aptamer for cancer treatment. Carbohydr Polym. 2018;201:131–40. https://doi.org/10.1016/j.carbpol.2018.08.060.

Vilela CA, Correia C, da Silva Morais M, Santos TC, Gertrudes AC, Moreira ES, Frias AM, Learmonth DA, Oliveira P, Oliveira JM, Sousa RA, Espregueira-Mendes JD, Reis RL. *In vitro* and *in vivo* performance of methacrylated gellan gum hydrogel formulations for cartilage repair. J Biomed Mater Res Part A. 2018;106:1987–96. https://doi.org/10.1002/jbm.a.36406.

Wang C, Xiong S, You J, Guan W, Xu G, Dou H. Dextran-based coacervate nanodroplets as potential gene carriers for efficient cancer therapy. Carbohydr Polym. 2020;231:115687. https://doi.org/10.1016/j.carbpol.2019.115687.

Yadav V, Sun L, Panilaitis B, Kaplan DL. In vitro chondrogenesis with lysozyme susceptible bacterial cellulose as a scaffold. J Tissue Eng Reger Med. 2015;9:E276–88. https://doi.org/10.1002/term.1644.

Ye G, Li G, Wang C, Ling B, Yang R, Huang S. Extraction and characterization of dextran from Leuconostoc pseudomesenteroides YB-2 isolated from mango juice. Carbohydr Polym. 2019;207:218–23. https://doi.org/10.1016/j.carbpol.2018.11.092.

Zarour K, Llamas MG, Prieto A, Ruas-Madiedo P, Duenas MT, de Palencia PF, Aznar R, Kihal M, Lopez P. Rheology and bioactivity of high molecular weight dextrans synthesized by lactic acid bacteria. Carbohydr Polym. 2017;174:646-657. https://doi.org/10.1016/j.carbpol.2017.06.113

Zhang L-K, Du S, Wang X, Jiao Y, Yin L, Zhang Y, Guan Y-Q. Bacterial cellulose based composites enhanced transdermal drug targeting for breast cancer treatment. Chem Eng J. 2019;370:749–59. https://doi.org/10.1016/j.cej.2019.03.216.

Zhang J-F, Wang Y, Lam ML, McKinnnie RJ, Claycomb WC, Xu X. Cross-linked poly(lactic acid)/dextran nanofibrous scaffolds with tunable hydrophilicity promoting differentiation of embryoid bodies. Mater Today Commun. 2017;13:306–16. https://doi.org/10.1016/j.mtcomm.2017.10.012.

Zhao X, Li P, Guo B, Ma PX. Antibacterial and conductive injectable hydrogels based on quaternized chitosan-graft-polyaniline/oxidized dextran for tissue engineering. Acta Biomater. 2015;26:236–48. https://doi.org/10.1016/j.actbio.2015.08.006.

Zheng Y, Liang Y, Zhang D, Sun X, Liang L, Li J, Liu Y-N. Gelatin-based hydrogels blended with gellan as an injectable wound dressing. ACS Omega. 2018;3:4766–75. https://doi.org/10.1021/acsomega.8b00308.

Zou P, Liu H, Li Y, Huang J, Dai Y. Surface dextran modified electrospun poly (3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) fibrous scaffold promotes the proliferation of bone marrow-derived mesenchymal stem cells. Mater Lett. 2016;179:109–13. https://doi.org/10.1016/j.matlet.2016.04.189.

Pharmaceutical and Biomedical Potential of Sulphated Polysaccharides from Algae

38

Alcina M. M. B. Morais, Ana Alves, Decha Kumla, and Rui M. S. C. Morais

Contents

Abstract

In recent years, sulphated polysaccharides (SPs) have emerged as an increasingly important class of biopolymers with potential innovative applications in the biomedical and pharmaceutical fields due to their diverse physicochemical

A. M. M. B. Morais · A. Alves · D. Kumla · R. M. S. C. Morais (✉)
CBQF – Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Porto, Portugal
e-mail: abmorais@porto.ucp.pt; aalves@porto.ucp.pt; dkumla@porto.ucp.pt; rcmorais@porto.ucp.pt

J. M. Oliveira et al. (eds.), *Polysaccharides of Microbial Origin*,
https://doi.org/10.1007/978-3-030-42215-8_49

properties and biological activities. The focus of the present chapter is to describe these biological activities and pharmacological properties of SPs and to highlight their potential application in the pharmaceutical and biomedical areas, as well. These compounds obtained from algae (macroalgae and microalgae) exhibit many beneficial biological activities, such as anticoagulant and/or antithrombotic, immunomodulator, antitumor and cancer preventive, hypoglycemic, antibiotics and anti-inflammatory, and antioxidant. In addition, the interest in using algal polysaccharides as biomedical vehicles for drug delivery has increased steadily. The isolation, structure elucidation, and bioactivity testing of algal SPs always bring perspectives for the discovery of new drugs and new applications, as well as an economic development, both by introducing a high diversity of new products and, eventually, a product with high added value.

Keywords

Sulphated polysaccharides · Biological activities · Macroalge · Microalgae · Biomedical applications

1 Introduction

Sulphated polysaccharides (SPs) comprise a complex group of macromolecules, consisting of single or different types of monosaccharides with one or more sulphate group attached in different positions, with a wide range of important biological properties (Cunha et al. 2009). Many organisms are very different sources of polysaccharides with several interesting functional properties, in which sulphate substitution patterns can provide specific biological functions.

In the last years, SPs from algae have emerged as an important class of natural biopolymers with applications of great interest. The truth is that the marine environment offers a tremendous biodiversity, and original polysaccharides have been discovered showing a high chemical diversity possessing variations in the carbohydrate backbone, location of the sulphate group(s), and degree of sulphation that is specific of the algae species, as mentioned above. Polysaccharides, in general, and sulphated exopolysaccharides, in particular, are produced by many species of algae (macroalgae and microalgae) and with a broad range of applications: bone joints and used in healthy foods, as well as other applications in various industrial areas (Muhamad et al. 2019).

A study on the biological properties of polysaccharides from marine eukaryotes and marine prokaryotes revealed that polysaccharides from the marine environment could provide a valid alternative to traditional polysaccharides. However, SPs are one of the most interesting biopolymers found in nature with the diverse range of potentials for use in medical, pharmaceutical, and biotechnological applications, such as antioxidant, anticoagulant, anti-inflammatory, antiviral, antitumor, and immunostimulatory agents. From the wide range of these compounds, the SPs of red algae have been extensively studied (Lee et al. 2012).

This review focuses on SPs from algae and their biological activities with potential for pharmaceutical and biomedical applications.

2 Structural Characteristics of Sulphated Polysaccharides Produced by Algae

Sulphated polysaccharides are compounds widespread from different sources in nature, having been isolated from animals, plants, and microorganisms. From previous researches it is possible to extract SPs from freshwater algae and seaweed, because their cellular walls are rich in SPs, such as carrageenan in red algae, fucoidans in brown algae, and ulvans in green algae. SPs have been reported to display main biological activities, such as anticoagulant, antiviral, antioxidant, antitumor, antiprotozoal, anti-inflammatory, anticomplementary, immunomodulatory, antibacterial, and antilipemic properties (Lee et al. 2012).

SPs isolated from algae also include sulphated galactan produced by red algae (*Gelidium crinale*), sulphated fucans produced by brown algae (*Ascophyllum nodosum*), and sulphated glucans and sulphated arabinogalactans produced by green algae (*Codium latum* and *Codium divaricatum*). Fucoidan and laminarin are SPs extracted from brown algae and showed for their antitumor antimicrobial, immunostimulatory, and anti-inflammatory activities (Dobrinčić et al. 2020).

2.1 Macroalgae/Seaweed

Marine macroalgae, or seaweeds, are plant-like organisms that grow attached to hard surfaces, such as dead coral or rock, or other hard substrates in coastal areas. Macroalgae are classified belonging to three major groups: green algae (phylum Chlorophyta, classes *Bryopsidophyceae*, *Chlorophyceae*, *Dasycladophyceae*, *Prasinophyceae*, and *Ulvophyceae*), brown algae (phylum Ochrophyta, class *Phaeophyceae*), and red algae (phylum Rhodophyta). Macroalgae represent an important role in the natural product field, because they have been reported as an important source of biomedical compounds, such as antimicrobial, antiviral, anticoagulant, anticancer, antifouling, and antioxidant. Recently, SPs have been developed and rosen interest as medical products, because SPs show many beneficial biological activities, such as anticoagulant, antiviral, antioxidative, antitumor, immunomodulating, antihyperlipidemic, and antihepatotoxic (Jesus et al. 2015).

2.1.1 Freshwater Algae (Macroalgae)

Freshwater algae as other sources of sulphated polysaccharides allow to explore interesting and important biological activities with potential health benefits. At present, SPs have only been reported from marine algae belonging to the genera *Ulva* (38%), *Enteromorpha* (14%), *Monostroma* (14%), and other (34%) (Wang et al. 2014). However, SPs have also been reported from freshwater algae (Table 1).

Table 1 Sources of sulphated polysaccharides from freshwater algae (macroalgae)

Algae group	Species
Green algae	*Cladophora glomerata*
	Cladophora surera
	Ulva flexuosa

Adapted from Ciancia et al. (2020)

2.1.2 Marine Algae (Macroalgae)

Marine macroalgae are potentially novel sources of sustainable biologically active secondary metabolites, which may exhibit various biotechnological applications. Among marine organisms, marine algae have been identified as simple unicellular or multicellular organisms, including seaweeds (macroalgae). Sulphated polysaccharides from marine macroalgae are commercially beneficial compounds with a wide range of pharmaceutical and biomedical applications. The SPs found mainly in marine seaweeds and their structures depend on the seaweed classes, Chlorophyta (green algae), Phaeophyta (brown algae), and Rhodophyta (red algae) (Ngo and Kim 2013). Well-known sulfated polysaccharides in seaweeds include galactans from red algae, ulvans from green algae, and fucans and fucoidans from brown algae. Therefore, SPs from different marine algae have been isolated and are summarized in Table 2.

2.2 Microalgae and Cyanobacteria

Microalgae and cyanobacteria are specifically attractive as natural sources of bioactive metabolites for drug discovery. Most bioactive compounds produced by microalgae include carotenoids, glycolipids, polysaccharides, and proteins. Furthermore, microalgae are valuable sources of structurally diverse bioactive compounds, including alkaloids, polyketides, cyclic peptides, polysaccharides, phlorotannins, diterpenoids, sterols, quinones, and lipids found in macroalgae as well (Talero et al. 2015).

Sulphated polysaccharides are found from many microalgae strains. Thus, microalgae display an important source of polysaccharides, the main producers, such as diatoms, chlorophytes, prasinophytes, haptophytes, rhodophytes, and dinoflagellates (Jesus et al. 2015).

2.2.1 Freshwater Microalgae

Currently, sulphated polysaccharides were found mostly in marine seaweeds and in the animal kingdom. Many researchers reported SPs from marine seaweeds and have studied their pharmacological activities, and some of these sulphate polysaccharides have been developed as new drugs for antitumor, antivirus, anticoagulant, and antihyperlipidemia treatments (Sigamani et al. 2016). Thus, some of freshwater algae are producers of SPs (Table 3).

Table 2 Sources of sulphated polysaccharides from different marine algae (macroalgae)

Algae group	Species
Red algae	*Acanthophora spicifera*
	Achanthophora muscoides
	Agardhiella ramosissima
	Ahnfeltiopsis flabelliformis
	Chondrus crispus
	Corallina officinalis
	Gloiopeltis furcate
	Gracilaria caudata
	Gracilaria cornea
	Gracilaria corticate
	Gracilaria cervicornis
	Gracilaria debilis
	Gracilaria rubra
	Gracilariopsis lemaneiformis
	Grateloupia filicina
	Grateloupia indica
	Grateloupia longifolia
	Grateloupia livida
	Hypnea musciformis
	Jania rubens
	Laurencia obtusa
	Laurencia papillosa
	Lithothamnion muelleri
	Mastocarpus stellatus
	Nemalion helminthoides
	Porphyra haitanensis
	Porphyra yezoensis
	Pterocladia capillacea
	Schizymenia dubyi
	Solieria filiformis
Brown algae	*Adenocystis utricularis*
	Ascophyllum nodosum
	Cystoseria barbata
	Cystoseira compressa
	Dictyopteris divaricata
	Dictyota cervicornis
	Dicty-ota mertensii
	Ecklonia cava
	Ecklonia kurome
	Fucus evanescens
	Fucus vesiculosus
	Hizikia fusiforme
	Kjellmaniella crassifolia

(continued)

Table 2 (continued)

Algae group	Species
	Laminaria japonica
	Lessonia vadosa
	Lobophora variegata
	Padina tetrastromatica
	Sargassum binderi
	Sargassum cristaefolium
	Sargassum duplicatum
	Sargassum fulvellum
	Sargassum fusiforme
	Sargassum hemiphyllum
	Sargassum honeri
	Sargassum pallidum
	Sargassum vulgare
	Spatoglossum schroederi
	Turbinaria conoides
	Turbinaria turbinata
	Undaria pinnatifida
Green algae	*Capsosiphon fulvescens*
	Caulerpa racemosa
	Codium cylindricum
	Codium divaricatum
	Codium fragile
	Codium pugniformis
	Enteromorpha intestinalis
	Enteromorpha prolifera
	Monostroma angicava
	Monostroma latissimum
	Monostroma oxysperma
	Ulva armoricana
	Ulva conglobata
	Ulva fasciata
	Ulva lactula
	Ulva rigida
	Undaria pinnatifida

Adapted from Silva et al. (2012), Ghazali et al. (2017), Manlusoc et al. (2019)

2.2.2 Marine Microalgae

Most of the marine microalgae have been of great interest because they were shown to produce a huge variety of bioactive compounds with biotechnological potential, particularly in the biomedical, pharmaceutical, nutraceutical, and cosmetic areas. Sulphated polysaccharides derived from microalgae have shown to possess a variety of bioactivities with potential applications in the pharmaceutical field, but also in the

Table 3 Sources of sulphated polysaccharides from freshwater algae (Microalgae)

Algae group	Species
Green algae	*Chlamydomonas debaryana*
	Chlorella stigmatophora
	Chlorella vulgaris
	Chlorella variabilis
	Chlorella sp.
	Haematococcus pluvialis
	Tetraselmis sp.

Adapted from Sigamani et al. (2016)

Table 4 Sources of sulphated polysaccharides from different marine algae (Microalgae)

Algae group	Species
Red algae	*Ahnfeltia tobuchiensis*
	Cochlodinium polykrikoides
	Gyrodinium impudicum strain KG03
	Gyrodinium impudicum
	Porphyridium cruentum
	Porphyridium purpureum
	Porphyridium sp.
	Rhodella reticulata
Green algae	*Isochrysis galbana*
	Navicula directa
	Navicula sp.
	Tetraselmis suecica
Diatom	*Phaeodactylum tricornutum*

Adapted from Talero et al. (2015)

food and cosmetic industries (Muhamad et al. 2019). SPs found in marine microalgae are, for example, red and green algae (Table 4).

2.2.3 Cyanobacteria (Blue-Green Algae)

Cyanobacteria or blue-green algae (Table 5) are an ancient and diverse group of photosynthetic microorganisms, and live in different environments, being known to produce extracellular and intracellular secondary metabolites. Many of secondary metabolites from cyanobacteria have been reported to present biological activities, for example, antibacterial, antifungal, algaecide, immunosuppressive, and antiviral (Jesus et al. 2015). Cyanobacteria also constitute an important source of SPs. Thus, some of cyanobacteria are producers of SPs (Table 5).

2.3 Other Sulphated Polysaccharides Produced by Animals, Plants, or Other Microorganisms

Sulphated polysaccharides have been reported mostly from marine algae sources, such as microalgae and macroalgae. Actually, SPs may be isolated not only from

Table 5 Sources of sulphated polysaccharides from cyanobacteria (blue-green algae)

Algae group	Species
Blue-green algae	*Anabaena* sp.
	Aphanocapsa sp.
	Aphanothece halophytica
	Arthrospira platensis (formely *Spirulina platensis*)
	Cyanothece sp.
	Gloeothece sp.
	Nostoc sp.
	Nostoc flagelliforme
	Phormidium sp.
	Synechocystis sp.

Adapted from Raposo et al. (2013)

marine algae, but also from other organisms such as bacteria, fungi, plants, and animals, for example, sulphated polysaccharide from fungi, such as *Antrodia cinnamomea*, *Agaricus blazei*, *Hypsizigus marmoreus*, and *Poria cocos* (Cheng et al. 2012). In addition, four SPs have been identified from plants, including two species of mangrove, *Avicennia schaueriana* and *Rhizophora mangle,* and three species of marine angiosperms, *Ruppia maritima*, *Halophila decipiens*, and *Halodule wrightii* (Aquino et al. 2011). Later on, SPs have been reported in three freshwater plants, *Eichhornia crassipes*, *Hydrocotyle bonariensis*, and *Nymphaea ampla*; moreover, SPs have also been isolated from *Gossipium hirsutum* L (cotton) and *Helianthus tuberosus* (sunroot), *Eleocharis dulcis* (water chestnut), *Psyllium ovata* (blond plantain), and *Moringa oleifera* (drumstick) (Mukherjee et al. 2019).

Animals also are natural sources of SPs, such as three species of sea urchins, *Echinometra lucunter*, *Arbacia lixula*, and *Lytechinus variegatus*, *Meretrix petechialis* (marine clam), *Haliotis discus hannai* Ino (abalone shell), and *Solen marginatus* (grooved razor shell) (Souissi et al. 2019).

Unfortunately, there is scarce information available in the literature about the structural elucidation of the SPs isolated from different organisms in order to allow highlighting the differences.

3 Bioactivity of Sulphated Polysaccharides from Algae. Relation with Chemical Features of Their Structures and Mechanisms of Action

3.1 Polysaccharides from Marine Algae

Polysaccharides are natural polymers, which consist of a single or several types of monosaccharides (Cunha et al. 2009). Sulphated polysaccharides comprise a complex group of macromolecules, whose specific structures are strongly related to a wide range of important biological properties. Thus, these compounds have gained

prominence due to their great applicability, mainly in the areas of foods, biomedical, pharmaceutical, and cosmetics (Lee et al. 2012; Muhamad et al. 2019).

Despite the various sources of polysaccharides, those with industrial applications are essentially extracted from plants (including algae), animals, and fungi, or are obtained via microbiological fermentation (Cunha et al. 2009). SPs from algae are rich sources to obtain various types of polysaccharides and are known as one of the most promising substances for drug administration. This is due to the various low-cost sources, their relative abundance, biocompatibility, biodegradation, unique chemical composition, low toxicity, and low immunogenicity. Thus, SPs obtained from algae have emerged as an important class of natural biopolymers with potential pharmacological applications (Lee et al. 2012).

In general, aquatic and marine algae have been of great interest as excellent sources of nutrients. However, polysaccharides are the main components of algae, although they can vary significantly in seaweed (4–76% w/w) and microalgae (8–64% w/w), according to the species and time of harvest. Therefore, much attention was paid to the isolation and characterization of seaweed polysaccharides due to their number of health benefits (Muhamad et al. 2019). Algae polysaccharides are important sources of raw materials used in food, pharmaceutical, and nutraceuticals industries. In the food industry, the search is for a gelling agent. In the pharmaceutical area, the following biological activities are relevant: antiviral, antithrombotic, anticoagulants, antioxidants, antitumor and antithrombotic agents, and other activities. As algae are routinely harvested in large quantities to be used as food, they become a cheap resource worldwide. Historically, as algae have been consumed for centuries without any identifiable toxicity, they have also gained interest at other industrial levels (Patil et al. 2018). Thus, there has been a focus on red and brown algae for the extraction of polysaccharides of interest (Cunha et al. 2009). However, it is important to note that the polysaccharides from seaweed exhibit structural variations depending on the species, geographic origin, time of harvest, extraction method, processing conditions, and environmental conditions. Structural variations can be in the form of position and degree of sulphation and/or composition of monosaccharides and percentage of uronic acid (Muhamad et al. 2019).

In general, there is a high degree of heterogeneity in size, sulphation, and branching in many polysaccharides used in natural sources. Thus, marine-based polysaccharides have a wide variety of structures that are still underexplored and should, therefore, be considered an extraordinary source of chemical diversity for many applications.

3.1.1 Green Algae Versus Red Algae

The sulphated polysaccharides of green algae are by far the least studied in terms of biological activities. These compounds present more heterogeneity, which makes it difficult to understand the relationship of the polysaccharide with its biological activity (Costa 2008). In addition, green algae remain a poorly explored biomass, particularly in areas where other polysaccharides of algal origin have already proved their value, such as the impressive commercial success of carrageenan (Silva et al. 2012). Although the number of works carried out with these polysaccharides is still minimalist, these

compounds have shown pharmacological importance since they present interesting biological activities, as can be seen in Table 6. The SPs of green algae are rich in galactose, mannose, xylose, arabinose, and/or uronic acids, but there are homopolysaccharides, such as arabinans and galactans. In recent years, the number of studies on SPs of green algae has increased, and several of these studies have reported their molecular structures, which may facilitate the elucidation of the mechanisms of action of these compounds, as well as the activity/structure relationship. For example, ulvan, a sulphated polysaccharide extracted from the green algae *Ulva lactuta*, had low antioxidant power; however, when modified by the addition of benzoyl and acetyl groups, it started to show excellent antioxidant activity, mainly for the superoxide radicals, which was even higher than that observed for vitamin C (Lahaye and Robic 2007).

Table 6 Algae sulphate polysaccharides and their biological activity

Types of polysaccharides	Species	Biological activities
HWE	*Caulerpa recemosa*	Antiviral
Arabinogalactans	*Codium fragile*	Anticoagulant
	Codium vermilara	Anticoagulant
	Codium isthmocladum	Anticoagulant
	Codium cillindricum	Antiangiogenic
	Codium latum	Antiviral
Ulvan	*Ulva pertusa*	Antihyperlipidemic
	Ulva lactuta	Antitumor, antiviral
	Ulva rígida	Immunomodulatory
Ulvan	*Ulva reticulata*	Antihepatotoxic
S-rhamnan	*Monostroma sp*	Anticoagulant, antiviral
Heterorahmnans	*Gayralia oxysperma*	Antiviral: Herpes
Carrageenans	*Maristella gelidium*	Antiviral: Dengue and herpes
	Hypnea musciformis	Antiviral: Dengue and herpes
	Gymnogongrus griffthsiae	Antiviral: Dengue and herpes
	Botryocladia occidentais	Antiviral: Dengue and herpes
Agarans	*Gracilaria birdiae*	
	Gracilaria domingensis	
	Gracilaria cornea	
	Porphyra spiralis	
	Bostrychia montagnei	Antiviral: Herpes
	Acanthophora spicifera	Antiviral: Herpes
Hybrid galactans	*Cryptonemia crenulata*	Antiviral: Dengue and herpes
Fucans	*Dyctyota menstrualis*	
	Dyctyota mertensii	
	Padina gymnospora	
	Spatoglossum schröederi	Anticoagulant, antithrombotic
	Sargassum stenophyllum	Antiviral: Herpes, antitumor
	Sargassum wightii	Antimicrobial

Adapted from Costa (2008), Cunha et al. (2009)

On the other hand, in red algae (Rhodophyta) the most characteristic SPs in their composition are the sulphated galactans, which are subdivided into carrageenans and agarans. Carrageenans are found, essentially, in the species *Soliera*, *Eucheuma*, *Maristiella*, and *Callophucis*, while the agarans in the species *Gracilaria*, *Gelidium*, and *Pterocladias* (Cunha et al. 2009). There are several reports in the literature on pharmacological/biological activities attributed to red algae, one of the most studied is the antiviral activity. All of these studies demonstrate that polysaccharides extracted from Rhodophyta (red algae), essentially galactans, exhibit antiviral activity against a broad spectrum of viruses, including human pathogens, such as human immunodeficiency virus (HIV), herpes simplex virus (HSV), vesicular stomatitis virus (VSV), and cytomegalovirus (CMV), in which the modes of action of these compounds are generally attributed to blocking some early stages of the virus replication cycle (Matsuhiro et al. 2005). However, other types of polysaccharides can also be found in these algae, as we can see in Table 6.

Phaeophyceas, a class of brown algae, have in their constitution a single group of SPs: fucans. These polymers are characterized by the presence of sulphated L-fucose in their structure and can be found in the form of homo- or heteropolysaccharides (Costa 2008). Fucans are assigned a wide range of pharmacological activities. In addition to those previously reported, many other biological activities are shown in the following table (Table 7).

3.2 Bioactivities of Sulphated Polysaccharides from Algae

Polysaccharide bioactivities are closely correlated with their chemical properties, such as molecular sizes, molecular weight, rheological properties, types and proportions of constituent monosaccharides, and characteristics of glycosidic bonds. Algae-derived polysaccharides with clearly elucidated compositions/structures identified cellular activities and desirable physical properties have shown the potential that can create new opportunities to exploit them to the fullest to serve as therapeutic tools for biomedical applications, such as immunoregulatory agents or pharmaceutical delivery vehicles. Thus, a basic understanding of the physicochemical properties and biological activities of algal polysaccharides is essential for successful application and will help to access their multifunctional applications (Lee et al. 2012).

The therapeutic potential of natural bioactive compounds, such as polysaccharides, combined with natural biodiversity will allow the development of a new generation of therapies. In particular, algal polysaccharides have real potential for the natural discovery of therapeutic applications, of which sulphated fucans and sulphated galactans are the most studied (Muhamad et al. 2019).

3.2.1 Antiviral, Antibacterial, and Antifungal Activities

Sulphated polysaccharides, in particular carrageenan, fucoidan, and ulvan, have shown to be of great interest, due to their ability to act as antimicrobial agents against human pathogens (Pierre et al. 2011; Jun et al. 2018). Antiviral activity is probably the most studied activity exhibited by SPs from marine microalgae. Its

Table 7 Pharmacological activities associated with fucans obtained from algae

Biological activities	Algae
Angiogenic	*Fucus vesiculosus*
Anticomplement	*Ascophyllum nodosum* *Fucus evanescens* *Laminaria cichorioide* *Laminaria japonica*
Anti-immigration	*Ascophyllum nodosum* *Fucus vesiculosus*
Antiadhesive	*Ascophyllum nodosum* *Laminaria brasiliensis* *Sargassum schröederi* *Sargassum stenophyllum*
Anticoagulant	*Ascophyllum nodosum* *Dictyota menstrualis* *Eisenia bicyclis* *Ecklonia kurome* *Padina gmynospora*
Antioxidant	*Fucus vesiculosus* *Laminaria japónica*
Antiproliferative	*Ascophyllum nodosum*
Antithrombotic	*Ascophyllum nodosum*
Antitumor	*Ascophyllum nodosum* *Eisenia bicyclis* *Graffenrieda caudata* *Sargassum thumbergii*
Anti-ulcer	*Cladosiphon okamuranus*
Antiviral	*Cystoseira indica* *Sargassum horneri*
Antimetastatic	*Fucus vesiculosus*
Cell-cell binding block via selectin	*Fucus vesiculosus*
Sperm-epithelium binding block in the oviduct	*Fucus vesiculosus*
Fibrinolytic	*Ecklonia kurome* *Fucus vesiculosus*

Adapted from Costa (2008)

inhibitory effects on viral replication have been reported for more than six decades. Polysaccharides and SPs have shown viral activity against a wide variety of viruses involved, including human papilloma virus (HPV), myocarditis encephaloma virus, hepatitis virus (types A and B), dengue virus and yellow fever, and the human immunodeficiency virus (HIV) (type 1 and 2) (Wijesinghe and Jeon 2011; Muhamad et al. 2019). The action of inhibiting the sulphated polysaccharide involves blocking the virus from binding to the surface of the host cell, inhibiting virus-induced syncytium formation or even inhibiting the replication of the virus involved, such as HIV, human cytomegalovirus (HCMV), and the respiratory syncytial virus (RSV), inhibiting, therefore, virus adsorption or virus entry into host cells (Wijesinghe and Jeon 2011). Some SPs are effective only if applied simultaneously to the virus or

immediately after infection. In general, and as mentioned in the previous paragraph, in relation to HIV, SPs act preventing the replication of the virus, even at low temperatures, whereas antiviral activity increases with the level of sulphation and the molecular weight (MW). Anti-HIV activity has also been reported for several algae, such as *Schizymenia pacifica*, *Eucheuma cottoni*, and *Nothogenia fastigiate.* A more detailed study demonstrated that the antiviral activity of sulphated galactans from *Chondrus ocellatus* was related to MW, those of smaller size presenting higher activity. However, further studies are needed to prove this theory (Zhou et al. 2004).

An article published by Matsuhiro et al. reported antiviral activity of a sulphated galactan isolated from *Spirulina binderi*. This compound was highly selective against herpes simplex virus types 1 and 2, with selectivity indexes (cytotoxicity/antiviral activity) higher than 1000 for all tested virus strains (Matsuhiro et al. 2005). In addition, no cytotoxic effects were observed when cell viability was assessed. The authors thought these results regarding the high selectivity of the compound were directly related to its unusual sulphation pattern. Bouhlal et al. evaluated the antiviral activity of SPs isolated from two species of red algae: *Sphaerococcus coronopifolius* and *Boergeseniella thuyoides*. The results were positive regarding the inhibition of the replication of the HSV-1 and HIV-1 viruses in vitro at concentrations that have no effect on cell viability. The HSV-1 adsorption stage in the host cell appears to be the specific target of the polysaccharide action. While for HIV-1, these results suggest a direct inhibitory effect on HIV-1 replication, controlling the appearance of new generations of viruses and having a potential virucidal effect (Bouhlal et al. 2011). Also, Gomaa and Elshoubaky developed a study on the antiviral activity of algal polysaccharides. This study showed that the natural product carrageenan, a polysaccharide from red algae *Acanthophora specifira* and brown algae *Hydroclathrus clathratus,* could inhibit the herpes simplex virus (HSV-1) and the rift valley fever virus (RVFV), and presented low cytotoxicity (Gomaa and Elshoubaky 2016).

Certain SPs from marine algae are also known for their antimicrobial activities against human bacterial pathogens, in addition to their physiological benefits. Thus, the antimicrobial and antibiofilm activities of SPs from different marine algae have been evaluated in several studies (Jun et al. 2018). For example, Pierre et al. tested, in vitro, the antimicrobial activity of green marine algae *Chaetomorpha aerial*. The polysaccharide isolated from this alga, composed of 18% arabinose, 24% glucose, and 58% galactose, exhibited selective antibacterial activity against three gram-positive bacteria, including *Staphylococcus aureus* (ATCC 25923) (Pierre et al. 2011).

In another research, Berri et al. reported the study of an aqueous extract of marine sulphated polysaccharide, prepared from the green macroalgae *Ulva armoricana*, regarding the antibacterial activity against 42 bacterial strains and isolates found in animals. The results show that the growth of gram-positive and gram-negative bacteria was affected, the most susceptible pathogens being *Pasteurella multocida*, *Mannheimia haemolytica*, *Erysipelothrix rhusiopathiae*, *Staphylococcus aureus*, and *Streptococcus suis*, with the minimum inhibitory concentration ranging from 0.16 to 6.25 mg mL^{-1} (Berri et al. 2016). Also, Jun et al. reported that among several sulphated polysaccharide obtained from seaweed, the fucoidan isolated from *Fucus vesiculosus* showed remarkable antimicrobial activity against dental plaque bacteria,

as well as some foodborne pathogenic bacteria. The minimum inhibitory concentrations were from 125 to 1000 μg mL^{-1}. Regarding the antibiofilm activity, the fucoidan at concentrations above 250 μg mL^{-1} completely suppressed the biofilm formations and planktonic cell growths of *Streptococcus mutans* and *S. sobrinus*. However, no eliminative effect on the completed biofilm was observed (Jun et al. 2018). Regarding the antifungal activity, studies on SPs are scarce and their activity against human pathogens is poorly documented.

3.2.2 Antiproliferative, Tumor Suppressor, Apoptotic, and Cytotoxicity Activities

There has been an increasing demand for natural therapies with bioactive compounds against cancer, since the side effects of synthetic chemicals and other types of treatment against tumor are sometimes very aggressive for the patient (Raposo et al. 2013). There are some works in the literature that have shown SPs from marine algae to present antitumor activities. These sulphated compounds can directly affect tumor cells by acting through the inhibition of metastasis and/or the proliferation of tumor cells by binding to growth factors and cell adhesion molecules. On the other hand, other sulphated polysaccharide can induce apoptosis and differentiation of tumor cells, although the mechanism is uncertain. Thus, the antitumor mechanism of sulphated polysaccharide can, in part, be explained by the direct effect on tumor biology (Manlusoc et al. 2019).

In addition to all the activities mentioned so far in relation to sulphated fucoidans, these compounds may also exhibit antiproliferative activity. For fucans, the blocking of the cell cycle preventing the proliferation of tumor cells and the activation of different apoptosis pathways, especially via caspases seem to be the main antitumor mechanisms. However, other studies show fucans acting through different mechanisms, in particular by potentiating NK cells and increasing the production of IFN (interferon gamma) in T cells or preventing cell adhesion to the extracellular matrix (Maruyama et al. 2006).

Another study, carried out by Zhou et al., highlights the antitumor activity of galactans, but, this time, isolated from the seaweed *Chondrus ocellatus*. The tests were performed in vivo on the S-180 sarcoma and H-22 hepatoma strains. The results demonstrate a positive correlation between the tumor inhibition and an increased activity of NK (Natural Killer) cells and lymphocyte proliferation (Zhou et al. 2004).

3.2.3 Anticoagulant and Antithrombotic Activities

Polysaccharides derived from algae may act as an anticoagulant, as they can inhibit thrombin, activate antithrombin III, or prolong the duration of intrinsic (via activation of contact of all components present in the blood) and extrinsic (via the tissue factor TF) pathways. However, some research attested that the molecules were involved in the prothrombin pathway, resulting in effect only in the intrinsic pathway. On the other hand, SPs play a more important role, as they contain sulphate, in the processes of coagulation and/or platelet aggregation. Anticoagulant activity depends on the type of polysaccharides, for example, sulphated galactans depend

on the nature of the sugar residue, the sulphating position and the sulphating content (Ngo and Kim 2013).

Red algae polysaccharides are by far the most studied for anticoagulant potential, since 1941, when this potential was discovered in extracts of algae of the genus *Laminaria* (Costa 2008). SPs from algae, as well as those obtained from invertebrate animals, have been investigated as anticoagulant and antithrombotic agents, due to the structural similarity with heparin (Cunha et al. 2009). Although heparin is a potent anticoagulant widely used in the clinic area, it has some limitations, which include pharmacokinetic varieties, residual hemorrhagic effect, thrombocytopenia, and inhibition of platelet function, among others. These limitations have led to the development of new anticoagulant agents, and among the alternative sources we have the SPs of marine algae, such as fucans (Lee et al. 2012).

Studies carried out on seaweed present their SPs as one of the main groups of compounds with anticoagulant/antithrombotic potential. Thus, these compounds are seen as promising drugs for the replacement of heparin, mainly due to the high structural diversity, which provides the possibility of presenting a different mechanism of action than heparin, but also because they reduce the possibility of contamination by viral particles. These polysaccharides are prone to form soluble complexes with plasma proteins, such as fibrinogen, and their mechanism of action occurs mainly through thrombin inhibition. For example, SPs isolated from the Rhodophyta *B. occidentalis* and *G. crinale* showed different biological effects, which are depending of their structural variations. Such structural variations produced anti- and procoagulant actions, as well as anti- and prothrombotic (Fonseca et al. 2008).

Due to their high sulphate content, SPs, in particular carrageenan, also show interest in coagulation processes. Patil et al. presented the hypothesis that highly sulphated groups of carrageenan could act as heparin sulphate and be a potent anticoagulant. However, they failed to find a conclusive link between carrageenan and coagulation. Carrageenan was suggested to have an antithrombin effect and disrupted platelet aggregation (Patil et al. 2018).

Fucoidans exhibit anticoagulant activity that is largely dependent on the seaweed from which they are extracted. For most fucoidans, increases in aPTT and thromboplastin time (TT) were observed on a scale comparable or superior to heparin; many fucans can also act indirectly in altering the coagulation process, such as promoting the release of TFPI (tissue factor pathway inhibitor) or heparan sulphate, a sulphated glycosaminoglycan produced by endothelial cells that has antithrombotic action. Therefore, these compounds have the potential to be a natural alternative to heparin Also, rhamnan sulphate, an equally important but less studied compound than other marine polysaccharides, has shown some anticoagulant properties. A low MW form has shown to have higher anticoagulant activity than heparin at high concentrations, as determined by the aPTT test (Wang et al. 2012; Manlusoc et al. 2019).

Although green algae polysaccharides are the least explored, there are reports in the literature on their anticoagulant activity. The first report was by Deacon-Smith, who worked with 45 species of *Codium* and found that the polysaccharides of these

algae, mostly galactans, showed anticoagulant activity (Costa 2008). In general, these algae synthesize homo- and sulphated heteropolymers rich in galactose, arabinose, mannose, or glucose. However, the presence of these monosaccharides was detected in several ketone fractions, which indicates that the alga does not synthesize a single type of polysaccharide formed by galactose or arabinose, but two families of sulphated galactans and of sulphated arabinans.

3.2.4 Antilipidemic Activities

Sulphated polysaccharides from seaweeds are potent inhibitors of human pancreatic cholesterol esterase, an enzyme that promotes its absorption at the intestinal level; this inhibitory effect is enhanced by higher MW and degree of sulphation.

Qi and Sun conducted an in vivo study on a sulphated polysaccharide derivative obtained from the species *Ulva pertusa* (*Chlorophyta*) in order to assess its antioxidant activity (it will be described in the Sect. 3.2.6) (Qi and Sun 2015). The study carried out on the liver of hyperlipidemic rats allowed to conclude that this SP can be used as an antihyperlipidemia agent.

3.2.5 Anti-Inflammatory and Immunomodulatory Activities

Inflammation is part of the complex biological response of the immune system to harmful stimuli, such as pathogens, damaged cells, and toxic compounds. Algal polysaccharides can reduce the pro-inflammatory state or other harmful conditions, such as allergic reactions to this activity during the intrinsic immune response. The immunomodulatory properties of algal polysaccharides can make the immune system to function better and can be strengthened in the right way to respond to certain infections. These properties can be demonstrated in several ways depending on the polysaccharide, its source, and type as well as the inflammation site (Muhamad et al. 2019).

Fucoidans are a complex heterogeneous group of SPs that have strong anti-inflammatory effects. Fucoidan has been established as an inhibitor of L-selectin, which mediates leukocyte rolling and neutrophil adhesion to inflamed endothelial cells, and it was used as a blocking agent that could reduce inflammation. Recently, Fernando et al. carried out a study on fucoidans from two species of brown algae, *Chnoospora minimal* (CMF) and *Sargassum polycystum* (SPF). Both species showed good dose-dependent anti-inflammatory effects, reducing the levels of the nitric oxide (NO) inflammation mediator. In addition, the corresponding increase in the viability of the cells tested meant that these fucoidans had protective effects against inflammation induced by lipopolysaccharides (LPS). The reduction in NO levels was related to the reduced production or activity of iNOS. In addition, fucoidan samples regulated the dose of PGE2, thus revealing its anti-inflammatory effects as inhibitors of prostaglandin production, which was related to the reduction of COX-2 levels. The ability to downregulate pro-inflammatory cytokines TNF-α, IL1β, and IL6 was another interesting feature of these compounds. These observations reflected that both CMF and SPF had a wide range of anti-inflammatory effects (Fernando et al. 2018).

Many animal models submitted to anti-inflammatory treatments are centered on the ability of carrageenan to induce local inflammation, playing the role of a pro-inflammatory agent. This is normally associated with the number of sulphate groups. However, unlike other modes of inflammation, histamine and 5-hydroxytryptamine do not participate in inflammation induced by carrageenan.

Purified polysaccharides extracted from an edible green alga, *Caulerpa lentillifera,* were evaluated in vitro for their anti-inflammatory activity. One of the tested compounds had a better anti-inflammatory effect, which might probably be related to the presence of sulphate groups; another study carried out on polysaccharides obtained from this algae species identified a new type of xylogalactomannans, which differ in MW, composition of monosaccharides, and in the content of uronic acids and sulphate groups leading to various activities, including immunomodulatory activity. The in vitro evaluation of immunostimulatory activity revealed that all fractions significantly stimulated macrophages, improving phagocytosis, NO production, and acid phosphatase activity in macrophages (Sun et al. 2020).

In addition, Pérez-Recalde et al. tested the SPs extracted from the red algae *Nemalion helminthoide* to determine, in vitro and in vivo, their immunomodulatory activities. Two fractions of xylomanan induced in vitro proliferation of macrophages from the murine cell line RAW 264.7 and significantly stimulated the production of NO and cytokines (IL-6 and TNF-α) in the same cells. On the other hand, the fraction of mannan did not exhibit this effect. In tests carried out in vivo, xylomanans were also shown to be immunomodulatory agents of interest (Pérez-Recalde et al. 2014).

3.2.6 Antioxidant Activities and Sequestration of Free Radicals

Recently, studies on polysaccharides of marine origin have shown that these polymers play an important role in the sequestration of free radicals responsible for a wide range of disorders (Fig. 1), including cardiovascular diseases, ischemia, and Alzheimer's disease, in addition to being directly involved in inflammation, aging, and cancer formation.

The main mechanism of antioxidant action of algal sulphated polysaccharide consists in the elimination or in the inhibition of appearance of free radicals (free radicals of superoxide, hydroxyl, 1,1-diphenyl-2-picryl-hydrazil (DPPH)). In addition, they have total antioxidant capacity and a strong capacity as reducing agents and ferrous chelators (Wang et al. 2014). Ulvans are water-soluble sulfated heteropolysaccharides obtained from the cell walls of green marine macroalgae of genus *Ulva*; this natural polymer can be chemically modified, an example of which is the addition of functional groups to promote its antioxidant abilities: acetylated and benzoylated ulvan exhibited stronger antioxidant effects compared to natural ulvan. High sulphate content and ulvan MW led to an increased antioxidant activity. The sulphate content appears to be especially important in the antioxidant effect of ulvan. Also, Qi and Sun concluded that, due to the antioxidant activity, a polysaccharide derivative with a high sulphated content obtained from the species *U. pertusa* (*Chlorophyta*) was effective in protecting the liver tissue of hyperlipidemic rats (Qi and Sun 2015).

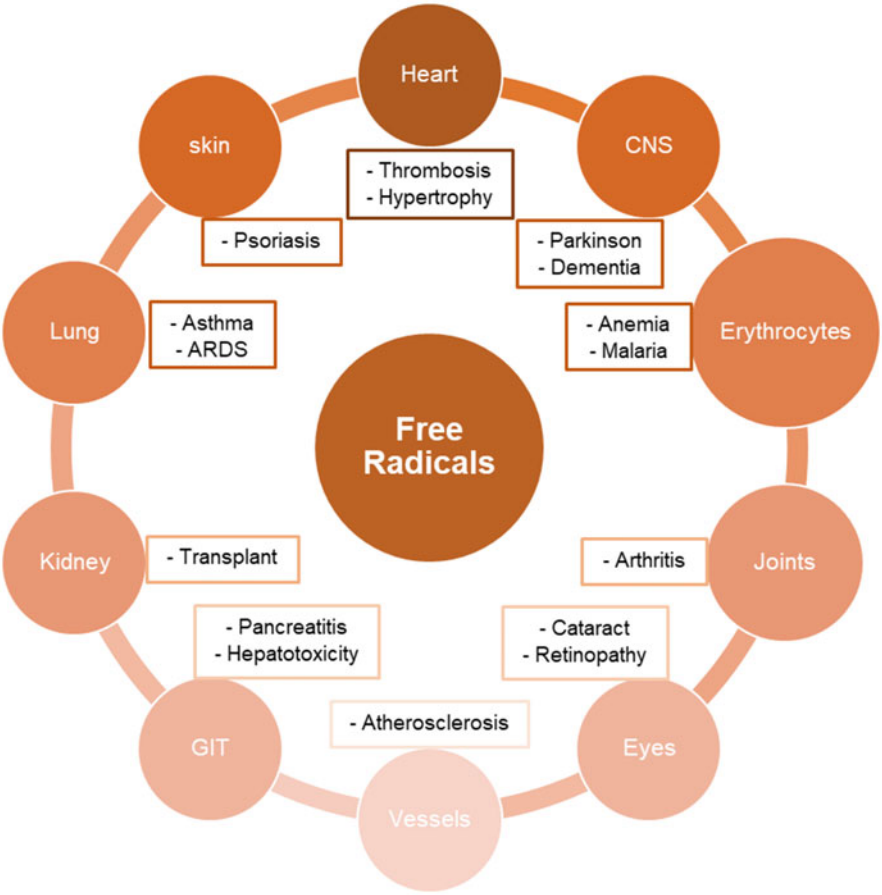


Fig. 1 Dysfunctions associated with free radicals. (Adapted from Costa 2008)

Antiaging Activity

The collagen and elastin fibers produced by fibroblasts are two of the main components of fibrous connective tissues that maintain the smooth and healthy appearance of human skin. The degradation of these fibers is mainly mediated by matrix metalloproteases (MMPs), which include the enzymes collagenase, serine protease, and elastase (Fig. 2). Thus, assessing the inhibition of these enzymes is a general approach for assessing the anti-wrinkle potential of a substance. Fucoidan, one of the most known SPs, is an effective inhibitor of collagenase and elastase.

For example, Wijesinghe and Jeon reported that the incorporation of fucoidans in cosmetic preparations showed improvements of the skin or in the cosmetic action, preventing and relieving skin aging conditions, such as freckles, wrinkles, and diseases (Wijesinghe and Jeon 2011). Fucoidan can effectively deregulate matrix metalloproteases (MMPs) involved in the degradation of connective tissues by activating MMP inhibitors. In addition, fucoidans exert mixed reversible

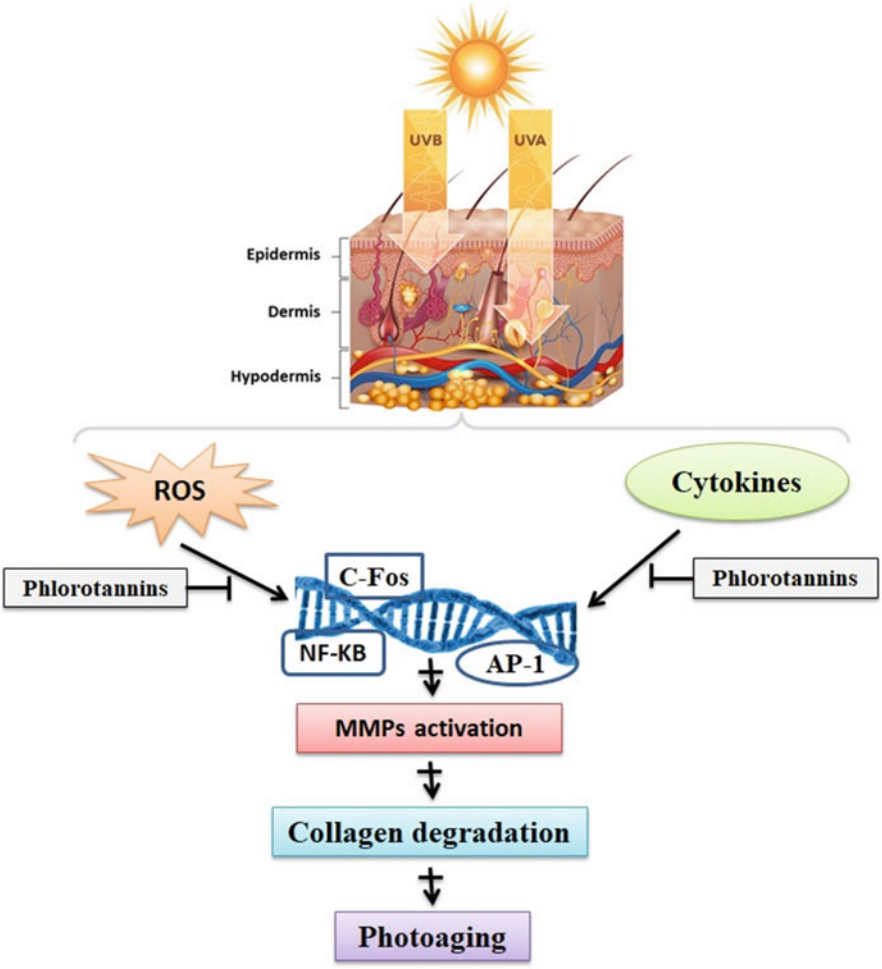


Fig. 2 Schematic example of one extrinsic factor, the UV radiation, that promotes aging. It penetrates into epidermal tissues, causing exfoliation of the dermal matrix. Degradation is caused by the activation of cytokines and enzymes collagenase and elastase. These enzymes further accelerate the generation of ROS, which in turn generate DNA damage. The combined effect of UVA and UVB leads to ROS generation, which directly activate MMPs, following multiple cascade pathways transcribing pro-inflammatory and/or proapoptotic genes. (Adapted from Thomas and Kim 2013)

(competitive and noncompetitive) inhibitory actions on tyrosinosis, which is a critical enzyme mediator in melanin biosynthesis. The antimelanogenic activity will be discussed in Sect. 3.2.7.

Wang et al. evaluated the antiaging activity by comparing the absorption and moisture retention properties of polysaccharide extracts from five different species of seaweed. With this study they concluded that the polysaccharides extracted from

brown seaweed (more precisely the fucoidan obtained from *Saccharina japonica*) exhibited the best capacity for absorption and moisture retention, while the green ones showed worse results. This ability of polysaccharides was influenced by their sulphated content, MW (chain length), and type of algae from which they are extracted (Wang et al. 2014). Also, Fernando et al. concluded that SPs from brown algae had antioxidant and anti-inflammatory properties and were capable of inhibiting collagenase and elastase, showing potential as anti-wrinkle cosmeceutical ingredients (Fernando et al. 2018).

3.2.7 Other Biological Activities

Other pharmacologic/biological activities attributed to the presence of sulphated polysaccharides in marine algae have been documented, especially in vivo tested. Wang et al. described fucoidan tyrosinase inhibiting activity as being of a reversible mixed type, and in silico studies have revealed that fucoidan pentameric and hexameric units have the best binding interactions with the localized copper ion tyrosinase active site. Thus, once the activity of the tyrosinase enzyme is inhibited, there is a greater control of the early reactions of the melanin synthesis pathway, preventing the appearance of spots and/or the darkening of the skin (Wang et al. 2012). Fernando et al. also developed some studies, which showed that fucoidans isolated from brown algae, *Chnoospora minima* and *Sargassum polycystum*, exert a direct inhibitory effect on tyrosinase, as well as the inhibition of tyrosinase during the synthesis of melanin in keratinocytes (Fernando et al. 2018). Thus, these compounds are promising to be incorporated as skin-lightening agents in cosmeceuticals due to their biocompatible properties. Among the different sources of polysaccharides, algal polysaccharides, such as fucoidans and, especially, their low MW derivatives, may play an important role in the future development of cell therapy and regenerative medicine. Thus, the desirable physical properties of polysaccharides derived from algae can create new opportunities that can serve as therapeutic tools for biomedical applications, such as immunoregulatory agents or medication delivery vehicles (Muhamad et al. 2019).

3.3 Case Study: Atherosclerosis

It was the year 1970 that boosted the interest in polysaccharides derived from seaweed as lipid-lowering drugs and inhibitors of the formation of atherosclerotic plaques. Currently, there is a great clinical need for therapies that can reduce the incidence and progression of atherosclerotic vascular disease beyond what is already possible with current treatments. Polysaccharides are, thus, seen as attractive therapies for atherosclerosis due to their low cost, availability, and low toxicity. SPs have potential against risk factors for atherosclerosis, including lipid reduction, coagulation, oxidative stress, inflammation, and modulation of the microbiome. In particular, fucoidan is a polysaccharide of great interest in this area. A potential mechanism of action for fucoidan activity in atherosclerosis is modified lipid uptake and metabolism, where levels of total cholesterol, triglycerides, and low-density

lipoprotein (LDL) were significantly reduced, while levels of high-density lipoprotein (HDL) suffered an increase. On the other hand, it has also been shown that fucoidan altered signaling through ROS. As ROS can modify LDL and LOX-1 is regulated under pro-inflammatory conditions, its decreased expression under treatment with fucoidan suggested that this marine polysaccharide reduced oxidative stress in atherosclerosis. Although the reduction of oxidative stress and inflammatory markers in the aorta might be one of the side effects of hepatic lipid metabolism, ROS was known to cause an interruption in the liver homeostasis. However, there was no consensus on the mechanism of action. Nevertheless, due to the additional effect of fucoidan on dyslipidemia, the liver was identified as a possible target for research focused on hepatic lipid metabolism. This suggested that fucoidan modified the development of atherosclerotic plaque by increasing lipid metabolism and decreasing lipid synthesis and absorption. With respect to carrageenan, it is considered to be a complex candidate for the treatment of atherosclerosis, including against the formation of atherosclerotic plaques (Patil et al. 2018).

4 Potential Medical/Biomedical Applications of Sulphated Polysaccharides from Marine Algae

The materials found in the marine environment are of great interest, since its chemical and biological diversity is unique. The biomedical field is constantly looking for new biomaterials, with innovative properties. Thus, natural polymers appear as the materials of choice for this purpose due to their biocompatibility and biodegradability. In particular, SPs have received increasing attention for their applicability in health-related fields (Silva et al. 2012). SPs may be isolated from plants, animals, and microbial organisms. Marine algae (red, brown, and green) are rich sources of SPs and they may display many health beneficial nutraceutical effects, such as anticancer, antioxidant, antitumor, anti-allergic, immunity enhancement, anticoagulant, antiviral, antihyperlipidemic, and antihepatotoxic activities. Therefore, SPs are a multipurpose group of materials widely explored to develop biomaterials for fulfilling various biomedical applications, such as drug delivery, tissue engineering scaffolds, wound healing materials, and anticancer agents (Ngo and Kim 2013).

Thus, in marine algae, the presence of sulphate radicals and their positions in the chemical structure have been prerequisites for these macromolecules to exhibit important biological activities of interest in biomedicine.

4.1 Tissue Engineering

As a response to trauma or tissue disease, the human body responds in order to remodel the injured tissue. However, when these efforts are not achieved the result starts in a dysfunctionality and may culminate in tissue failure. To solve this problem, science has focused on developing new alternative therapeutic solutions to overcome the drawbacks of current clinical practices. Thus, tissue engineering

emerges as a multidisciplinary approach that uses the principles of engineering and life sciences to create a new tissue that will be implanted at the patient's injury site. Thus, it is necessary that certain properties, especially biocompatibility, both in the implanted form and after degradation, are present throughout the process and that it occurs in a controlled way. Regenerative medicine has brought new hope to the treatment of numerous diseases and conditions.

In this context, several techniques and a wide range of materials have been proposed, including natural and synthetic polymers, or a combination of both. It should be noted that the degree of sulphation, MW, and structural composition may influence cellular behavior, with changes being observed in different physiological but also pathological processes. These are important aspects to be considered in the process of tissue regeneration using polymers as alternative therapy material. Due to the enormous potential of sulphate groups, scientific communities have been looking for alternative ways to obtain them. These polysaccharides can be obtained from marine organisms, in particular, macroalgae (Jesus et al. 2015).

As an underexploited resource, marine environments are an extraordinary source of biomaterials and, in particular, SPs. In addition to their biological activity and potential pharmaceutical use, SPs from green algae can also be used for biomedical applications, in areas as demanding as regenerative medicine. In this sense, several researches have already been carried out related to the processing and development of biomaterials as well as work inherent to the modification of polysaccharides, mainly using ulvan as starting material (Silva et al. 2012).

Fucoidan is one of the most studied SPs in terms of tissue engineering. This compound, generally extracted from brown seaweed, in combination with different polymers, such as chitosan or polycaprolactone (PCL), and processed in hydrogels, scaffolding films and nanofibers, has an enormous potential for cell support systems. An example of this is a study by Sezer et al. who propose the use of a fucoidan-chitosan hydrogel as a fast healing agent. The use of chitosan is, thus, proposed due to its hydrogel-forming properties and advantageous use in applications as dressings, adding to the excellent anticoagulant activity of fucoidan, among other properties (Sezer et al. 2008). Another study addresses a rapid prototyping methodology resulting in a mixture of fucoidan with polyesters (PCL). This procedure resulted in a porous structure suitable for bone tissue regeneration, where low MW fucoidan promoted cell proliferation in addition to osteoconductive properties, including alkaline phosphatase activity, type I collagen expression, and deposition minerals (Lee et al. 2012).

However, not all polysaccharides are suitable for tissue engineering, mainly due to their gelatinous consistency and insufficient mechanical properties. Thus, and as mentioned in this topic, SPs are generally combined with other natural or synthetic polymers, or reinforced with inorganic substances.

4.2 Drug Delivery

Sulphated polysaccharides for drug delivery substances are usually not administered as they are in their pure state, but rather as part of a formulation where they are

frequently combined with other agents (excipients). Often, SPs used for excipients act as simple inert supports of the active molecule(s). Currently, the specific application of some polysaccharides and/or SPs are in pharmaceutical formulations; for example, they are used in the manufacture of solid monolithic matrix systems, implants, films, beads, microparticles, nanoparticles, inhalable, injectable systems, and hydrogel formulations. Fucoidan is a SP with application in pharmaceutical, as fucoidan-based drug carriers, such as nanoparticles, microparticles, and hydrogels, while chitosan sulphates have been used to design microcapsules, as carrier systems for drug delivery. Nevertheless, Sezer and Akbuğa reported fucoidan from brown seaweed *Fucus vesiculosus* for the development of a new microspheres delivery system based on cross-linking of fucoidan with chitosan named fucosphere, and evaluated it as a drug carrier with extent of drug release being dependent on the concentrations of the polymers and protein (Sezer and Akbuğa 2006). In addition, Tziveleka et al. discovered that ulvan, a sulphated polysaccharide isolated from the green algae *Ulva rigida*, showed antibacterial activity with gram-positive bacterial strain *Staphylococcus aureus*, while this compound has potential for the development of drug delivery nanoplatforms (Tziveleka et al. 2018).

4.3 Anticancer Agents

Currently, there are more than 200 different types of cancers, such as lung cancer, breast cancer, liver cancer, cervical carcinoma, and pancreatic cancer. Chemotherapy and radiotherapy are cancer treatments, but they can cause many adverse secondary reactions in the patients. Thus, sulphated polysaccharides can be an important and nontoxic natural source for potential use in cancer treatments. Some SPs have already been reported to demonstrate anticancer and cancer-preventive properties. Fucoidan isolated from the seaweed *Cladosiphon novae-caledoniae* was capable of enhancing the anticancer effect of a chemotherapeutic drug against MDA-MB-231 and MCF-7 breast cancer cells (Zhang et al. 2013). Another fucoidan isolated from the brown seaweed *Fucus vesiculosus* displayed cytotoxic effects on MCF-7 and MDA-MB-231 human breast cancer cell lines in in vitro tests. Moreover, sulphated polysaccharide isolated from brown seaweeds, *Saccharina japonica* and *Undaria pinnatifida,* showed high antitumor activity and inhibited the proliferation and colony formation of human breast cancer T-47D and melanoma SK-MEL-28 cell lines (Vishchuk et al. 2011). In addition, sulphated polysaccharide with a MW of 197 kDa isolated from the filamentous algae *Tribonema* sp. showed significant anticancer activity on HepG2 cells with a 66.8% inhibition at a concentration of 250 μg/mL (Chen et al. 2019).

4.4 Immune Function

Nowadays, immunomodulatory studies on sulphated polysaccharides from marine algae have been performed, mainly from *Undaria pinnatifida*, *Caulerpa lentillifera*,

and *Hypnea spinella*. On the other hand, SPs from microalgae, *Phaeodactylum tricornutum*, *Chlorella stigmatophora*, *Pavlova viridis*, *Thraustochytriidae*, and *Spirulina,* have been studied regarding the human immune system (Muhamad et al. 2019). Fucoidan from the brown alga *Undaria piaantifida* has a variety of immunomodulatory effects on lactic acid bacteria (LAB), by stimulating cytokine production from antigen-presenting cells (APCs). The results indicated that fucoidan could enhance a variety of beneficial effects of LAB on immune functions (Kawashima et al. 2012). Karnjanapratum et al. showed that sulphated polysaccharides isolated from *Capsosiphon fulvescens* strongly stimulated macrophage cells, RAW264.7 cell line, producing considerable amounts of nitric oxide (NO), prostaglandin E_2 (PGE_2), and cytokines, which suggested that they could be strong immunostimulators (Karnjanapratum et al. 2012). In other in vitro and in vivo tests, sulphated xylomannans, a water-soluble sulphated polysaccharide from the red seaweed *Nemalion helminthoides*, exhibited strong immunomodulators (Pérez-Recalde et al. 2014). In addition, a sulphated polysaccharide named UP2-1 isolated from *Ulothrix flacca* exhibited significant in vitro immunomodulating activity and another sulphated polysaccharide isolated from microalgae *Tribonema* sp. exhibited significant immune-modulatory activity on cytokines, including upregulating interleukin 6 (IL-6), interleukin 10 (IL-10), and tumor necrosis factor α (TNF-α), by enhancing macrophage cell (Chen et al. 2019).

4.5 Wound Healing

Natural polysaccharides can stimulate the extracellular matrix synthesis and facilitate the wound healing. In general, wound healing concerns different phases, for example, hemostasis, inflammation, proliferation, angiogenesis, and scar formation. Sulphated polysaccharides are emerging as high-value drugs and biomaterials for use in wound management and tissue engineering. Some SPs have shown therapeutic potential for using in wound healing. Sezer et al. reported a chitosan film with fucoidan from *Fucus vesiculosus* for the treatment of dermal burns on rabbits, the results showing that chitosan-fucoidan films might be a potential treatment system for dermal burns (Sezer et al. 2008). In another study, fucoidan polysaccharides were isolated from two species of seaweeds, *Padina tetrastromatica* and *Padina boergesenii*, which exhibited significant wound healing effects (Kordjazi et al. 2017).

4.6 Antipathogenic and Anti-Inflammatory

In a study of Chen et al., fucoidan isolated from the brown seaweed *Ulva pinnatifida* inhibited the invasion of erythrocytes by *Plasmodium falciparum* (Chen et al. 2009). However, fucoidan extracted from the brown seaweed *Fucus vesiculosus* displayed anti-inflammatory effects, through the inhibition of production of inflammatory mediators, nitric oxide (NO), and prostaglandin E2 (PGE2), in LPS-stimulated BV2 microglia, by suppressing nuclear factor kappa B (NF-κB), p38 mitogen-

activated protein kinase (MAPK), and Akt pathways. In addition, fucoidans-sulphated polysaccharide from brown algae, *Chnoospora minima* and *Sargassum polycystum,* showed anti-inflammatory effects on LPS-stimulated RAW 264.7 cell macrophages (Fernando et al. 2018). Sanjeewa et al. reported the anti-inflammatory effect of sulphated polysaccharide purified from *Sargassum horneri,* using LPS-stimulated RAW 264.7 cells. This sulphated polysaccharide inhibited the LPS-induced NO production and PGE_2 production, by inhibiting the MAPK and NF-kB signaling pathways (Sanjeewa et al. 2017).

5 Clinical Trials

A phase II of clinical trial involved oral administration of seaweed extracts (*Fucus vesiculosus* 10%, w/w; *Macrocystis pyrifera* 10%, w/w; *Laminaria japonica*, 5% w/w; zinc, vitamin B6 and manganese). After 12 weeks, the results revealed a decrease in the dose-dependent osteoarthritis in five female and seven male participants, with no adverse health effects (Myers et al. 2010). Carrageenan a sulphated polysaccharide was incorporated into four food items and distributed to 20 human volunteers to determine its effects on cholesterol, blood, and lipid levels. The results showed that carrageenan could significantly reduce serum cholesterol and triglyceride levels, presenting anticoagulant properties and also a regulatory role, mainly in growth factors (Panlasigui et al. 2003). In addition to the benefits of regular algae consumption, many studies have indicated the health benefits of algae supplementation along with a regular diet. Hence these organisms continue to be increasingly explored in the areas of nutrition, pharmacology, cosmetics, and many other.

6 Conclusions

Unlike many artificial chemical inhibitors in certain pharmaceutical and cosmetic products, sulphated polysaccharides are biocompatible polymers with a wide range of desirable functionalities. SPs from seaweed and microalgae exhibit many beneficial biological activities such as anticoagulant and/or antithrombotic, immunomodulator, antitumor and cancer preventive, hypoglycemic, antibiotics and anti-inflammatory, and antioxidant properties. In addition, the interest in using algal polysaccharides as biomedical vehicles for drug delivery has increased steadily. Thus, the structural analyses of polysaccharides from algae, as well as specific biological tests on them, are useful tools to investigate molecular mechanisms of possible biological activities, in addition to their obvious practical implications. Therefore, the isolation of a new algal sulphated polysaccharide always brings perspectives for the discovery of new drugs, as well as an increased economic development, by introducing a higher diversity of products and, eventually, products with high added value.

Acknowledgments This work was supported by National Funds from FCT – Fundação para a Ciência e a Tecnologia through Project UID/Multi/50016/2019 and project EXTRATOTECA-COMPETE2020 POCI-01-0247-FEDER-033784.

References

Aquino RS, Grativol C, Mourão PA. Rising from the sea: correlations between sulfated polysaccharides and salinity in plants. PLoS One. 2011;6(4):18862.

Berri M, Slugocki C, Oliver M, et al. Marine-sulfated polysaccharides extract of *Ulva armoricana* green algae exhibits an antimicrobial activity and stimulates cytokine expression by intestinal epithelial cells. J Appl Phycol. 2016;28(5):2999–3008.

Bouhlal R, Haslin C, Chermann JC, et al. Antiviral activities of sulfated polysaccharides isolated from *Sphaerococcus coronopifolius* (Rhodophytha, Gigartinales) and *Boergeseniella thuyoides* (Rhodophyta, Ceramiales). Mar Drugs. 2011;9(7):1187–209.

Chen JH, Lim JD, Sohn EH, et al. Growth-inhibitory effect of a fucoidan from brown seaweed *Undaria pinnatifida* on *Plasmodium parasites*. Parasitol Res. 2009;104(2):245–50.

Chen X, Song L, Wang H, et al. Partial characterization, the immune modulation and anticancer activities of sulfated polysaccharides from filamentous microalgae *Tribonema* sp. Molecules. 2019;24(2):322.

Cheng JJ, Chang CC, Chao CH, et al. Characterization of fungal sulfated polysaccharides and their synergistic anticancer effects with doxorubicin. Carbohydr Polym. 2012;90(1):134–9.

Ciancia M, Fernández P, Leliaert F. Diversity of sulfated polysaccharides from cell walls of coenocytic green algae and their structural relationships in view of green algal evolution. Front Plant Sci. 2020;11:554585. https://doi.org/10.3389/fpls.2020.554585.

Costa LS. Atividades biológicas de polissacarídeos sulfatados extraídos da alga vermelha *Gracilaria caudata*. Universidade Federal do Rio Grande do Norte, Brazil; 2008.

Cunha PLR, Paula RCM, Feitosa JPA. Polissacarídeos da biodiversidade brasileira: uma oportunidade de transformar conhecimento em valor econômico. Química Nova. 2009;32:649–60.

Dobrinčić A, Balbino S, Zori Z, et al. Advanced technologies for the extraction of marine brown algal polysaccharides. Mar Drugs. 2020;18(3):168.

Fernando IPS, Sanjeewa KKA, Samarakoon KW, et al. The potential of fucoidans from *Chnoospora minima* and *Sargassum polycystum* in cosmetics: antioxidant, anti-inflammatory, skin-whitening, and antiwrinkle activities. J Appl Phycol. 2018;30(6):3223–32.

Fonseca RJC, Oliveira ANM, Melo FR, et al. Slight differences in sulphation of algal galactans account for differences in their anticoagulant and venous antithrombotic activities. Thromb Haemost. 2008;99(3):539–45.

Ghazali FC, Aroyehun AQB, Razak SA. Acknowledging sulfated polysaccharides from marine macroalgae multi-functional properties. Haya: Saudi J Life Sci. 2017;2:269–83.

Gomaa HH, Elshoubaky GA. Antiviral activity of sulfated polysaccharides carrageenan from some marine seaweeds. Int J Curr Pharm Rev Res. 2016;7(1):34–42.

Jesus RMF, Morais AM, Morais RM. Marine polysaccharides from algae with potential biomedical applications. Mar Drugs. 2015;13(5):2967–3028.

Jun JY, Jung MJ, Jeong IH, et al. Antimicrobial and antibiofilm activities of sulfated polysaccharides from marine algae against dental plaque bacteria. Mar Drugs. 2018;16(9):301.

Karnjanapratum S, Tabarsa M, Cho M, et al. Characterization and immunomodulatory activities of sulfated polysaccharides from *Capsosiphon fulvescens*. Int J Biol Macromol. 2012;51(5):720–9.

Kawashima T, Murakami K, Nishimura I, et al. A sulfated polysaccharide, fucoidan, enhances the immunomodulatory effects of lactic acid bacteria. Int J Mol Med. 2012;29(3):447–53.

Kordjazi M, Shabanpour B, Zabihi E, et al. Investigation of effects of fucoidan polysaccharides extracted from two species of Padina on the wound-healing process in the rat. Turk J Vet Anim Sci. 2017;41(1):106–17.

Lahaye M, Robic A. Structure and functional properties of ulvan, a polysaccharide from green seaweeds. Biomacromolecules. 2007;8(6):1765–74.
Lee JS, Jin GH, Yeo MG, et al. Fabrication of electrospun biocomposites comprising polycaprolactone/fucoidan for tissue regeneration. Carbohydr Polym. 2012;90(1):181–8.
Manlusoc JKT, Hsieh CL, Hsieh CY, et al. Pharmacologic application potentials of sulfated polysaccharide from marine algae. Polymers. 2019;11(7):1163.
Maruyama H, Tamauchi H, Iizuka M, et al. The role of NK cells in antitumor activity of dietary fucoidan from *Undaria pinnatifida sporophylls* (Mekabu). Planta Med. 2006;72(15): 1415–14157.
Matsuhiro B, Conte AF, Damonte EB, et al. Structural analysis and antiviral activity of a sulfated galactan from the red seaweed *Schizymenia binderi* (Gigartinales, Rhodophyta). Carbohydr Res. 2005;340(15):2392–402.
Muhamad II, Zulkifli N, Selvakumaran SAP, et al. Bioactive algal-derived polysaccharides: multifunctionalization, therapeutic potential and biomedical applications. Curr Pharm Des. 2019;25(11):1147–62.
Mukherjee S, Ghosh K, Hahn F, et al. Chemically sulfated polysaccharides from natural sources: assessment of extraction-sulphation efficiencies, structural features and antiviral activities. Int J Biol Macromol. 2019;136:521–30.
Myers SP, O'Connor J, Fitton JH, et al. A combined phase I and II open label study on the effects of a seaweed extract nutrient complex on osteoarthritis. Biologics. 2010;4:33–44.
Ngo DH, Kim SK. Sulfated polysaccharides as bioactive agents from marine algae. Int J Biol Macromol. 2013;62:70–5.
Panlasigui LN, Baello OQ, Dimatangal JM, et al. Blood cholesterol and lipid-lowering effects of carrageenan on human volunteers. Asia Pac J Clin Nutr. 2003;12(2):209–14.
Patil NP, Le V, Sligar AD, et al. Algal polysaccharides as therapeutic agents for atherosclerosis. Front Cardiovasc Med. 2018;5:153.
Pérez-Recalde M, Matulewicz MC, Pujol CA, et al. *In vitro* and *in vivo* immunomodulatory activity of sulfated polysaccharides from red seaweed *Nemalion helminthoides*. Int J Biol Macromol. 2014;63:38–42.
Pierre G, Sopena V, Juin C, et al. Antibacterial activity of a sulfated galactan extracted from the marine alga *Chaetomorpha aerea* against *Staphylococcus aureus*. Biotechnol Bioprocess Eng. 2011;16(5):937–45.
Qi H, Sun Y. Antioxidant activity of high sulfate content derivative of ulvan in hyperlipidemic rats. Int J Biol Macromol. 2015;76:326–9.
Raposo MFJ, Morais RMSC, Morais AMMB. Bioactivity and applications of sulphated polysaccharides from marine microalgae. Mar Drugs. 2013;11(1):233–52.
Sanjeewa KKA, Fernando IPS, Kim EA, et al. Anti-inflammatory activity of a sulfated polysaccharide isolated from an enzymatic digest of brown seaweed *Sargassum horneri* in RAW 264.7 cells. Nutr Res Pract. 2017;11(1):3–10.
Sezer A, Akbuğa J. Fucosphere-new microsphere carriers for peptide and protein delivery: preparation and *in vitro* characterization. J Microencapsul. 2006;23(5):513–22.
Sezer AD, Cevher E, Hatipoglu F, et al. Preparation of fucoidan-chitosan hydrogel and its application as burn healing accelerator on rabbits. Biol Pharm Bull. 2008;31(12):2326–33.
Sigamani S, Ramamurthy D, Natarajan H. A review on potential biotechnological applications of microalgae. J Appl Pharm Sci. 2016;6(8):179–84.
Silva TH, Alves A, Popa EG, et al. Marine algae sulfated polysaccharides for tissue engineering and drug delivery approaches. Biomatter. 2012;2(4):278–89.
Souissi N, Boughriba S, Abdelhedi O, et al. Extraction, structural characterization, and thermal and biomedical properties of sulfated polysaccharides from razor clam *Solen marginatus*. RSC Adv. 2019;9(20):11538–51.
Sun Y, Liu Z, Song S, et al. Anti-inflammatory activity and structural identification of a sulfated polysaccharide CLGP4 from *Caulerpa lentillifera*. Int J Biol Macromol. 2020;146:931–8.
Talero E, García MS, Ávila RJ, et al. Bioactive compounds isolated from microalgae in chronic inflammation and cancer. Mar Drugs. 2015;13(10):6152–209.

Thomas NV, Kim SK. Beneficial effects of marine algal compounds in cosmeceuticals. Mar Drugs. 2013;11(1):146–64.

Tziveleka LA, Pippa N, Georgantea P, et al. Marine sulfated polysaccharides as versatile polyelectrolytes for the development of drug delivery nanoplatforms: Complexation of ulvan with lysozyme. Int J Biol Macromol. 2018;118:69–75.

Vishchuk OS, Ermakova SP, Zvyagintseva TN. Sulfated polysaccharides from brown seaweeds *Saccharina japonica* and *Undaria pinnatifida*: isolation, structural characteristics and antitumor activity. Carbohydr Res. 2011;346(17):2769–76.

Wang ZJ, Si YX, Oh S, et al. The effect of fucoidan on tyrosinase: computational molecular dynamics integrating inhibition kinetics. J Biomol Struct Dyn. 2012;30(4):460–73.

Wang L, Wang X, Wu H, et al. Overview on biological activities and molecular characteristics of sulfated polysaccharides from marine green algae in recent years. Mar Drugs. 2014;12(9): 4984–5020.

Wijesinghe W, Jeon YJ. Biological activities and potential cosmeceutical applications of bioactive components from brown seaweeds: a review. Phytochem Rev. 2011;10(3):431–43.

Zhang Z, Teruya K, Yoshida T, et al. Fucoidan extract enhances the anti-cancer activity of chemotherapeutic agents in MDA-MB-231 and MCF-7 breast cancer cells. Mar Drugs. 2013;11(1):81–98.

Zhou G, Sun YS, Zhang Y, et al. *In vivo* antitumor and immunomodulation activities of different molecular weight lambda-carrageenans from *Chondrus ocellatus*. Pharmacol Res. 2004;50(1): 47–53.

Natural Polymers for Biophotonic Use

39

Rita Rebelo, Mariana Caldas, Miguel A. D. Neves, Subhas C. Kundu, Rui L. Reis, and Vitor Correlo

Contents

Rita Rebelo and Mariana Caldas contributed equally with all other contributors.

R. Rebelo · S. C. Kundu · R. L. Reis
3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Zona Industrial da Gandra, Barco, Guimarães, Portugal

ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães, Portugal

M. Caldas · M. A. D. Neves · V. Correlo (✉)
Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, I3B's Research Institute on Biomaterials Biodegradables and Biomimetics, Universidade do Minho, Barco, Guimarães, Portugal

ICVS/3B's – PT Government Associated Laboratory, Braga/Guimarães, Portugal
e-mail: vitorcorrelo@i3bs.uminho.pt

J. M. Oliveira et al. (eds.), *Polysaccharides of Microbial Origin*,
https://doi.org/10.1007/978-3-030-42215-8_50

Abstract

The constant development of optical techniques for medical diagnostics and therapies has led to a growing need to develop and optimize biophotonic applications. Through biophotonics, it is possible to precisely control light propagation, scattering, and emission, enabling a different range of applications, from light-activated therapies to sensing and drug delivery technologies. Particularly, the use of biocompatible materials with desired mechanical, optical, biological, and chemical properties is essential to enable clinically relevant biophotonic devices. Thus, natural polymers such as chitosan, cellulose, keratin, silk, and agarose are promising building blocks for the development of multifunctional biophotonic structures due to their biocompatibility, mechanical characteristics, renewability, and surface chemistry.

In this chapter is presented an overview of recent advances in medical biophotonics applications, using natural polymers, from imaging to sensing and phototherapy uses. Herein, the light phenomena in biophotonics, the most used natural polymer properties and state-of-the-art biophotonics applications will be explored.

Keywords

Natural polymers · Optical applications · Biocompatibility · Photonic structures

1 Introduction

Photonics is the science of photon (light) generation, detection, and processing. The term originated somewhere in the 1960s and 1970s with the integration of the first practical semiconductor light emitters with optical fibers. The emergence of low intensity loss optical fibers led to the replacement of copper wires for most long distance and transcontinental telecommunications with fiberoptic wires, which now serve as the backbone of high-speed data transmission networks (Quimby 2006).

Biophotonics is the science of production and use of photons or light to study optical procedures in biological systems. It results from the integration of four major technologies: lasers, photonics, biotechnology, and nanotechnology and it is applied in several fields. Biomedical applications include light interactions in medicine and biology for the purposes of health care. More specifically, biophotonics can be used for imaging and detecting cells and tissues, drug delivery systems, sensing devices, and injection of fluorescent markers, to track cells or molecules. Additionally, biophotonics can also be used for light scattering applications, in a micro- or macroscale, such as for microscopy or tomography, respectively.

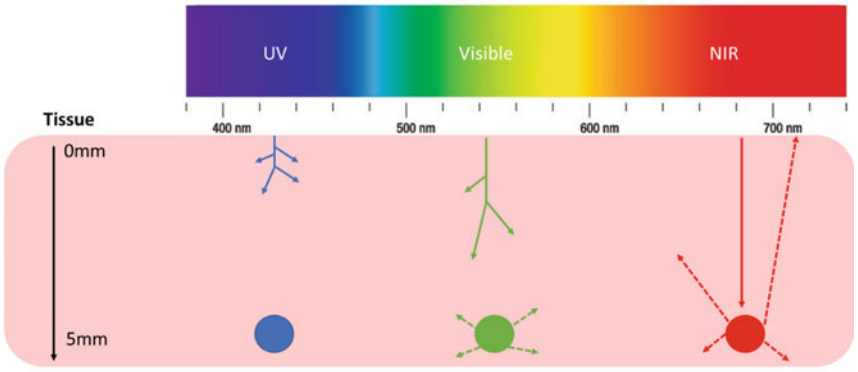
The development of this technology allows for monitoring the progression of and early detection of diseases, light-guided and light-activated therapies that allow a better understanding of the pathobiology (Shan et al. 2018). These techniques are able to precisely control light propagation, scattering, and emission via hierarchical structures and chemistry, allowing biophotonic applications for transparency, camouflaging, protection, mimicking, and signaling (Xiong et al. 2020). The features that affect the most the potential of biophotonic structures are the optical properties, mechanical properties, chemical structures, and the biological functionalities. However, the most fundamental requirement for optical materials suitable for biophotonic is its capability to achieve high-efficiency light delivery with diminished loss. In the current materials, silica is the most used of the optical material platform due to its considerable optical properties that include a high transparency over a large spectral range from visible to near-infrared (NIR), which makes it suitable for different possible applications (Tong et al. 2003). Nevertheless, its fragile properties make it a risk to use in biological tissues; it is also nonbiodegradable and, as the traditional silica, has low biocompatibility. Therefore, there is a need to find better materials that are biodegradable and biocompatible, and natural occurring polymers can be a great substitute as next-generation materials in optical sensing (Shan et al. 2017). For biophotonics applications, the materials used are essential to produce optical structures, such as waveguides, i.e., a physical structure that guides electromagnetic waves in the optical spectrum, and lenses, which are optical components that are designed to either focus or diverge light that will transmit, detect, and transform light. Thus, an optical material can be defined as the material that can control or alter the electromagnetic radiation in the ultraviolet, visible, and infrared spectral region (Shan et al. 2018). The most important property of this material is its degree of transparency and its refractive index, so when a material has high transparency, it will have low reflection, absorption, and scattering, which are the desired features. Polymeric biomaterials are typically nonhomogeneous due to a somewhat disordered network of polymer chains and macromolecules and usually are not considered for optical devices applications (Shanmugam and Sahadevan 2018). Nevertheless, despite the relatively high optical loss, polymeric optical biomaterials can still meet the distance of interest between the network spaces in most biomedical applications. Therefore, optically transparent polymeric biomaterials are emerging as key materials, as they often have advantages over inorganic silica like soft mechanical properties, biocompatibility, biodegradability, as well as desired chemical and biological functionalities. Furthermore, polymeric optical biomaterials can be easily manufactured into complex structures with high optical efficiency. The advantages mentioned above have motivated researchers to pay more attention to the development of advanced natural polymer structures for biophotonic applications.

2 Applications of Biophotonics

One of the branches of biophotonics is optical bioimaging, which refers to cellular imaging techniques that acquire images of biological matter using fluorescent probes, labels, or nanomaterials. These can be small fluorescent organic molecules, fluorescent proteins, and nanoparticles; the last of which has been gaining major focus over the last decade (Yang et al. 2019a). When it comes to fluorescent probes, they must have emission in a suitable range of detection (400–800 nm), a specific excitation wavelength, a high quantum yield, molar absorption coefficient, and photostability. However, a fluorophore can lose its ability to fluoresce when excited several times or for long durations, a phenomenon known as photobleaching. The loss of fluorescence is caused by the absorbed excitation energy, rather than being emitted as fluorescence, is absorbed by the molecule causing the cleavage of covalent bonds or nonspecific covalent reactions with surrounding molecules. This phenomenon is a major problem in the use of these molecules for high-intensity excitation and long-lifetime applications, such as imaging or sensing (Ma et al. 2017). Some nature-inspired optical systems based on bacteria have been produced to overcome photobleaching. However, these systems present several disadvantages such as: inherent variability, limited sources, and restrictive engineering potential. Such disadvantages led to the use of other natural or synthetic polymers and inorganic materials for the development of biophotonic systems (Shan et al. 2018; Prasad 2004).

Therefore, nanoparticles and nanomaterials have arisen as a promising alternative. The majority of these fluorescent nanoparticles and nanomaterials are inorganic; however many have been constructed of inorganic natural (bio)polymer hybrids and natural polymers. When compared to conventional small molecule fluorophores, these materials present good emission, contrast, and photostability, along with their nanometric size, allow for efficient and fast cellular internalization, similar to that of small molecule fluorophores (Owens et al. 2015). Thus, optical bioimaging is a noninvasive, high-sensitivity, and high-resolution tool that is able to perform effective and low-cost diagnostics of different wide-ranging diseases and imaging in wide-ranging cells and tissues. The techniques range from the traditional luminescence to more recent, state-of-the-art techniques, such as photoacoustic imaging and surface-enhanced Raman spectroscopy (SERS); where biophotonic techniques are used as a mean to diagnose several diseases using light-responsive materials (Son et al. 2019), natural biopolymer biphotonic materials have shown to provide improved biocompatibility over inorganic and hybrid materials, hence are preferred for certain applications, which has led to increased development (Ma et al. 2017).

Luminescence is based on the light (energy) emission of certain materials by either chemical or physical processes and includes photoluminescence (fluorescence or phosphorescence), chemiluminescence, and electroluminescence. Regarding these, the fluorescence imaging is the most representative on biophotonics because



Photon Traveling	Complete Absorption by Tissue	Absorption & Scattering	Deep Tissue Imaging
Wavelength (nm)	< 400	400-650	650-900
Spectrum	Ultraviolet	Visible	Near-infrared
Excitation laser	None	Low	High
Absorption & Scatter	Very High	High	Low
Photon Emission	None	Low	High
Autofluorescence	High	High	Low
Cyanine Fluorophores	None	Mono-/Trimethine	Penta-/Heptamethine

Fig. 1 In vivo optical properties of injected fluorophores along with wavelength

it uses light from microscope imaging to be used in intraoperative image-guided surgery. The major problems regarding this technique are the autofluorescence of the endogenous biomolecules, which is the light emitted from the background tissues that leads to an increase of excitation light, and the depth of the tissue penetration. One way to counterbalance this effect is to use NIR, which wavelength of the electromagnetic radiation spectrum ranges 650–900 nm (Owens et al. 2015). NIR fluorescence can be advantageous for biophotonics because of the low absorption of endogenous hemoglobin and water that can disperse the light, the scattering effect, and also minimize the autofluorescence, resulting in a better tissue penetration of NIR light (Fig. 1).

It is important to understand that fluorophores are at the ground state level on energy (S0) in a singlet state and, when they absorb light, they move to an excited state (Sn) (Fig. 2). When they return to the S0 level of energy is when fluorescence is emitted; therefore, the controlling of energy and retention in the excited state is very important for imaging purposes (Son et al. 2019).

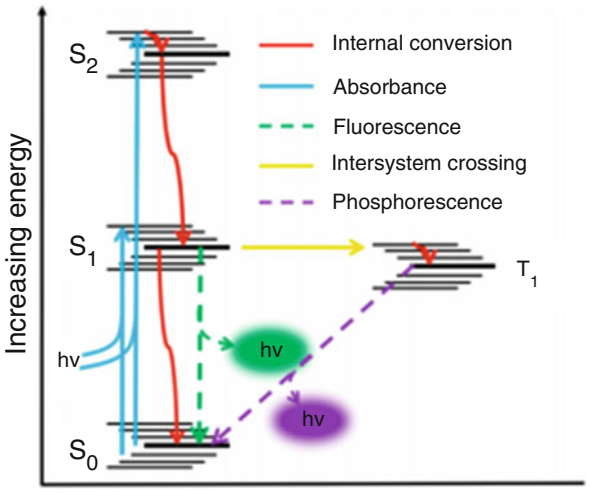


Fig. 2 A Simplified Jablonski diagram. (Owens et al. 2016). (Figure reproduced with permission from American Chemical Society)

2.1 Photoacoustic Imaging

A hybrid technique based on the generation of acoustic signals by the absorption of optical energy, photoacoustic imaging (PAI), has emerged to overcome the limitations of current optical imaging, such as light diffusion and photon penetration depth. This process consists in the absorption of energy by endogenous chromophores within a tissue (or in some cases an injected tracer); these chromophores convert the absorbed light energy into heat energy that is thermoelastically converted into acoustic waves able to be transduced by an ultrasound probe into a signal (Xia et al. 2014). This technique greatly outperformed the aforementioned optical imaging methods on account of the increased sensitivity, resolution, and the superior depth achieved by the nonionizing laser light source. PAI is a noninvasive technique and can be performed without using any contrast agents because of the endogenous molecules, such as hemoglobin, melanin, lipids, and water, which will absorb electromagnetic energy and generate acoustic waves. Each one of these chromophores has their own characteristic absorption spectra, so multiple wavelengths allow their relative quantification and analyze physiological changes. Nevertheless, the use of exogenous probes (contrast agents), such as fluorophores, IR active molecules (i.e., indocyanine green), polymers, carbon, or gold nanoparticles, will enhance the photoacoustic (PA) signal if the endogenous contrast is not sufficient due to the similarity of absorption spectra of one or more chromophore or in the case of monitoring circulation (Attia et al. 2019; Steinberg et al. 2019; Chaudhary et al. 2019).

2.2 Surface-Enhanced Raman Spectroscopy

Surface-enhanced Raman spectroscopy (SERS) is a noninvasive analytical tool that combines molecular fingerprint information provided by Raman scattering

with the signal-enhancing power of the metallic nanostructures of gold or silver. The Raman effect consists of inelastic light scattering by chemical species with a change in the wavelength of light that occurs when a light beam is deflected by molecules. Through SERS it is possible to obtain qualitative and quantitative chemical information of biomolecules in situ without the need of any external labeling and reach single-molecule detection levels (De Marchi et al. 2019; Fan et al. 2020). In vitro applications of SERS include primarily detection of infectious pathogens, including virus and bacteria via their building blocks, such as DNA and proteins, using hybridization or immunoassay concepts. Another application is cancer diagnostics by analyzing proteins, nucleic acids, circulating tumor cells, immunophenotyping of cancer cells, as well as the detection of several tumor biomarkers produced by cancer cells. SERS is advantageous for its rapid screening, capability of facile multiplexing, and for high sensitivity. Regarding in vivo applications, clinical SERS can be used for guided intraoperative imaging for tumor resection and endoscope-based imaging (Prochazka 2016).

2.3 Optical Coherence Tomography

Optical coherence tomography (OCT) is a noninvasive diagnostic technique that provides cross-sectional images of biological structures based on the optical properties of a different tissue. It is an interferometric technique that uses NIR light waves that reflect off the internal microstructure in a way that, in principle, is analogous to an ultrasonic pulse echo. It is possible to obtain real-time images with excellent axial resolution. OCT offers tomographic imaging of internal tissues with high-resolution 2D or 3D cross-sectional images. In particular, OCT has been highly useful in ophthalmology to analyze optic nerves, the retinal nerve layer and for measuring the stiffness of the cornea (Katkar et al. 2018). When small nanorods, nanostructured objects with a rod shape, are located in the matrix, their Brownian movements are changed by the intermittent collision with neighboring macromolecules. The polarized light scattering produced can reveal the nanotopology of the tissue by OCT. For example, gold nanorods can provide anisotropic optical scattering, which makes it easier to track in native tissue using polarization-sensitive OCT, and in a tissue where there is increased collagen and cell density, the polarization is decreased (Son et al. 2019).

Another possible application is biosensing, where analytical devices enable the sensing of molecular interactions and convert them into a detectable electrical signal (Solaimuthu et al. 2020). Therefore, optical biosensors use optical responses, like intensity, wavelength, or polarization variations, as a result of chemical or biological changes that are correlated with the physiological disorder. Using these devices, it is possible to obtain real-time multiplexed information. To date, many biosensors have been developed to detect biomarkers of innumerous cancer types such as breast cancer, lung cancer, or melanoma and other diseases including Alzheimer's disease (Jayanthi et al. 2017; Yang et al. 2019b; Eftekhari et al. 2019; Carneiro et al. 2020).

2.4 Photodynamic Therapy (PDT)

An area of biophotonics that has received a great deal of attention and development is light-activated therapy. Photodynamic therapy (PDT) is a modern noninvasive therapy, utilized for the treatment of several diseases including a variety of cancers (Beack et al. 2015; Kim et al. 2020; Sun et al. 2019). The technique relies on photosensitive compounds, photosensitizers, which accumulate in the pathological tissues. The photosensitizers absorb the light at a specific wavelength and initiate the activation process, which leads to the destruction of surrounding cells. It is a selective therapy as the photo-cytotoxic reaction only occurs in the pathological tissues where the agent has been administered. Under laser irradiation, the photosensitizer is excited into the excited state leading to the production of singlet oxygen species and free radicals in the surrounding tissue causing damage to local cells; for example, if the photosensitizer accumulated in a tumor, excitation would lead to tumor cell death in the local area around the photosensitizer (Zhang and Li 2018). Photosensitizers accumulate in higher concentrations in cancer cells due to their tendency to combine preferentially with low density lipoproteins (LDL), which supply cholesterol to create cellular membranes. In cancer cells, the division and mitotic process is accelerated where an increased uptake of LDL takes place and in that way the LDL can act as a transporter of the photosensitizer to the tumor. If cancer cells escape the photo-cytotoxic effect directly, they can be destroyed by the indirect influence of PDT on tumor vessels, as a result of reactive oxygen species damage of blood vessel endothelial cells. Furthermore, this blood vessel damage leads to an inflammatory state causing platelet activation and aggregation resulting in the formation of platelet thrombi, which causes local hypoxia resulting in cell death (Kwiatkowski et al. 2018). PDT is proven effective alone or in combination with chemotherapy for the treatment of many cancers, including skin and breast cancer and leukemia (Beack et al. 2015; Kim et al. 2020; Sun et al. 2019).

2.5 Photothermal Therapy

Photothermal therapy (PTT) is another form of biophotonic light-responsive targeted therapy, where the photosensitizer applies heat for tumor ablation. PTT has emerged as a minimally invasive approach to kill cancer cells without damaging the healthy tissue around the pathogenic cells. This strategy is possible as the absorbance of energy by the photosensitizer causes it to release a large amount of vibrational energy, which turns into heat energy (Batul et al. 2017; Cheng et al. 2014). Literature reports indicate that, to cause the destruction of cancer cells, the local temperature should be maintained for a duration of 15–60 min at approximately 43 °C, or for 5 min at over 50 °C (Habash et al. 2006). Owing to the superior tissue penetration and high-resolution adjustability in both duration and three-dimensional space, NIR has been extensively utilized for PTT, as precise control can be achieved. The possibility of inducing heat by NIR light irradiation at the specific tumor site improves the targeting and efficacy of the therapy. Due to the low heat tolerance

of tumor cells, the high temperature is able to destroy them while avoiding damage to normal healthy cells (Wei et al. 2019).

Light-responsive agents, such as nanoparticles, are useful for photo-triggered drug release upon irradiation, which commonly utilized photo-degradable materials, or a photo-induced decrease in hydrophobicity. These strategies allow the stable storage of drugs in these agents while in blood circulation and controlled in situ drug release only in the specific tissue at the desired time. A large number of light-responsive materials that have been developed respond to UV light. However, UV light has a limited tissue penetration depth due to light scattering and absorption by water or other biological substances. Furthermore, UV light is harmful for all cells, both pathogenic and health, which greatly limits applications. Alternatively, NIR can penetrate deep into tissues without damage and, therefore, has been gaining an increased interest for on-demand therapeutic and drug delivery purposes (Cho et al. 2015).

To achieve the full potential of biophotonic applications, the characteristics of the material used are of crucial importance, influencing the overall performance of the system (Shan et al. 2018).

3 Natural Polymers in Biophotonics – Current Uses and Applications

Besides the optical properties needed for biophotonics applications, the biological, chemical, and mechanical properties are also crucial. Thus, optically transparent polymers are emerging as key materials for biophotonics, since they present advantages over traditional optical materials (e.g., silica), including soft mechanical properties, biodegradability, biocompatibility, as well as desired chemical and biological functionalities.

Natural polymers are produced by animals, plants, and microorganisms and present excellent biocompatibility and biodegradability. Additionally, many of those polymers also present good optical properties and are constructed into several well-organized photonic structures (Xiong et al. 2020). These structures offer good mechanical properties, are lightweight, renewable, and of low-cost to produce at large scale. Moreover, many natural polymers present strong adhesion and good responsive properties, can be self-healing, and have tunable properties. Furthermore, natural polymers can suppress the major disadvantage of synthetic polymers: non-degradability and lack of biocompatibility (Xiong et al. 2020; Shan et al. 2018).

The natural polymers most used in biophotonics, as well as their main properties and applications, are presented in Table 1.

3.1 Chitosan

Chitosan, a natural biopolymer, is a cationic polysaccharide produced by deacetylation of chitin, a polysaccharide found in the exoskeleton of almost all vertebrates, obtained from an alkalizing process at high temperatures (Fig. 3) (Younes and Rinaudo 2015).

Table 1 Summary of polymeric biomaterials, their properties and applications for biophotonics

Natural polymers	Natural source	Photonic structure	Optical mechanisms	Properties	Applications	Reference
Chitosan	Insect cuticles/ wings, mollusk shells	Chiral nematic structures, multilayered structure, disordered networks	Chiral photonic crystals, multilayer interference, light scattering, diffraction gratings	Antibacterial effect Biocompatibility Biodegradability Nontoxicity High humidity absorption Optical clarity Mechanical stability	Sensing	Chen et al. (2012), Voznesenskiy et al. (2013), Mironenko et al. (2017) and Razali et al. (2020)
Cellulose	Fruits, flowers	Chiral nematic structures, gratings	Chiral photonic crystals, diffraction gratings	High transmittance of visible light Favorable permeability for water and ions High tensile and compressive strength Nontoxic Biodegradability	Photonic and plasmonic Raman spectroscopy Sensing	Mu and Gray (2014), Espinha et al. (2018), Orelma et al. (2020) and Peng et al. (2011)
Keratin	Avian feathers	Multilayered structure, photonic crystals	Multilayer interference	Durability High toughness and modulus High content of amino acids	Photonic and plasmonic	Zhu et al. (2019)
Silk	Silkworm	Photonic crystals	Crystals	Good mechanical strength and	Eco-dye and multifunctionalization of fabric	Diao et al. (2013), Burke et al. (2016), Arsenault et al. (2007), Colusso et al.

				toughness Flexibility Biocompatibility High transparency	Sensing Photothermal therapy Imaging	(2017), Li et al. (2017), He et al. (2019) and Xu et al. (2018)
Agarose	Seaweeds	Photonic crystals	Crystals	High gel strength Agarose gels are thermo reversible Nontoxic	Drug delivery Sensing Imaging	Jain et al. (2012), Fujiwara et al. (2020) and Gao et al. (2014)

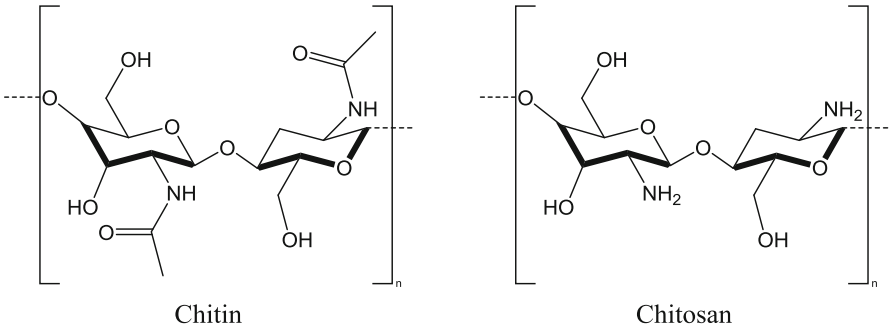


Fig. 3 Schematic representation of chitin and chitosan chemical structures. (Figure reproduced from Younes and Rinaudo (2015))

Chemically, chitin is a cationic amino polysaccharide polymer randomly composed of β-1,4 linked N-(acetyl-D-glucosamine). As chitosan is produced after partial deacetylation of chitin, it presents in its structure D-glucosamine as the major constituent (80%) and N-(acetyl-D-glucosamine) as the minor one (20%). Thus, chitin and chitosan are structurally very similar, with the exception of the less acetyl groups present in the later form (Younes and Rinaudo 2015; Kumar and Kumar 2017; Rebelo et al. 2017).

Widely known for its antimicrobial properties, chitosan is also biodegradable and highly biocompatible. Furthermore, chitosan is polycationic; it can easily interact with polyanionic polymers, is insoluble in water but soluble in weakly acidic aqueous solutions, it is non-immunogenic and mucoadhesive. Due to such versatile properties, chitosan has been broadly used in different forms (membranes, gels, particles, films, or scaffolds) in several applications: from biomedical to industrial areas (Kumar and Kumar 2017; Rebelo et al. 2017).

Chitosan has been used to coat an optical fiber tip (Fig. 4a) to detect Pb^{2+} at 0–70 ppm levels, in water. Chitosan has excellent adsorbent properties for Pb^{2+} due to metal chelating moieties in its structure (as amine and hydroxyl groups). As a result, chitosan refractive index (RI) is modulated by the amount of Pb^{2+} present in the analyte and, consequently, modulates the Fresnel reflectivity at the fiber tip region. The use of the chitosan coating greatly enhanced the sensitivity for the detection of Pb2+; seven-fold higher than the uncoated sensor (Fig. 4b) (Jain et al. 2012). Moreover, chitosan was also applied to form the waveguide as a sensing element of a relative humidity sensor. Different forms of chitosan (salt and neutral) were evaluated, and both led to the production of integrated-optical sensors based on waveguide films with high sensitivity and short response time (He et al. 2019).

3.2 Cellulose

Cellulose is one of the most abundant natural polymers on earth; generally produced by plants, but it can also be synthetized by some bacteria. This natural polymer is a

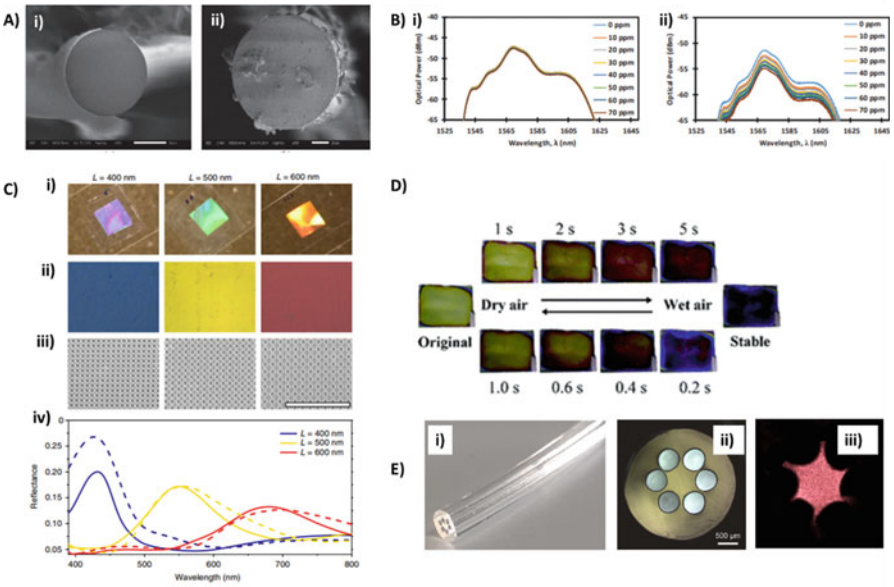


Fig. 4 (**a**) SEM images of sensor (i) before chitosan coating and (ii) after chitosan coating; (**b**) Sensing spectrum of (i) uncoated sensor and (ii) chitosan-coated sensor (Razali et al. 2020); (**c**) (i) Photographs of the hydroxypropyl cellulose (HPC) photonic films (square lattice of imprinted cylindrical holes), for lattice parameters of 400, 500, and 600 nm. Square lateral size is 1 cm, (ii) images of the HPC photonic films acquired with an optical microscope, (iii) SEM micrographs of the HPC photonic films (top view). Scale bar, 5 μm, (iv) Specular reflectance characterization of the samples (solid curves), along with theoretical modeling calculations (dashed curves) (Espinha et al. 2018); (**d**) A cycle test of the color change between humidity levels of ~10% and ~90%. The change can take place in 5 s, with recovery in 1 s upon pumping wet or dry air (Li et al. 2017); and (**e**) (i) agarose-based structured optical fiber: (ii) cross-section view of the end-face and (iii) output speckle field of the core-guided modes. The fiber has 60 mm length, diameters of 0.64 mm, 2.5 mm, and 0.5 mm for core, cladding, and holes, respectively, and bridges of ~0.08 mm width (Fujiwara et al. 2020). (Figures reproduced with permission from AIP Publishing, Springer Nature and Royal society of chemistry)

homopolymer of glucose, in which the monomers are joined by β-1,4 linkages. Due to its long and linear molecular structure, cellulose does not dissolve readily in water. Cellulose chains are arranged in microfibrils, which are organized into fibrils. This arrangement not only provides stability for the plant structures but also infers the high strength and superior mechanical properties of cellulose, when compared with other natural polymers (Torok and Dransfield 2017; Pandey et al. 2019; Nishiyama 2009).

The hydrogen atoms of cellulose are all in the axial position, whereas all the hydroxyl groups are equatorial. These equatorial hydroxyl groups can hydrogen-bond with their nearest neighbors, allowing cellulose to readily crystallize. Additionally, cellulose can be functionalized with many modifiers through these hydroxyl groups in order to form cellulose derivatives with useful properties, like cellulose esters of cellulose butyrate, cellulose acetate, or cellulose triacetate (Pandey et al. 2019; Andreana and Stolow 2014).

Moreover, cellulose features a high transmittance of visible light and favorable permeability for water and ions, which can be useful in biophotonic applications, like optical fibers and sensors (Shan et al. 2018; Andreana and Stolow 2014).

Regenerated cellulose and cellulose acetate have been used as optical sensing fibers. These fibers were prepared by coating regenerated cellulose filaments with cellulose acetate. While cellulose acetate passed the whole light wavelength range, regenerated cellulose absorbed UV light, passing the visible light wavelengths. The prepared optical fiber guided light in the range of 500–1400 nm and when fiber was placed in water, a clear attenuation in the light intensity was observed (Younes and Rinaudo 2015).

In a different strategy, hydroxypropyl cellulose was used to produce photonic and plasmonic structures (Fig. 4c), using soft lithography. This led to a structural color change in this material, which was obtained by exploiting its chiral nematic phase. Patterned cellulose membranes possessed tunable colors and may be used to boost the photoluminescence of a host organic dye. Additionally, metal coating these cellulose photonic structures resulted in plasmonic crystals that presented excellent optical properties and can act as SERS substrates (Gao et al. 2014).

3.3 Keratin

Keratin represents a family of structural proteins that are abundant in nature, specifically in the outer layer of skin, hair, nails, horns, and feathers of animals. Keratin is rich in half cysteine and it possesses the ability to self-assemble into bundles of fibers. Within these fiber bundles, individual strands are further crosslinked through disulfide bonds, mediated by the cysteine side chains. This high sulfur content introduces several inter-/intra-molecular disulfide bridges, allowing a complex hierarchical structure of keratinous materials with polypeptide chains and filament-matrix, forming lamellar/sandwich structures (D'Alba et al. 2011; Xiong et al. 2020).

Keratin can be classified as: α-pattern, β-pattern, feather-pattern, or amorphous pattern, determined by X-ray diffraction. α-pattern keratin can be found in mammals, for example, in wool, hair, and nails, while β-pattern keratin are present in feathers (Xiong et al. 2020; Wang et al. 2016).

Thus, keratin forms durable insoluble structures that are of particularly high toughness (5.6–22.8 kJ/m^2 to 10–80 kJ/m^2) and modulus (10 MPa to 2.5 GPa), which are among the strongest nonmineralized tissues found in nature (Xiong et al. 2020; Wang et al. 2016; Singh et al. 2017).

The capability to pattern natural polymers at different scales is extremely important in several biomedical applications, including biophotonics. Zhu et al. used wool keratin for production of precise protein microarchitectures. Through straightforward biochemical processes, modified wool keratin proteins become intrinsically photoreactive without major changes in protein function or structure. The resultant photoreactive keratin can be photo-crosslinked using a photomask and UV-light. The un-crosslinked water-soluble area can be removed, leaving periodic microstructures for creating brilliant structural colors (Rebelo et al. 2017).

3.4 Silk

Silks are natural protein fibers produced by insect larvae of silkworms, moths, butterflies, and spiders. The raw silk thread, produced by silkworms, is composed of a fibroid core, silk fibroin, and a glue-like coating consisting of sericin proteins. Their hydrophobic β-sheet structures grant high mechanical strength and toughness while providing comparable in vitro and in vivo biocompatibility with other commonly used biomaterials, like collagen. This behavior can be attributed to the β-sheet configuration of silk fibroin, in which van der Waals forces and strong hydrogen bonds generate a structure, which is thermodynamically stable (Shan et al. 2018; Rebelo et al. 2017).

The high flexibility of silk allows the production of several silk forms: films, gels, or scaffolds. Moreover, molecular designability and moderate aqueous processing permits the incorporation of growth factors, cells, or peptides into the silk structures, which is extremely useful in biomedical applications. Regarding biophotonics, silk films with 20 and 100 μm of thickness were found to be great candidates for optical devices, due to their low surface roughness (<5 nm rms) and high transparency (>95%) across the visible spectrum (Shan et al. 2018; Khalid et al. 2020).

For instance, Li et al. used silk fibroin to develop sensing applications. Silk fibroin films were produced by spin coating and demonstrated bright color and high sensitivity to humidity. The optical properties of silk fibroin can be easily tuned by the coating rates. Moreover, due to the high hydrophilicity of silk fibroin, the film presented fast responses to humidity, in just few seconds (Fig. 4d). At humidity above 80%, the red shift of the reflectance peak in the visible spectrum was even larger than 130 nm, corresponding to a significant color change as distinct as yellow to violet. Thus, the silk fibroin film is superior to several other multilayered or photonic crystal-based humidity sensors (Li et al. 2017). In a different application, a silk fibroin nanofiber hydrogel system complexed with upconversion nanoparticles and nano-graphene oxide was developed for upconversion luminescence imaging and photothermal therapy. The injectable system showed excellent upconversion luminescence imaging properties and a photothermal therapy effect under NIR laser irradiation (He et al. 2019).

3.5 Agarose

Agarose is a nontoxic marine-based polysaccharide, derived from agar and extracted from red seaweeds, that has been widely used as a biocompatible material for cell encapsulation, DNA electrophoresis, and tissue regeneration. Agarose is composed by an alternating copolymer of β-1,3-linked d-galactose and α-1,4-linked 3,6-anhydro-α-l-galactose residues. It is a thermally gelling polymer: at temperatures below 35 °C the gelling process occurs due to the formation of an infinite network of 3D agarose fibers, and at temperatures above 85 °C the networks of agarose hydrogel disassemble. In solid state, agarose is brittle, but maintains its shape for a long period of time at a broad range of temperatures. Moreover, agarose is an attractive natural polymer for optical

applications, since it is possible to tune the optical refractive index of agarose hydrogels according to concentration (Shan et al. 2018; Gao et al. 2014; Giuseppe et al. 2019).

Recently, Fujiwara et al. used agarose to develop an optical fiber able to be used in sensing and imaging applications. This fiber was produced by pouring food-grade agar into a mold with stacked rods, forming a solid core surrounded by air holes, in which the fiber geometry and refractive index can be tuned through the mold design and agarose solution composition, respectively (Fig. 4e). The fiber exhibited practical transmittance at 633 nm, in comparison to other hydrogel waveguides, and can also be used for chemical sensing either by detecting volume changes, due to agar swelling or contraction (dehydration), or by modulation of transmitted light, through the insertion of fluids into the air holes (Fujiwara et al. 2020).

4 Conclusion and Future Perspectives

Natural polymers have been used to establish many important technologies in the application of biophotonics. Biomaterials composed of natural chitosan, cellulose, keratin, melanin, silk, and agarose have demonstrated key advantages including biocompatibility, degradability, and suitable mechanics, leading to the development of several optical biomedical devices. The major advancements of natural polymers in biophotonics have focused on diagnostics and treatments, and are featured in sensing, imaging, drug delivery, and therapeutic technologies. However, despite these great features there are still some limitations, namely, the reproducibility and high spatial resolution regarding the current processing of naturally based materials.

Biophotonics is a quickly growing field, driven by the increasing number of patients with chronic diseases in developed countries. According to a report by Grand View Research, San Francisco, CA, it is estimated that the biophotonics market will reach $91.31 billion by 2024 (Grand View Research, San Francisco, C 2016).

Currently, there are still are some limitations that hamper market growth, like the high price and complexity of biophotonics technologies and the inherently slow commercialization process of medical devices. However, these developments in optical technologies and the increasing demand for early diagnosis and home-based point-of-care devices is expected to spur the growth of the market. In future, it will be necessary to bring together the current medical techniques with the broad spectrum of possibilities offered by biophotonics. Laser systems and light-emitting nanostructures will be just few of the biophotonic options. Biophotonics will continue to be a highly active research field, and results will lead to new applications in clinical diagnosis and therapies (Krafft 2016).

The ability to use several natural polymers in different optical applications leads to enormous opportunities and opens new areas in the use of these materials in photonics and in the biomedical field. For that, a deeper understanding of the optical properties of natural polymers will be required for scale-up procedures, therefore allowing reproducibility and lower manufacturing costs. With that, biphotonic natural polymers demonstrate that personalized medicine with green renewable materials can be achieved.

Acknowledgments R. Rebelo would like to acknowledge BREAST-IT PTDC/BTM-ORG/28168/2017 project. M. Caldas would like to acknowledge PD/BD/150476/2019 FCT Grant and V.M. Correlo would like to acknowledge SofTE- PTDC/EMD-EMD/31590/2017 project.

References

Andreana M, Stolow A. Multimodal nonlinear optical microscopy: from biophotonics to geophotonics. Opt Photon News 2014;25:42–49.

Arsenault A, Puzzo DP, Manners I, Ozin GA. Photonic-crystal full-colour displays subjects. Nat Photonics. 2007;1:1–11.

Attia ABE, Balasundaram G, Moothanchery M, Dinish US, Bi R, Ntziachristos V, Olivo M. A review of clinical photoacoustic imaging: current and future trends. Photo-Dermatology. 2019;16:100144. https://doi.org/10.1016/j.pacs.2019.100144.

Batul R, Tamanna T, Khaliq A, Yu A. Recent progress in the biomedical applications of polydopamine nanostructures. Biomater Sci. 2017;5:1204–29. https://doi.org/10.1039/c7bm00187h.

Beack S, Kong WH, Jung HS, Do IH, Han S, Kim H, Kim KS, Yun SH, Hahn SK. Photodynamic therapy of melanoma skin cancer using carbon dot – chlorin e6 – hyaluronate conjugate. Acta Biomater. 2015;26:295–305. https://doi.org/10.1016/j.actbio.2015.08.027.

Burke KA, Brenckle MA, Kaplan DL, Omenetto FG. Evaluation of the spectral response of functionalized silk inverse opals as colorimetric immunosensors. ACS Appl Mater Interfaces. 2016;8:16218–26. https://doi.org/10.1021/acsami.6b02215.

Carneiro P, Morais S, do Carmo Pereira M. Biosensors on the road to early diagnostic and surveillance of Alzheimer's disease. Talanta. 2020;211:120700. https://doi.org/10.1016/j.talanta.2019.120700.

Chaudhary Z, Khan GM, Abeer MM, Pujara N, Wan-Chi Tse B, McGuckin MA, Popat A, Kumeria T. Efficient photoacoustic imaging using indocyanine green (ICG) loaded functionalized mesoporous silica nanoparticles. Biomater Sci. 2019;7:5002–15. https://doi.org/10.1039/c9bm00822e.

Chen LH, Li T, Chan CC, Menon R, Balamurali P, Shaillender M, Neu B, Ang XM, Zu P, Wong WC, et al. Chitosan based fiber-optic fabry-perot humidity sensor. Sensors Actuators B Chem. 2012;169:167–72. https://doi.org/10.1016/j.snb.2012.04.052.

Cheng L, Wang C, Feng L, Yang K, Liu Z. Functional nanomaterials for phototherapies of cancer. Chem Rev. 2014;114:10869–939. https://doi.org/10.1021/cr400532z.

Cho HJ, Chung M, Shim MS. Engineered photo-responsive materials for near-infrared-triggered drug delivery. J Ind Eng Chem. 2015;31:15–25. https://doi.org/10.1016/j.jiec.2015.07.016.

Colusso E, Perotto G, Wang Y, Sturaro M, Omenetto F, Martucci A. Bioinspired stimuli-responsive multilayer film made of silk-titanate nanocomposites. J Mater Chem C. 2017;5:3924–31. https://doi.org/10.1039/c7tc00149e.

D'Alba L, Saranathan V, Clarke JA, Vinther JA, Prum RO, Shawkey MD. Colour-producing β-keratin nanofibres in blue penguin (Eudyptula minor) feathers. Biol Lett. 2011;7:543–6. https://doi.org/10.1098/rsbl.2010.1163.

De Marchi S, Bodelón G, Vázquez-Iglesias L, Liz-Marzán LM, Pérez-Juste J, Pastoriza-Santos I. Surface-enhanced Raman scattering (SERS) imaging of bioactive metabolites in mixed bacterial populations. Appl Mater Today. 2019;14:207–15. https://doi.org/10.1016/j.apmt.2018.12.005.

Diao YY, Liu XY, Toh GW, Shi L, Zi J. Multiple structural coloring of silk-fibroin photonic crystals and humidity-responsive color sensing. Adv Funct Mater. 2013;23:5373–80. https://doi.org/10.1002/adfm.201203672.

Eftekhari A, Ahmadian E, Salatin S, Sharifi S, Dizaj SM, Khalilov R, Hasanzadeh M. Current analytical approaches in diagnosis of melanoma. TrAC Trends Anal Chem. 2019;116:122–35. https://doi.org/10.1016/j.trac.2019.05.004.

Espinha A, Dore C, Matricardi C, Alonso MI, Goñi AR, Mihi A. Hydroxypropyl cellulose photonic architectures by soft nanoimprinting lithography. Nat Photonics. 2018;12:343–8. https://doi.org/10.1038/s41566-018-0152-1.

Fan M, Andrade GFS, Brolo AG. A review on recent advances in the applications of surface-enhanced Raman scattering in analytical chemistry. Anal Chim Acta. 2020;1097:1–29. https://doi.org/10.1016/j.aca.2019.11.049.

Fujiwara E, Cabral TD, Sato M, Oku H, Cordeiro CMB. Agarose-based structured optical fibre. Sci Rep. 2020;10:1–8. https://doi.org/10.1038/s41598-020-64103-3.

Gao R, Jiang Y, Ding W. Agarose gel filled temperature-insensitive photonic crystal fibers humidity sensor based on the tunable coupling ratio. Sensors Actuators B Chem. 2014;195:313–9. https://doi.org/10.1016/j.snb.2014.01.061.

Giuseppe O, Lorenzo P, Ricordi C, Robert JS, Rainer G. Transplantation, Bioengineering, and Regeneration of the Endocrine Pancreas. Elsevier; 2019. ISBN 9780128148310.

Grand View Research, San Francisco, C. Biophotonics market by end-use (tests and components, medical therapeutics, medical diagnostics, non-medical application), by application (see-through imaging, microscopy, inside imaging, spectro molecular, analytics sensing, light therapy, surface imagin. 2016.

Habash RWY, Bansal R, Krewski D, Alhafid HT. Thermal therapy, part 1: an introduction to thermal therapy. Crit Rev Biomed Eng. 2006;34:459–89.

He W, Li P, Zhu Y, Liu M, Huang X, Qi H. An injectable silk fibroin nanofiber hydrogel hybrid system for tumor upconversion luminescence imaging and photothermal therapy. New J Chem. 2019;43:2213–9. https://doi.org/10.1039/c8nj05766d.

Jain A, Yang AHJ, Erickson D. Gel-based optical waveguides with live cell encapsulation and integrated microfluidics. Opt Lett. 2012;37:1472. https://doi.org/10.1364/ol.37.001472.

Jayanthi VSPKSA, Das AB, Saxena U. Recent advances in biosensor development for the detection of cancer biomarkers. Biosens Bioelectron. 2017;91:15–23. https://doi.org/10.1016/j.bios.2016.12.014.

Katkar RA, Tadinada SA, Amaechi BT, Fried D. Optical coherence tomography. Dent Clin N Am. 2018;62:421–34. https://doi.org/10.1016/j.cden.2018.03.004.

Khalid A, Peng L, Arman A, Warren-Smith SC, Schartner EP, Sylvia GM, Hutchinson MR, Ebendorff-Heidepriem H, McLaughlin RA, Gibson BC, et al. Silk: a bio-derived coating for optical fiber sensing applications. Sensors Actuators B Chem. 2020;311. https://doi.org/10.1016/j.snb.2020.127864.

Kim Y-J, Lee H-I, Kim J-K, Kim C-H, Kim Y-J. Peptide 18-4/chlorin e6-conjugated polyhedral oligomeric silsesquioxane nanoparticles for targeted photodynamic therapy of breast cancer. Colloids Surf B: Biointerfaces. 2020;189:110829. https://doi.org/10.1016/j.colsurfb.2020.110829.

Krafft C. Modern trends in biophotonics for clinical diagnosis and therapy to solve unmet clinical needs. J Biophotonics. 2016;9:1362–75. https://doi.org/10.1002/jbio.201600290.

Kumar A, Kumar A. Chitosan as a biomedical material: properties and applications. Biopolym Struct Perform Appl. 2017;7:139–54.

Kwiatkowski S, Knap B, Przystupski D, Saczko J, Kędzierska E, Knap-Czop K, Kotlińska J, Michel O, Kotowski K, Kulbacka J. Photodynamic therapy – mechanisms, photosensitizers and combinations. Biomed Pharmacother. 2018;106:1098–107. https://doi.org/10.1016/j.biopha.2018.07.049.

Li Q, Qi N, Peng Y, Zhang Y, Shi L, Zhang X, Lai Y, Wei K, Kim IS, Zhang KQ. Sub-micron silk fibroin film with high humidity sensibility through color changing. RSC Adv. 2017;7:17889–97. https://doi.org/10.1039/c6ra28460d.

Ma X, Sun X, Chen J, Lei Y. Natural or natural-synthetic hybrid polymer-based fluorescent polymeric materials for bio-imaging-related applications. Appl Biochem Biotechnol. 2017;183:461–87. https://doi.org/10.1007/s12010-017-2570-9.

Mironenko AY, Sergeev A, Nazirov AE, Voznesenskiy SS, Bratskaya SY, Mirochnik AG. Highly sensitive chitosan-based optical fluorescent sensor for gaseous methylamine detection. Prog Chem Appl Chitin Deriv. 2017;22:159–65. https://doi.org/10.15259/PCACD.22.16.

Mu X, Gray DG. Formation of chiral nematic films from cellulose nanocrystal suspensions is a two-stage process. Langmuir. 2014;30:9256–60. https://doi.org/10.1021/la501741r.
Nishiyama Y. Structure and properties of the cellulose microfibril. J Wood Sci. 2009;55:241–9. https://doi.org/10.1007/s10086-009-1029-1.
Orelma H, Hokkanen A, Leppänen I, Kammiovirta K, Kapulainen M, Harlin A. Optical cellulose fiber made from regenerated cellulose and cellulose acetate for water sensor applications. Cellulose. 2020;27:1543–53. https://doi.org/10.1007/s10570-019-02882-3.
Owens EA, Lee S, Choi J, Henary M, Choi HS. NIR fluorescent small molecules for intraoperative imaging. Wiley Interdiscip Rev Nanomed Nanobiotechnol. 2015;7:828–38. https://doi.org/10.1002/wnan.1337.
Owens EA, Henary M, El Fakhri G, Choi HS. Tissue-specific near-infrared fluorescence imaging. Acc Chem Res. 2016;49:1731–40. https://doi.org/10.1021/acs.accounts.6b00239.
Pandey A, Larroche C, Dussap C-G, Gnansounou E, Khanal SK, Ricke S. Biofuels: alternative feedstocks and conversion processes for the production of liquid and gaseous biofuels. 2nd ed. Elsevier; 2019. ISBN 9780128168561.
Peng L, Yang X, Yuan L, Wang L, Zhao E, Tian F, Liu Y. Gaseous ammonia fluorescence probe based on cellulose acetate modified microstructured optical fiber. Opt Commun. 2011;284:4810–4. https://doi.org/10.1016/j.optcom.2011.06.015.
Prasad PN. Polymer science and technology for new generation photonics and biophotonics. Curr Opin Solid State Mater Sci. 2004;8:11–9. https://doi.org/10.1016/j.cossms.2004.01.011.
Prochazka M. Medical applications of SERS; 2016. p. 149–211. https://doi.org/10.1007/978-3-319-23992-7_7.
Quimby RS. No title. 2006. https://doi.org/10.1002/0471791598.
Razali NM, Zaidi NFA, Ja'Afar PNSS, Hamzah A, Ahmad F, Ambran S. Optical fibre tip sensor coated with chitosan for lead ion detection. AIP Conf Proc. 2020;2203. https://doi.org/10.1063/1.5142127.
Rebelo R, Fernandes M, Fangueiro R. Biopolymers in medical implants: a brief review. Proced Eng. 2017;200:236–43. https://doi.org/10.1016/j.proeng.2017.07.034.
Shan D, Zhang C, Kalaba S, Mehta N, Kim GB, Liu Z, Yang J. Flexible biodegradable citrate-based polymeric step-index optical fiber. Biomaterials. 2017;143:142–8. https://doi.org/10.1016/j.biomaterials.2017.08.003.
Shan D, Gerhard E, Zhang C, Tierney JW, Xie D, Liu Z, Yang J. Polymeric biomaterials for biophotonic applications. Bioact Mater. 2018;3:434–45. https://doi.org/10.1016/j.bioactmat.2018.07.001.
Shanmugam K, Sahadevan R. Bioceramics – an introductory overview. In: Fundamental biomaterials: ceramics, Woodhead Publishing Series in Biomaterials. Elsevier; 2018. p. 1–46. ISBN 9780081022030.
Singh SP, Alam H, Dmello C, Mamgain H, Vaidya MM, Dasari RR, Krishna CM. Identification of morphological and biochemical changes in keratin-8/18 knock-down cells using Raman spectroscopy. J Biophotonics. 2017;10:1377–84. https://doi.org/10.1002/jbio.201600249.
Solaimuthu A, Vijayan AN, Murali P, Korrapati PS. Nano-biosensors and their relevance in tissue engineering. Curr Opin Biomed Eng. 2020;13:84–93. https://doi.org/10.1016/j.cobme.2019.12.005.
Son J, Yi G, Yoo J, Park C, Koo H, Choi HS. Light-responsive nanomedicine for biophotonic imaging and targeted therapy. Adv Drug Deliv Rev. 2019;138:133–47. https://doi.org/10.1016/j.addr.2018.10.002.
Steinberg I, Huland DM, Vermesh O, Frostig HE, Tummers WS, Gambhir SS. Photoacoustic clinical imaging. Photo-Dermatology. 2019;14:77–98. https://doi.org/10.1016/j.pacs.2019.05.001.
Sun M, Zhang Y, He Y, Xiong M, Huang H, Pei S, Liao J, Wang Y, Shao D. Green synthesis of carrier-free curcumin nanodrugs for light-activated breast cancer photodynamic therapy. Colloids Surf B: Biointerfaces. 2019;180:313–8. https://doi.org/10.1016/j.colsurfb.2019.04.061.

Tong L, Gattass RR, Ashcom JB, He S, Lou J, et al. Subwavelength-diameter silica wires for low-loss optical wave guiding. Nature. 2003;426:816–9.

Torok B, Dransfield T. Green chemistry. Elsevier; 2017. ISBN 9780128092705.

Voznesenskiy SS, Sergeev AA, Mironenko AY, Bratskaya SY, Kulchin YN. Integrated-optical sensors based on chitosan waveguide films for relative humidity measurements. Sensors Actuators B Chem. 2013;188:482–7. https://doi.org/10.1016/j.snb.2013.07.043.

Wang B, Yang W, McKittrick J, Meyers MA. Keratin: structure, mechanical properties, occurrence in biological organisms, and efforts at bioinspiration. Prog Mater Sci. 2016;76:229–318. https://doi.org/10.1016/j.pmatsci.2015.06.001.

Wei W, Zhang X, Zhang S, Wei G, Su Z. Biomedical and bioactive engineered nanomaterials for targeted tumor photothermal therapy: a review. Mater Sci Eng C. 2019;104:109891. https://doi.org/10.1016/j.msec.2019.109891.

Xia J, Yao J, Wang L. V Photoacoustic tomography: principles and advances. Electromagn Waves (Camb). 2014;147:1–22.

Xiong R, Luan J, Kang S, Ye C, Singamaneni S, Tsukruk VV. Biopolymeric photonic structures: design, fabrication, and emerging applications. Chem Soc Rev. 2020;49:983–1031. https://doi.org/10.1039/c8cs01007b.

Xu HL, ZhuGe DL, Chen PP, Tong MQ, Lin MT, Jiang X, Zheng YW, Chen B, Li XK, Zhao YZ. Silk fibroin nanoparticles dyeing indocyanine green for imaging-guided photo-thermal therapy of glioblastoma. Drug Deliv. 2018;25:364–75. https://doi.org/10.1080/10717544.2018.1428244.

Yang Y, Wang L, Wan B, Gu Y, Li X. Optically active nanomaterials for bioimaging and targeted therapy. Front Bioeng Biotechnol. 2019a;7:320.

Yang G, Xiao Z, Tang C, Deng Y, Huang H, He Z. Recent advances in biosensor for detection of lung cancer biomarkers. Biosens Bioelectron. 2019b;141:111416. https://doi.org/10.1016/j.bios.2019.111416.

Younes I, Rinaudo M. Chitin and chitosan preparation from marine sources. Structure, properties and applications. Mar Drugs. 2015;13:1133–74. https://doi.org/10.3390/md13031133.

Zhang Q, Li L. Photodynamic combinational therapy in cancer treatment. J BUON. 2018;23:561–7.

Zhu S, Zeng W, Meng Z, Luo W, Ma L, Li Y, Lin C, Huang Q, Lin Y, Liu XY. Using wool keratin as a basic resist material to fabricate precise protein patterns. Adv Mater. 2019;31:1–7. https://doi.org/10.1002/adma.201900870.

Part VI

Tissue Engineering Applications

Polysaccharides-Based Biomaterials for Surgical Applications

40

Garima Agrawal and Anuj Kumar

Contents

Abstract

Polysaccharides are highly abundant natural polymers and can be obtained from three mainly different renewable resources such as plants, animal, and microorganisms. These polysaccharides have shown a great potential in biomedical applications, especially tissue engineering due to their high availability in nature, cost-effective production, ease of processing, nontoxicity, excellent

G. Agrawal
School of Basic Sciences, Indian Institute of Technology Mandi, Kamand, India,

A. Kumar (✉)
School of Chemical Engineering, Yeungnam University, Gyeongsan, South Korea

J. M. Oliveira et al. (eds.), *Polysaccharides of Microbial Origin*,
https://doi.org/10.1007/978-3-030-42215-8_51

biocompatibility, and intrinsic immunogenic ability. Among them, microbial polysaccharides have received a great attention in tissue engineering or surgical applications. In this chapter, an introduction of surgical tissue regeneration and used biomaterials are briefly discussed. Further, an overview of different polysaccharides, their processing and modifications (especially microbial polysaccharides with other polysaccharides) as well as their applications in various surgical procedures are reviewed and discussed precisely.

Keywords

Polysaccharides · Biomaterials · Hydrogels · Surgical applications · Tissue adhesives · Sealants

1 Introduction

In the last decade, tremendous efforts have been made to explore various natural and synthetic polymers to design remarkable biomaterials for targeted biomedical applications (Agrawal et al. 2017, 2019; Birkholz et al. 2016; Wiemer et al. 2017; Doermbach et al. 2014). In this regard, natural polymers such as polysaccharides are particularly interesting owing to their inherent biocompatibility. Hence, increasing attention has been given to develop polysaccharides-based biomaterials that can perform the desired task under complex biological environment that is encountered especially during or post-surgery. The biggest challenge of surgical process is the proper closure of surgical wounds by efficient stabilization of wound margins in their desired position and achieving required mechanical support during surgical wound healing. Sutures, surgical tape, staple, and clips have been frequently explored so far for wound healing (Pillai and Sharma 2010; Taira et al. 2010; Shantz et al. 2013). However, time-consuming procedure, leakage, tissue scarring, and infections are some of the disadvantages here (Jain and Wairkar 2019). For example, lung tissue wound closed by sutures might encounter gaseous leakage while surgery of traumatic injury with sutures might lead to lesions agglomeration (Foster et al. 2010). Hence, significant efforts are being made for developing surgical glues to address the abovementioned issues and achieve minimally invasive surgery.

Surgical glues are often used to seal the injured tissue or wounds and maintain stabilization of wound margins in required position, thus influencing the success of any surgical procedure. To achieve the desired outcome, these surgical glues should have high binding affinity, elasticity, biocompatibility, and biodegradability along with being economical and easy to use. Surgical glues can be classified into three categories based on their mode of action: (1) hemostatic agents which form clot in blood; (2) sealants are used to seal the wound opening to prevent leakage and blood loss; and (3) adhesives which merge the margins of wound site together (Jain and Wairkar 2019). A variety of surgical glues are being developed as their market is growing fast globally from $4 billion in 2012 to $7 billion in 2017 (Annabi et al. 2015). Despite significant efforts for fabricating novel surgical glues, most of the

developed systems still require a unique combination of elasticity and adhesion for being suitable for both hard and soft tissue. Recently, an extensive research has been done to develop surgical glues based on both natural (e.g., fibrin, collagen, gelatin, albumin, chitosan, dextran, etc.) and synthetic (e.g., polyurethanes, polyethylene glycols, polycyanoacrylates, etc.) polymers (Chao and Torchiana 2003; Gruber-Blum et al. 2010; Hidas et al. 2006; Jeon et al. 2017; Berger et al. 2001; Thorn et al. 2004; Ates et al. 2014; Osbun et al. 2012; Leggat et al. 2007). Tissucol®, Beriplast®, Bolheal®, and Biocol® are some of the examples of commercially available fibrin-based tissue adhesives (Janmey et al. 2009). However, their widespread use is limited due to poor adhesion efficiency and lower strength. Histoacryl® is an example of cyanoacrylate-based glue having good bonding strength but it suffers with cell toxicity, inflammation, and foreign body type giant cell reaction after usage (Shivamurthy et al. 2010; Mizrahi et al. 2011). On the other hand, GRF glue and BioGlue® suffer from the toxicity associated with their components formaldehyde and glutaraldehyde, respectively (Chao and Torchiana 2003; Shaffer and Belsito 2000). In quest to explore other options, different polysaccharides-based (e.g., chitosan and dextran) hemostatic agents such as Surgicel®, Celox™, HemCon® Bandage, and mRDH have been developed and are currently available in the market (Valeri and Vournakis 2011; Pozza and Millner 2011; Matsumura et al. 2014; Wang et al. 2012; Li et al. 2011; Hyon et al. 2014).

In this chapter, we provide the readers with a brief overview of different polysaccharides-based biomaterials that have been reported in the literature for surgical applications. We will also focus on different types of polysaccharides, their processing and modification (especially microbial polysaccharides), and their use for various surgical procedures.

2 Polysaccharides

Polysaccharides represent an important class of natural polymers that have been widely explored for biomedical applications (Sauraj et al. 2020). These natural polymers consist of different monosaccharide units connected via o-glycosidic linkages. Cellulose, starch, chitin/chitosan (CS), chondroitin sulfate, hyaluronic acid (HA), dextran, and xanthan gum are some of the examples of widely used polysaccharides (Fig. 1). These polysaccharides exhibit a wide range of physico-chemical properties based on different monosaccharide units present in the polymer chain, chemical composition, and source of extraction. In general, polysaccharides are obtained from renewable sources including plants, animals, and microorganisms.

2.1 Plant-Origin Polysaccharides

Plant cell wall is one of the major sources of polysaccharides, namely cellulose and starch, and their extraction mainly depends on the structure of the cell wall. Starch is

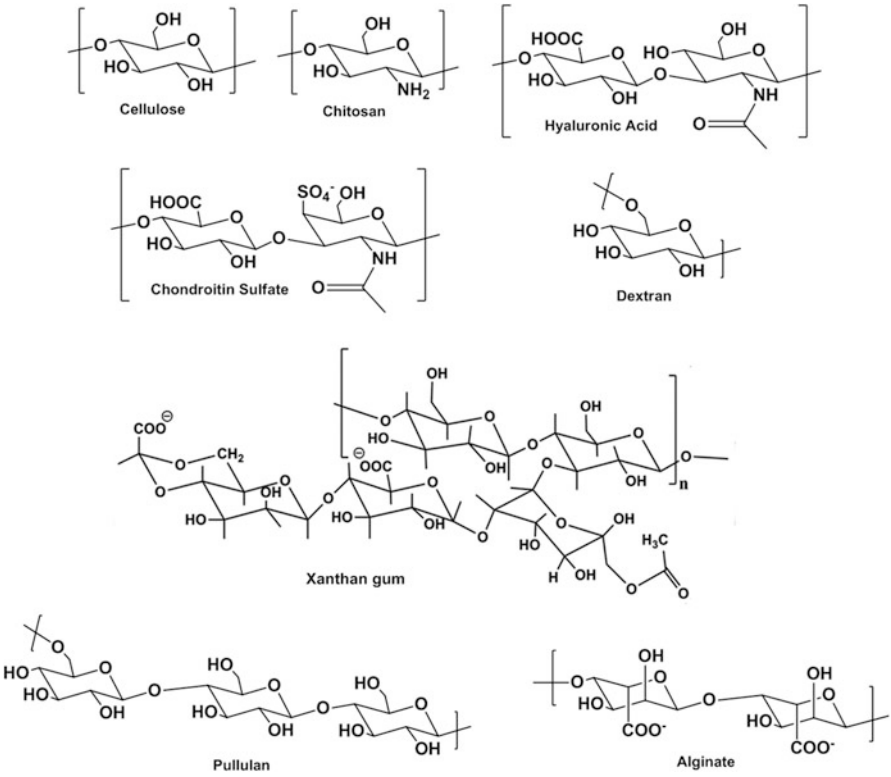


Fig. 1 Schematic representation of chemical structures of various natural polysaccharides

considered as the storage polysaccharide, while cellulose is considered as structural polysaccharide.

Cellulose is the most abundant natural polymer found in the cell wall of plants and can also be produced from microorganisms. It is a linear polysaccharide consisting of D-glucose units linked via β(1→4) glycosidic linkages. In general, oxidized cellulose obtained by partial oxidation of hydroxyl groups is used as surgical glue. Surgicel® consisting of oxidized regenerated cellulose (ORC) is widely used to achieve hemostasis during surgery (Breech and Laufer 2000). It has been successfully used in different surgeries due to its nonadherence to medical equipment, and possibility to crop it as required based on the wound site. Although ORC-based hemostatic agents are broadly used, yet they encounter the problem of relatively poor hemostasis, acidity, and low biodegradability (Cheng et al. 2018). It is believed that the hemostatic action of oxidized regenerated cellulose is due to the incorporation of negative charge caused by carboxylic group leading to thrombus formation via platelets activation (Ryšavá et al. 2003). Platelet activation makes the platelet glycoprotein (GPIIb/IIIa) receptor active for binding soluble fibrinogen and thus releasing clotting factors (Cheng et al. 2013).

Starch is another naturally occurring polysaccharide obtained from plants such as potato, cereal grain, chickpeas, etc. Similar to cellulose, starch is also a polymer of glucose molecules. However, starch consists of only α-glucose while cellulose is made up of only β-glucose. Starch contains both (1→4) and (1→6) glycosidic linkages. Starch is formed by photosynthesis in plants and it functions as an energy storage unit. Arista AH and Quickclean are commercially available hemostatic agents prepared by physical/chemical modification of starch (Murat et al. 2006; Zhu et al. 2019). They are made up of microporous polysaccharide hemosphere which can absorb blood components leading to concentration of clotting proteins and thus enhancing blood clotting (Lewis et al. 2015).

2.2 Animal-Origin Polysaccharides

Polysaccharides such as chitin-derived chitosan, chondroitin sulfate, and hyaluronic acid are found in various body parts of different animals. Among polysaccharides, chitin holds the position of second most abundant natural polymer after cellulose (Min et al. 2004). It is found in the exoskeleton and endoskeleton of crustaceans and molluscs, respectively (Hamed et al. 2016). It is obtained from arthropods such as crabs, lobsters, and shrimps along with cell wall of fungi. As the native chitin form is insoluble in water and difficult to process, it is converted into chitosan (CS) by deacetylation of chitin. Chitosan is a linear copolymer of D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit) linked via β(1→4) glycosidic linkages (Li and Hsieh 2006). The generated free amino groups not only impart solubility to chitosan in water and various organic solvent but also make it suitable for further chemical modification. Chitosan undergoes degradation in the presence of enzymes such as lysozyme, chitotriodidase, di-N-acetylchitobiase, and N-acetyl-β-D-glucosaminidase without generating harmful by-products, making it a suitable candidate for surgical applications (Lim et al. 2008). Amino group present in the polymer chain forms bonds with tissue collagen via electrostatic interaction, thus facilitating blood coagulation and showing great potential as tissue adhesives (Jain and Wairkar 2019). It has also been reported that chitosan-based biomaterials help in wound healing by releasing important growth factors (Okamoto et al. 2003).

Chondroitin sulfate is a sulfated glycosaminoglycan which is mainly found as a structural component of cartilage around joints in the body (Garnjanagoonchorn et al. 2007). This unbranched polysaccharide consists of alternating N-acetylgalactosamine and glucuronic acid bound together in a polymeric chain (Meziane-Tani et al. 2006). It is generally extracted from shark and cow cartilage. It contains several functional groups such as –OH, –COOH, sulfate, etc. which can be further modified into methacrylate-, –CHO-, and NHS-activated ester groups, thus providing enormous possibilities of functionalization. Based on its association with cartilage and other tissues, it has been explored for developing surgical glues for tissue regeneration and wound healing (Rainer et al. 2005; Strehin et al. 2010).

Hyaluronic acid (or hyaluronan) is a linear, nonsulfated glycosaminoglycan and it is a major component of extracellular matrix. It is composed of N-acetyl-d-glucosamine and d-glucuronic acid and it degrades in the presence of hyaluronidases (Flynn et al. 2011). It is also an important part of skin and plays a crucial role in tissue repair. It is generally extracted from rooster combs. It is biocompatible and has plenty of functional groups which make it a potential candidate for designing novel biomaterials for surgical applications (An et al. 2019; Liu et al. 2018; Luo et al. 2019).

2.3 Microbe-Origin Polysaccharides

Microbial origin polysaccharides are an important class of polysaccharides due to the possibility of producing them in high yield. Hence, different microbe-based polysaccharides such as dextran and xanthan have been widely used for designing functional biomaterials for biomedical applications. Dextran is a complex polysaccharide made up of glucose units connected via α(1→4) glycosidic linkages and having branches from α-1,3 linkages. As compared to chitosan, no amine functional groups are present in dextran. Hence, dextran is chemically modified to incorporate aldehyde or methacrylate functional groups. Dextran having a wide range of molecular weights is synthesized from sucrose mainly by the action of *Leuconostoc bacteroides* and *Streptococcus mutans* (Sarwat et al. 2008). The enzymes glucosyltransferase and dextran sucrase are involved in the synthesis of dextran (Devulapalle et al. 2004). These enzymes polymerize the glucose fraction of sucrose releasing fructose. It has been found that dextran exhibits enzymatic degradation in the spleen, liver, and colon (Khalikova et al. 2005).

On the other hand, xanthan is a high-molecular-weight polysaccharide which is commercially prepared by fermentation process using microorganism *Xanthomonas campestris*. Xanthan consists of glucose, mannose, and glucuronic acid. It is a good viscosity modifier and used as a stabilizer to avoid the segregation of components in a mixture (Franceschini et al. 2012; Bassetto et al. 2008; Kumar et al. 2018).

3 Processing and Modifications of Polysaccharides

Polysaccharides are emerged as potential and ideal candidate toward biomimicry of the native extracellular matrix (ECM). Particularly, microbial polysaccharides are considered better candidate due to their economical and sustainable benefits in fast and high-yield production processes. Therefore, microbial polysaccharides show an increasing potential in addition to plant- and animal-origin polysaccharide-based biomaterials in tissue engineering or surgical applications. However, they do not interact with human tissues biologically that limits their translation into model scaffold for in vitro/in vivo tissue regeneration. To overcome this, these polysaccharides should undergo modification through physical or chemical or in combination, enzymatic, and biological methods to improve the gelation behavior or functional

properties of the hydrogels/scaffolds. Further, the mixing of two or more polysaccharides/polymers facilitates in different characteristics and functional properties as compared to the properties of single polysaccharide/polymer in hydrogel network (Ng et al. 2020; Ahmad et al. 2015; Karaki et al. 2016). Therefore, the proper processing, modification, and characterization of polysaccharides are important factors in fabricating functional biomaterials for successful surgical applications. Microbial polysaccharides are gaining research interests in surgery due to their renewable nature, cost stability in production, and reproducible physicochemical properties of the microbial polysaccharides have shown a great potential as compared to the polysaccharides from plants (Sutherland 2001; Vanhaverbeke et al. 2003) and found applications in diverse fields in industrial and biomedical applications. Microbial polysaccharides can be categorized into three ways according to their particular morphological existence as: (1) intracellular, (2) cell wall, and (3) extracellular polysaccharides. Further, these polysaccharides can also be classified in two ways: (1) homo-polysaccharides and (2) hetero-polysaccharides (Ahmad et al. 2015; Mollet 1996). Homo-polysaccharides possess only one sugar unit, whereas hetero-polysaccharides possess two or more sugar units (Mollet 1996). These polysaccharides can have various lengths of side chains in some structures along with the existing linear molecular chains and lead to a broad range of possible complex shapes and architectures. In addition, the association of these polymeric chains having high molecular weight leads to the chain entanglements in complex network and thereby the physical properties in double-stranded (e.g., xanthan gum, gellan gum, etc.) or triple-stranded (e.g., schizophyllan) helical form (Atkins 1986; Sutherland 1994; Nishinari and Takahashi 2003). Therefore, depending on chemical and physical properties, the processing and the modification of polysaccharides can be decided for surgical applications. Among microbial polysaccharides, gellan gum, xanthan gum, dextran, pullulan, and bacterial cellulose are considered and used extensively in biomedical applications. Further, polysaccharides-based hydrogels lack of flexibility in their main molecular chains that lead to the higher viscosities (Ahmad et al. 2015; Morrison et al. 1999). Moreover, in surgical applications, hydrogels in the form of adhesive or glues or in other forms are expected to deliver desired components for proper tissue regeneration without affecting other properties. Here, some microbial polysaccharides, including their combinations with other polysaccharides, are discussed for their processing and modifications to achieve improved properties for tissue regeneration in surgical applications.

3.1 Bacterial Cellulose

Bacterial cellulose (BC), prepared by aerobic bacteria, has shown a great potential in tissue engineering applications due to its high values of purity, porosity, high water holding capacity, and mechanical strength, including excellent biocompatibility. With this objective, various processing and modification approaches have been used for different focused surgical applications (Pang et al. 2020a).

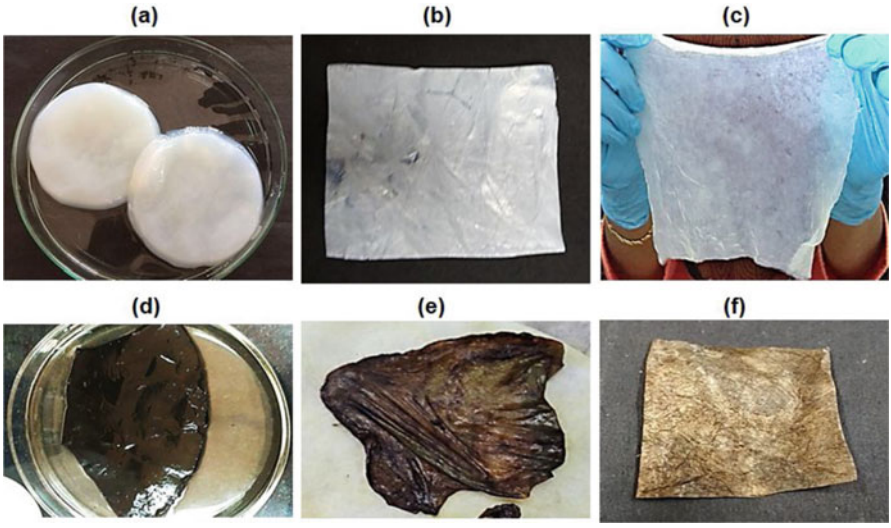


Fig. 2 Digital images of freshly prepared BC (**a**), dried BC (**b**), hydrated BC (**c**), PD-coated BC (**d**), BC-PDAg nanocomposite (**e**), and dried BC-PDAg nanocomposite (**f**). (Reproduced with permission from Elsevier, Jiji et al. 2020)

In a study, BC graft myringoplasty showed good results and can provide a rapid and safe surgery under local anesthesia in outpatient clinic within shorter time of surgery with better hearing and healing as compared to fat graft myringoplasty and temporalis fascia graft myringoplasty (Mandour et al. 2019). In another study for hernia repair surgery, the modification of BC with CS provided improved results as surgical meshes (BCS) for facilitating better absorption in native tissue with reduced risk of mesh-related infections. To analyze immunological responses due to hypersensitivity to the implants in rats, three types of meshes were used as (a) propylene mesh, (b) modified with BC only, and (c) BCS mesh for 1 and 3 months after intramuscular implantation. Here, BCS showed lowest immune response and highest degree of fibroplasia for 1 and 3 months of implantation. Moreover, toxicological analyses for BCS demonstrated negligible inflammation with no signs of sensitization or allergic responses (Piasecka-Zelga et al. 2018). Jiji et al. developed polydopamine (PD)-coated BC with silver nanoparticles (AgNPs) for third burn wound healing (Fig. 2). Here, AgNPs were successfully synthesized into BC matrix through catecholic redox method. This BC-PDAg nanocomposite exhibited an excellent biocompatibility, antibacterial activity against both gram-positive and gram-negative bacteria, and fast in vivo burn wound healing without scar formation in rat. Here, nanocomposite promoted re-epithelization and COLL deposition and gene expression analyses exhibited the upregulation of IL-10, VEGF-A, VEGF-B, and bFGF, while suppression of IL-1α and IL-3 transcripts. In addition, expression of TGF-β3 and SMAD-3 revealed the promotion of scarless wound healing (Jiji et al. 2020).

Enhancement in moisture-holding capacity or moist environment is a key factor in applying and economically preferable extended wear time. With this objective,

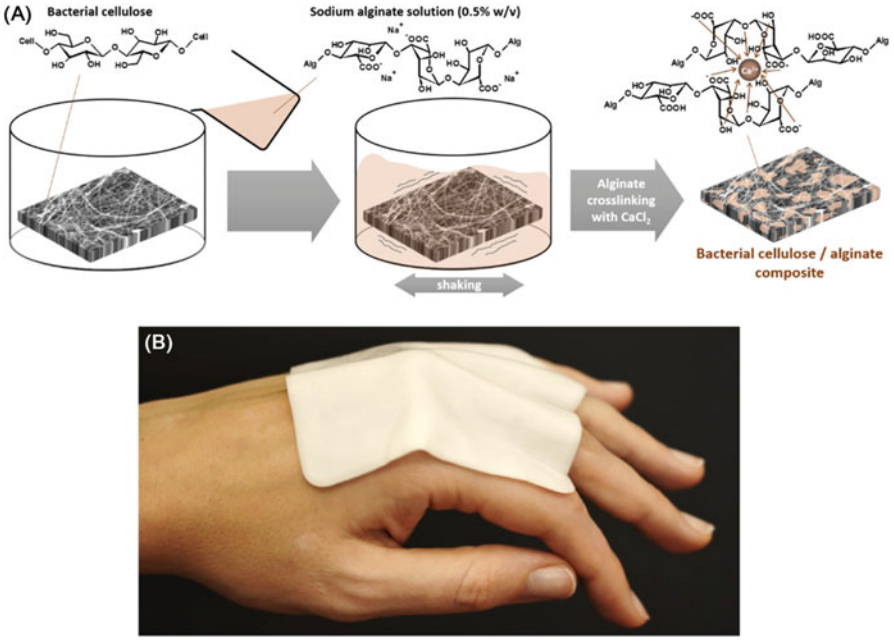


Fig. 3 Preparation method of BC/SA composite dressings (**a**) and digital image of BC/SA composite dressing showing flexibility (**b**). (Reproduced with permission from Elsevier, Sulaeva et al. 2020)

BC dressing was impregnated with sodium alginate as second hydrophilic domain (see Fig. 3). The developed crosslinked BC-SA composite dressing exhibited enhanced water retention properties through facilitating smooth dressing exchange in a wound-imitating model (Sulaeva et al. 2020).

3.2 Dextran

As demanding needs, an injectable gel or surgical adhesive to be used in different situation, in stopping bleeding and fastening tissues well together, is a major challenge. Further, the developed adhesive should also be biocompatible, biodegradable, nontoxic, economically viable, and easy to use in complex surgical circumstances or locations. With this objective, an injectable enzymatically crosslinked dextran tyramine (Dex-TA)/heparin tyramine (Hep-TA) hydrogels were prepared and showed good cell viability and proliferation followed by the improvement in the production of chondroitin sulfate and significant collagen as compared to only Dex-TA hydrogel (Jin et al. 2011).

In another approach, Dex was oxidized by sodium periodate ($NaIO_4$) and then developed a DDA sponge with appropriate absorption capacity of blood (47.7 g/g) and strong tissue adhesion property (~100 kPa) for hemorrhage control (Liu et al. 2019).

With this DDA, Araki et al. mixed DDA and poly(l-lysine) (PLL) to prepare a DDA/PLL hydrogel glue as surgical sealant and compared with a conventional fibrin glue. The obtained DDA/PLL glue (mean bursting pressure: 38.4 ± 4.6 cm H_2O) was found to be more effective in sealing of pulmonary air leakage than fibrin glue (mean bursting pressure: 32.1 ± 4.5 cm H_2O). Further, no adhesions or infections were observed in the application areas of the glue and degraded nearly 90% within 3 months, but complete degradation was not observed till 6 months as the fibrin glue. The product exhibited a strong sealing behavior and good biocompatibility to be used in lung surgery (Araki et al. 2007). In addition to this study, PLL (-NH_2) was modified by acylation and prepared DDA/a-PLL bioadhesive via Schiff base reaction. Further, the mechanical (from 120 Pa to 20 kPa) and degradation properties of bioadhesive DDA/a-PLL were controlled by the oxidation degree of Dex and the concentration or type of anhydride species in the a-PLL (Matsumura et al. 2014).

It is worth to note that self-crosslinking (Schiff base reaction) in hydrogels is more advantageous over the external chemical crosslinker, but the mechanical properties of the hydrogels can be enhanced further by incorporating nanoparticles. In a study by Pang et al., for self-crosslinking reaction, dextran as microbial polysaccharide was oxidized by using sodium periodate ($NaIO_4$) to incorporate dialdehyde functional groups as dextran dialdehyde (DDA) and chitosan was modified with carboxymethylation by using chloroacetic acid. Further, chitin nanowhiskers (ChtNWs) were prepared by sulfuric acid and incorporated into self-crosslinked carboxymethyl chitosan (CMCS)/oxidized dextran through Schiff base reaction that led to a mechanically strong tissue adhesive (Fig. 4). The optimal composition of complexed CMCS-DDA/ChtNWs hydrogel with ChtNWs showed 1.87-fold higher compressive strength as compared to noncomplexed hydrogel and 1.51 times higher adhesive strength on porcine skin. Also, this hydrogel system showed negligible cytotoxicity, optimum antibacterial and hemostatic abilities, in vivo degradability without long-term inflammatory responses, and could prevent severe postoperative adhesion and necrosis in case of wound repair (Pang et al. 2020b).

Similarly, in another study, N-carboxyethyl CS (CECS) was prepared by the modification with acrylic acid. Then this CECS and DDA were used to prepare in situ gelable hydrogels at physiological pH and body temperature (37 °C). The developed DDA/CECS gels showed improved wound healing when applied to mice full-thickness transcutaneous wound models (Weng et al. 2008). Du et al. designed and prepared multifunctional injectable and self-healable hydrophobically modified CS (hmCS)/DDA hydrogels with excellent hemostatic, antibacterial, and accelerated wound healing performances. Here, CS was hydrophobically modified with dodecyl aldehyde through Schiff base reaction and then prepared a tissue adhesive hmCS/DDA hydrogel that showed good potential for hemorrhagic and infected wound healing. The self-healing process, rheological properties, injectability, and in vivo gelation of the hydrogel are shown in Fig. 5 (Du et al. 2019).

In another study, photocrosslinkable tissue adhesive based on Dex was prepared. Here, first urethane dextran derivative was prepared by incorporating

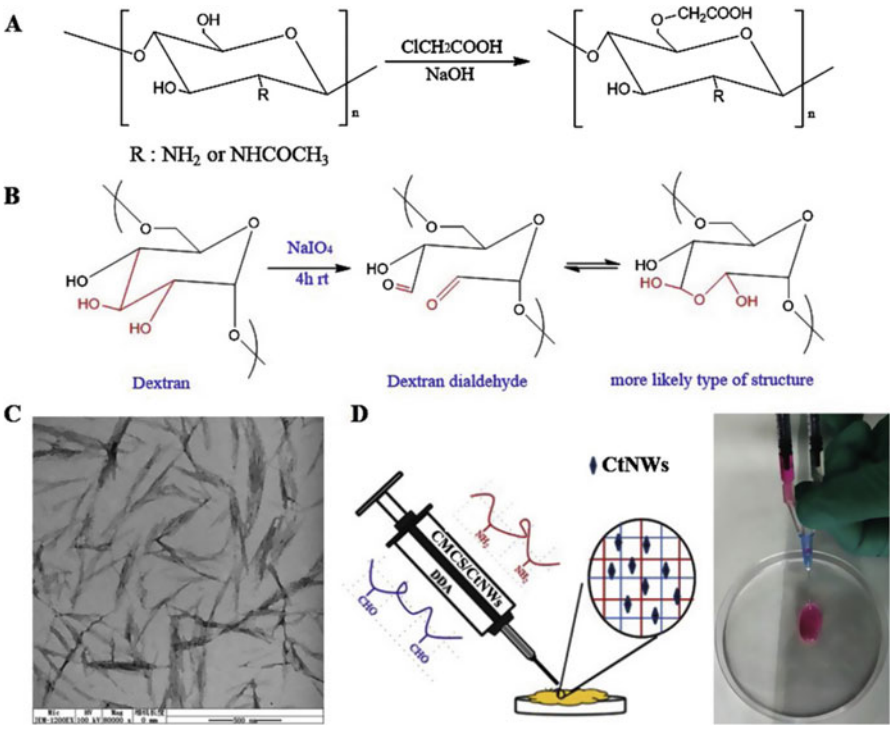


Fig. 4 Modification of CS to CMCS (**a**) and Dex to DDA (**b**), TEM image of CtNWs (**c**), schematic and digital image of complexed CMCS-DDA/CtNWs hydrogel formed by the extrusion by using double-barrel syringe (**d**). (Reproduced with permission from Elsevier, Pang et al. 2020b)

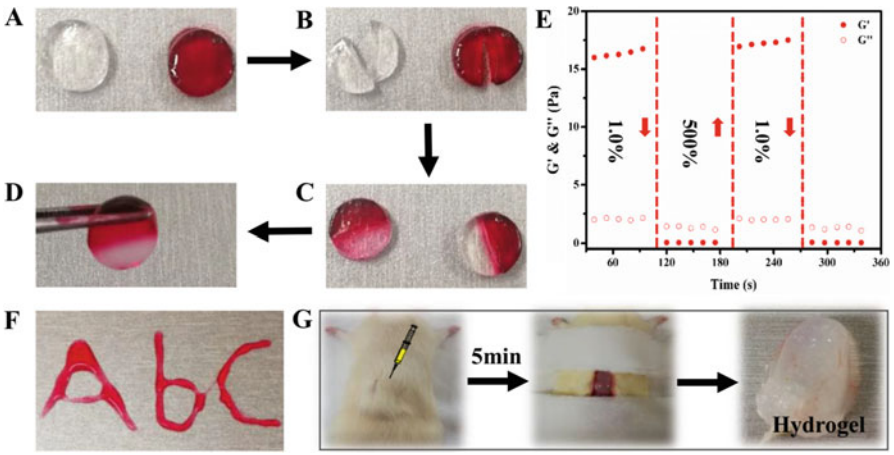


Fig. 5 (**a**–**d**) Digital images of self-healing process, (**e**) rheological properties, (**f**) injectability, and (**g**) in vivo gelation behavior of the hmCS/DDA hydrogel. (Reproduced with permission from Elsevier, Du et al. 2019)

2-isocyanatoethyl methacrylate (IEMA) onto dextran (Dex-U) that could be photocrosslinked under UV irradiation and progressively adhere to the surface of gelatin and mouse skin that encourage development of the human tissue. The highest adhesive strength (2.99 MPa) and burst pressure (35 mmHg) were observed, where adhesive strength was found to be higher than that of fibrin glue (Li et al. 2011). In addition to this study, a photocrosslinkable tissue adhesive composed of oxidized urethane dextran (Dex-U-AD) and gelatin was developed. Here, Dex-U was oxidized by using $NaIO_4$ that led to the final polymer as Dex-U-AD. The obtained Dex-U-AD was processed with gelatin to form hydrogel through Schiff base reaction followed by photocrosslinking under ultraviolet (UV) irradiation. The results showed good adhesive property to the surface of gelatin that simulated the human tissue in a control manner and maximum adhesion strength could be observed to 4.16 ± 0.72 MPa that was higher than that of fibrin glue significantly. Moreover, Dex-U-AD/gelatin gels were observed to be nontoxic and could be used as good tissue adhesive biomaterial (Wang et al. 2012). Further, a facile in situ approach was presented to prepare anionic inter-polymeric complex polysaccharide biomaterial, where sodium alginate (SA) and kappa-carrageenan (*k*-CG) were treated with silver salt and formed in soft nano-floral inter-polymeric complex with self-stability. The obtained polymeric complexes exhibited good resistance toward *S. aureus* and *E. coli* bacterium, and improved exudate absorption, thereby promoting effective proliferation and evolving the compact fibrous network and hair follicles (Zia et al. 2020).

In another study, amine-modified succinyl CS was prepared and used with DDA for preparing a series of hydrogels. The properties and rate of preparation of the hydrogel were dependent on the level of both amine and aldehyde-based precursors. By monitoring the reaction conditions, these levels could readily be altered and allowed good control over the gel properties. These gels exhibited excellent hemostatic properties and adhesion reduction in animal models (Liu et al. 2009).

In another study, thiolated-Dex (Dex-SH) was modified by thiolation reaction and prepared an in situ crosslinked hydrogel composed of Dex-SH and vinyl sulfone modified Pluronic 127 (PL-VS) or acrylated Pluronic 127 (PL-Ar) through Michael-type addition reaction. The results showed rapid formation of hydrogel in situ and exhibited a wide range of storage moduli (from 0.3 to 80 kPa) and thermosensitive behavior (from 10 to 37 °C), when increasing concentration of PL from 5% to 20 w/v %. Moreover, Dex-SH/PL-Ar hydrogels were observed to be degradable at physiological conditions and exhibited lower cytotoxicity compared to Dex-SH/PL-VS hydrogels (Lin et al. 2010).

3.3 Gellan Gum

Learmonth et al. developed a dopamine-modified gellan gum hydrogel (DGG) with enhanced physicochemical and biological properties, suitable for minimally invasive cell delivery and retention in terms of cartilage repair. The developed DGG hydrogels underwent rapid ionic crosslinking under physiologically relevant

mono- or divalent cations to prepare stable 3D hydrogels with excellent tissue adhesiveness. In addition, DGG hydrogels maintained mammalian cell viability and promoted upregulation of the expression of healthy chondrogenic ECM markers upon stimulation (Learmonth et al. 2020). In tissue regeneration, macrophages serve a critical role in regulating the host response to implanted materials. The macrophage phenotype tends to be dynamic throughout the host response and this phenotype should be balanced for favorable progression from injury to actual wound healing. Therefore, methacrylated GG (MGG) hydrogel was prepared and demonstrated the effect of hydrogel on macrophage phenotype and proliferation. Here, MGG hydrogels were prepared with various thiol-ene (SH/C=C) and different crosslinking mechanisms (crosslinked with chain growth or step growth or in combination) (Li and Bratlie 2019). In another study, GG was grafted with cinnamate (Cin) to prepare photocrosslinkable polymer (GG-Cin) under UV irradiation, where 14.7% of D-galacturonic residues of GG were reacted with Cin and exhibited maximum absorption at 254 nm. GG-Cin showed 82% crosslinking efficiency after 16 min of UV irradiation in preparing antiadhesion GG-Cin films. The prepared films showed high gel contents (88 ± 2%), appropriate mechanical properties, and highly promising antiadhesion activity, when implanted into rats, in two rats from ten rats without forming any tissue adhesion. Also, film could inhibit inflammatory response significantly in rats (Lee et al. 2012).

3.4 Carrageenan

To eliminate wound infection and accelerated wound healing on wound area, *k*-carrageenan (k-CG)-based sprayable adhesives with multifunctional properties was developed. Here, *k*-CG was modified by the methacrylation reaction to prepare methacrylated *k*-CG (k-CGMA). Further, zinc oxide nanoparticles (ZnO NPs) were synthesized by using aloe vera leaf extract and then modified with polydopamine to prepare ZnOPD NPs. Finally, visible-light crosslinked hydrogel was prepared by using k-CGMA and ZnOPD NPs. Furthermore, L-glutamic acid was added to this nanocomposite hydrogel network to accelerate wound healing. The results showed significant improvement in tensile strength (from 64.1 ± 10 to 80.3 ± 8 kPa) and elongation (from 20 ± 4% to 61 ± 5%) of nanocomposite hydrogel with the addition of 1 wt.% ZnOPD NPs. In addition, effectual blood clotting capacity and biocompatibility (more than 95% cell viability) was observed after 3 days of cell culture. L-glutamic acid in hydrogel exhibited significant acceleration in wound healing with superior granulation tissue thickness as compared to control in a full-thickness skin defect model (Tavakoli et al. 2020). In another study, an injectable hydrogel composed of k-CG and C-phycocyanin was developed through ionic crosslinking. Here, a pigmented protein as C-phycocyanin was used with *k*-CG due to its antimicrobial, antioxidant, anti-inflammatory, wound healing properties as well as fluorescence imaging ability in vivo.

The developed injectable hydrogel exhibited good hydrophilic nature, mechanical stiffness, and improved in vitro proliferation of dermal fibroblasts without

creating inflammation, including reduction in blood clotting time with no hemolysis. Moreover, hydrogel showed superior hemostatic abilities in traumatic injury situation and rapid wound healing process (Dev et al. 2020). Tytgat et al. prepared 3D printed scaffolds composed of methacrylated k-CG and methacrylated gelatin (GelMA). Here, k-CG and Gel were modified with methacrylic anhydride according to their alcohol and amine moieties. Then both modified polymers were blended to prepare hydrogel inks and printed hydrogel structures by using extrusion-based 3D printing. The developed only GelMA and Gel-MA/k-CGMA printed scaffolds were observed to be stable over time for 21 days, absorb high amounts of water, and showed mechanical performances comparable to that of native breast tissue (2 kPa). Further, both hydrogel scaffolds showed a similar adipose tissue–derived stem cell viability and proliferation rate after 14 days of incubation. Moreover, cells were able to be differentiated into adipogenic lineage on hydrogel scaffolds, but lower on Gel-MA/k-CGMA printed scaffold compared to only GelMa printed scaffold (Tytgat et al. 2019). In another study, dopamine-modified graphene oxide (GOPD) was used with k-CGMA and prepared injectable nanohybrid hydrogels. Here, shear-thinning property and injectability through the interaction of active catechol functional groups of PD with other moieties in hydrogel structure further promoted the mechanical properties of hydrogels and significant improvement in compressive strength (8 times), toughness (6 times), and recovery of the hydrogels was observed in case of 20 wt.% GOPD reinforcement. Furthermore, k-CGMA/GOPD20% hydrogel improved fibroblast cell proliferation and spreading was observed after 5 days of cell culture (Mokhtari et al. 2019).

3.5 Pullulan

PLN hydrogel (10%) was used in sutureless wounds in rats for analyzing its healing efficacy. Here, a PLN hydrogel-treated incision wound in rats was healed within 6 days, while wounds in positive control and control rats took 11 and 15 days, respectively, in complete healing (Fig. 6). Also, two times increase in tensile strength (3.63 MPa) was observed in PLN hydrogel-treated wounds as compared to positive control (1.34 MPa) or control (1.17 MPa) wounds. Further, more than 25% increase in shrinkage temperature was also evaluated as compared to control. Moreover, improved fibroblast cell proliferation followed by faster epithelialization of wounds in PLN hydrogel-treated rats was demonstrated (Priya et al. 2016).

To improve the properties of PLN, Choudhary et al prepared a composite hydrogel composed of GG and pullulan (PLN), without any chemical functionalization, and the developed GG/PLN hydrogel exhibited fast setting and maintained its elasticity at high frequency and temperature (Choudhury 2019). In another study, PLN was oxidized by using $NaIO_4$ and chondroitin sulfate (CHS) was modified with adipic dihydrazide, and both were used for self-crosslinked and injectable oxPLN/CHS-ADH hydrogels. This oxPLN/CHS-ADH hydrogel with 7/3 weight ratio could provide a best tissue-mimetic 3D microenvironment and maintained chondrocyte cell phenotype and improving chondrogenesis (Li et al. 2018). To prevent or reduce

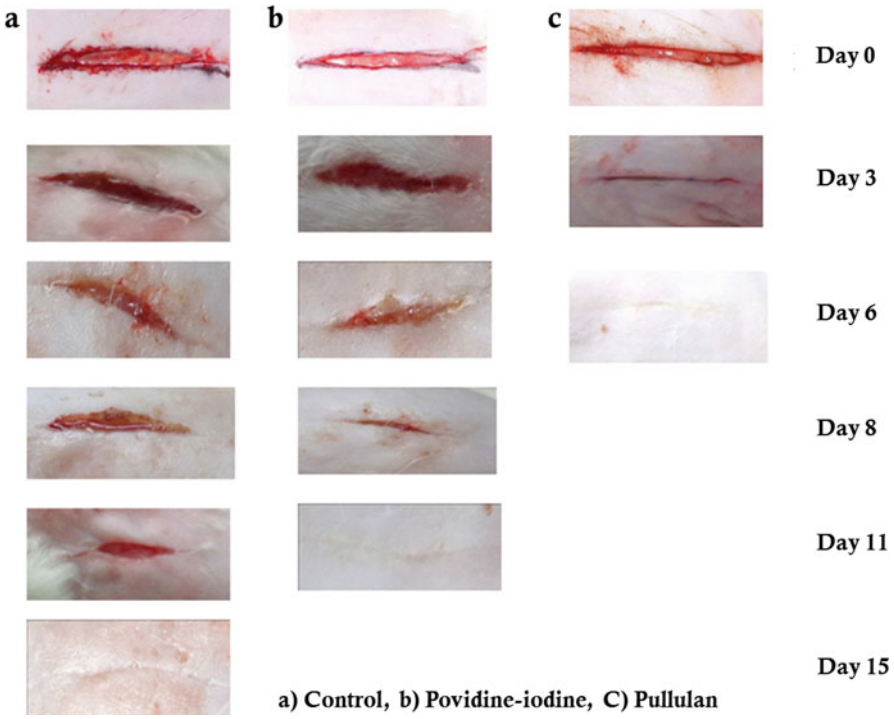


Fig. 6 Digital images showing healing rate in sutureless wounds. (Reproduced with permission from Elsevier, Priya et al. 2016)

postoperative abdominal adhesions is a major concern, where adhesion causes serious complications (e.g., postoperative pain, intestinal obstruction, and infertility). In recent times, various form of tissue adhesion barriers such as sprays, films, membranes, hydrogels, and knits have been developed. Among them, hydrogels possess various advantages and especially injectable hydrogels can fill and cover all spaces of any shape and do not need a surgical process for implantation. In this way, Bang et al. modified PLN with the reaction of 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO) to incorporate carboxyl groups followed by the addition of tyramine on carboxylated PLN with 1-ethyl-3-(3-dimethylamino-propyl)-carbodiimide (EDC) and N-hydroxyl-succinimide (NHS) to incorporate phenyl functional groups as crosslinking sites. Further, tyramine-grafted PLN-based hydrogel was prepared by an enzymatic reaction through horseradish peroxidase (HRP) and hydrogen peroxide (H_2O_2). PLN-Tyr hydrogel showed significant inhibition of cell proliferation and exhibited proper prevention of abdominal tissue adhesion (in an animal model) (Bang et al. 2016).

The control over resolution of hydrogel features, modification, and mechanical performances is very beneficial for providing possible natural tissue niches on cell growth and differentiation. With this aim, PLN-based cell adhesive hydrogel with

3D printable in different dimensions and tunable mechanical properties was processed through stereolithography technique. Here, methacrylated PLN (PLN-MA) was synthesized and printed by using multiscale light-assisted 3D printing techniques as visible stereolithography (SL) and two-photon lithography (TPL). Further, complex 3D shapes through spatially controlled irradiation (SL) were printed and mechanical properties were controlled by incorporating a bifunctional crosslinker and also enabled water absorption capacity of PLN-MA hydrogels (Della Giustina et al. 2019).

3.6 Xanthan Gum

Xanthan gum (XG) has widely been applied in different industrial (e.g., food and food packaging, paints, and cosmetics) and biomedical (drug delivery and tissue engineering) applications. Due to some disadvantages in processing and mechanical properties of only XG, it needs appropriate modification or processing to be used efficiently in surgical applications (Kumar et al. 2018).

XG-based bilayered mucoadhesive buccal patch with zolmitriptan drug was developed. Here, hydroxypropyl methylcellulose E-15 to improve film-forming ability and polyvinyl alcohol (PVA) to enhance the tensile strength of the mucoadhesive patches were used. The results exhibited modified rate of drug release and good bioadhesion due to XG and bilayered patches may prevent hepatic metabolism in a large extent possible (Shiledar et al. 2014). Generally, the resulting adhesion between surgical tendon and synovial sheath tends to cause poor functional repairing after tendon repair surgery. Seprafilm, a commercial product composed of hyaluronan (HA) and CMC hydrogels, has been used to prevent this adhesion during surgery. With this issue, Kuo et al. developed a new composite membrane composed of XG, GG, and HA and analyzed in a rat model (Kuo et al. 2014). Membranes of different formulations of XG/GG/HA (XGH) as well as Seprafilm were wrapped around repaired tendons and evaluated grossly and histologically after healing for 3 weeks. Certain designed XGH hydrogel membranes decreased tendon adhesion with equal ability without reducing the tendon strength as compared to Seprafilm. These membranes rapidly swelled and more readily and closely blanketed onto tendon tissue with slow degradation than that of Seprafilm (see Fig. 7) (Kuo et al. 2014).

After abdominal surgery, postoperative adhesion is a significant challenge. With this objective, various polymeric barriers have been applied to prevent adhesions, but complete prevention of adhesion was not observed in all situations. Therefore, Song et al. developed new XG-based biomaterial and investigated the estimation of its potential as injectable tissue adhesion barrier with various concentrations (0.5–2.0% w/v) and molecular weight (2.5×10^6 – 6.9×10^6 Da). Here, XG provides an antiadhesion property in the rat abdominal cavity. One percent of XG gel having high molecular weight (6.9×10^6 Da) was observed to be more effective to prevent adhesions as compared to commercial product (1.2% HA). In addition, XG gel exhibited no cytotoxicity in vitro to L929 cells and no side effect during

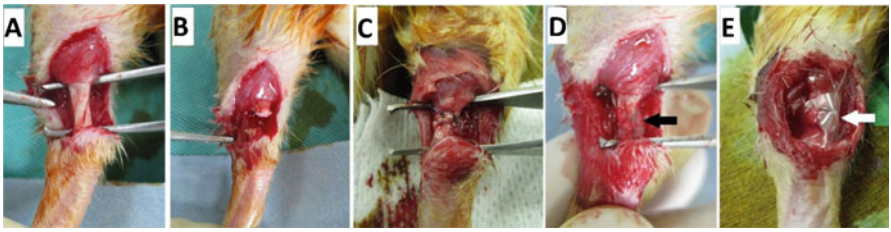


Fig. 7 Animal model: (**a**) Achilles tendon exposed by sharp dissection, (**b**) transected Achilles tendon (midpoint), (**c**) tendon treated with 5-0 Prolene sutures, (**d**) postrepaired tendon wrapped with XGH membrane (smooth fitting on tendon), and (**e**) tendon wrapped with Seprafilm (irregular deformation). (Reproduced with permission from Elsevier, Kuo et al. 2014)

wound healing. Moreover, XG showed good potential in preventing intra-abdominal adhesion. In this study, intra-abdominal adhesions were divided into two groups as insubstantial (grade 0 and 1) and substantial (grade 2–4) adhesions (Fig. 8). Here, substantial adhesions were difficult to separate and considered clinically significant high-grade adhesions (Song et al. 2019).

3.7 Glucan

Intra-abdominal infections are associated with the deposition of fibrin, which may result significant abscess formation and adhesions clinically. In this way, several agents have been applied in experimental and clinical studies to prevent postoperative adhesions. Further, nonspecific immune-stimulant beta-glucan was used for the reduction of intra-abdominal abscesses and adhesions. Here, beta-glucan did not have significant effect on mortality and abscess formation. However, it was capable in reducing the adhesion frequency (Bedirli et al. 2003). In another study, a new functional wound dressing composed of CS-glucan complex (GC) hollow fibers reinforced collagen (COLL) embedded with aloe vera (AV) to be used in especially infected chronic wound and ulcers for skin regeneration. Here, CSGC hollow fibers were prepared from mycelium beads and then AV aqueous solution was mixed with CSGC hollow fibers under high-speed homogenizer (12,000 rpm) to prepare CSGC@AV and further was mixed with COLL to prepare CSGC@AV/COLL suspensions, which were homogenized, centrifuged, and freeze-dried to prepare functional wound dressing (Abdel-Mohsen et al. 2020).

4 Surgical Applications of Polysaccharides

4.1 Orthopedic Surgery

Different surgical sealants having higher adhesion and bonding strength have been used for joining two surfaces in orthopedic surgery. An ideal sealant for orthopedic surgery should promote bone healing, should not hamper blood circulation in

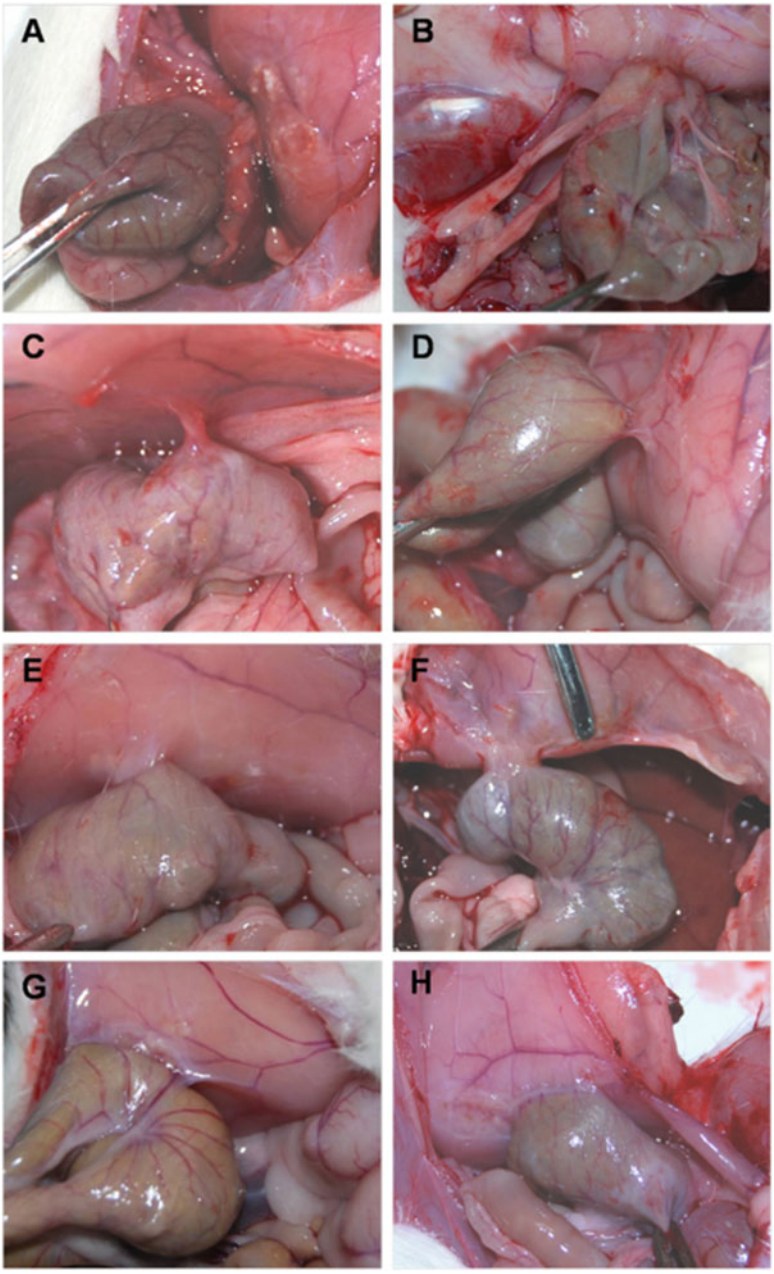


Fig. 8 Digital images of intra-abdominal adhesions with adhesion severity stages on postoperative day 8: stage 0 (**a**), stage 4 (**b**), stage 1 (**c** and **d**), stage 2 (**e** and **f**), and grade 3 (**g** and **h**). (Reproduced with permission from Elsevier, Song et al. 2019)

musculoskeletal tissue, and prevent gap formation in bones. Strehin et al. prepared chondroitin sulfate/polyethylene glycol (CS/PEG)-based tissue adhesive for cartilage surgery (Strehin et al. 2010). Here, chondroitin sulfate succinimidyl succinate (CS-NHS) was reacted with six arm polyethylene glycol amine (PEG–$(NH_2)_6$). The properties of the developed gel could be tuned by altering the polymer ratio and degree of NHS functionalization (Strehin et al. 2009, 2010). The gels showed ten times higher adhesive strength than fibrin glue and their stiffness and swelling properties could be adjusted by tuning the pH of starting materials. The gels displayed good sealing properties when used for treating collagen membranes in rats.

Wang et al. synthesized methacrylate and aldehyde functionalized chondroitin sulfate which was used as a sealant (Wang et al. 2007). This sealant could effectively form chemical bonds with tissue proteins and the incorporated photoinitiator led to the hydrogel formation. It was reported that the developed sealant could effectively achieve strong tissue adhesion and proteoglycan production when tested in vivo in rat model for cartilage tissue repair. Furthermore, Simson et al. fabricated bone marrow and chondroitin sulfate–based adhesives for meniscus tissue repair (Simson et al. 2013). Different adhesives samples were prepared by varying the ratio of bone marrow to chondroitin sulfate. It was found that bone marrow component helped in achieving fibrochondrocyte viability and its migration. On the other hand, chondroitin sulfate was useful for achieving a wide range of adhesive strength (60 ± 17 to 335 ± 88 kPa). The adhesives were tested in athymic rats and were found suitable for fusion of adhered meniscus. Li et al. synthesized chondroitin sulfate modified with glycidyl methacrylate to develop photocrosslinkable gels showing their high potential for cartilage tissue repair (Li et al. 2004). Chou et al. synthesized gelatin/hyaluronic acid/chondroitin-6-sulfate (GHC6S) tri-copolymer particles which were subsequently added to fibrin glue (Chou et al. 2007). The addition of these particles helped in achieving higher mechanical strength, efficient cell seeding, and distribution. The developed glues were tested to repair a damaged articular cartilage showing improved cartilage regeneration in vitro. For orthopedic and maxillofacial surgical applications, Fricain et al. prepared PLN/Dex scaffolds with or without nanohydroxyapatite (nHAp) (Fricain et al. 2013). In this study, in vitro analyses exhibited the formation of multicellular aggregates and expression of early and late bone-specific markers with human bone cells in the medium with deficient of osteoinductive factors. Further, only without cell-seeded PLN/Dex-nHAp scaffold showed maintained subcutaneously local growth factors, induced deposition of biological apatite formation, and favorable subcutaneously formation of a dense mineralized tissue in mice and well osteoid tissue after intramuscular implantation in goat. Further, the developed scaffold was implanted in three orthopedic preclinical models of critical size defects (e.g., small and large animals) in rat and goat. This scaffold induced highly mineralized tissue in all three models whatever the site of implantation, including osteoid tissue and regeneration of bone tissue (Fricain et al. 2013).

4.2 Neural Surgery and Plastic Surgery

Recently, polysaccharide-based biomaterials are gaining the attention for being used as surgical glue in neural and plastic surgery. Ereth et al. performed a comparative study to determine the safety and hemostatic efficiency of different commercially available formulations in neurosurgery (Ereth et al. 2008). In this study, a brain tissue defect was created in 228 rats followed by application of different hemostatic agents such as starch-based Arista AH, oxidized cellulose-based Surgicel, collagen-based Avitene, gelatin-based FloSeal, and kaolin. The rats were sacrificed and investigated at different time intervals and presence of residual material and foreign body reaction was determined. It was reported that Arista AH was not only able to stop bleeding efficiently buy also to degrade quickly without causing any inflammation.

Franceschini et al. used ORC (Tabotamp®) for breast conservative oncoplastic surgery (Franceschini et al. 2012). It was observed that ORC applied after oncoplastic surgery allowed not only to reduce the bleeding but also to lower the rate of infection at the surgical site. It also helped in optimizing cosmetic defects especially in small–medium size breast. Bassetto et al. reported the use of ORC (Tabotamp®) for hemostasis while performing a facelift procedure (Bassetto et al. 2008). It was showed that no local pain or skin damage was observed post-operation.

4.3 Wound Healing and Miscellaneous Applications

Wound healing requirements and challenges have led to the preparation of various tissue scaffolds or adhesives to resolve the problems associated with conventional sutures and staples for their time-consuming, tissue damage, immunological response, and insufficient reduction of fluid leakage from the wounds (Balakrishnan et al. 2017). With this objective, MGG hydrogels were developed and good cytocompatibility and an improved anti-inflammatory production of nitrites from naïve and classically activated macrophages by stiffer biomaterials as compared to the softer biomaterials. Further, arginine and CD206 expression markers for alternatively activated macrophages were inhibited by higher thiol content. Moreover, the introduced ionic crosslinking by using calcium ions (Ca^{2+}) had no influence on the proliferation or polarization for any of three macrophage phenotypes (Li and Bratlie 2019). Further, tyramine-modified carboxymethyl cellulose (CMC) (CMC-Tyr) was prepared and then injectable hydrogel was synthesized in situ by using an enzyme-mediated reaction of CMC-tyr with HRP and H_2O_2. Further, PLN was added to CMC-Tyr hydrogel solution to enhance adhesiveness to the wound area and acceleration of biodegradation. Moreover, in vivo analysis demonstrated significant reduction in postoperative tissue adhesion (Bang et al. 2017). In another study, Abdel-Mohsen et al. developed a functional CSGC@AV/COLL wound dressing that exhibited high hydrolytic stability with improved swelling properties, enhanced hemostatic effect, and excellent biocompatibility on human dermal fibroblast cells as compared to native COLL. Further, high antibacterial behavior against various bacteria (gram-positive/gram-negative) was observed by this functional

CSGC@AV/COLL wound dressing. Moreover, an improved percentage of wound closure was observed after 1 week treatment. This developed wound dressing showed good potential to be used in especially infected chronic wound and ulcers for skin regeneration (Abdel-Mohsen et al. 2020).

In recent years, different polysaccharides and their derivatives have been explored as surgical glues for various types of surgeries. Di Lello et al. used ORC for sutureless epicardial fixation of long aortocoronary saphenous vein grafts and compared its efficiency with fibrin glue (Di Lello et al. 1989). It was reported that (a) ORC gauze readily adheres to the surface wet with blood; (b) it has antibacterial properties; and (c) ORC gauze is more economical than fibrin glue.

Chakoli et al. prepared ORC-based composites by reinforcing with multiwalled carbon nanotubes (MWCNTs) (Chakoli et al. 2014). Cellulose films and fibers were oxidized by using nitrogen dioxide and carbon tetrachloride. Further, ORC fibers and films were reacted with amine functionalized MWCNTs using 1-ethyl-3-(3-dimethylamino-propyl)-carbodiimide (EDC)/N-hydroxyl-succinimide (NHS) system. The developed composites were crosslinked using glutamic acid. The incorporation of MWCNTs led to enhanced hydrophilicity and hemostatic efficiency of ORC gauze. Cheng et al. developed hemostatic composite gauze consisting of carboxymethyl chitosan and ORC (Cheng et al. 2016). It was reported that these composite gauzes not only show enhanced hemostatic behavior in rabbit liver injury model but also good antibacterial activity against both gram-positive and gram-negative bacteria. The animal experiments indicated that the composite gauze could stop the bleeding in 90 s showing rapid coagulation as compared to pure ORC which took 4 min to achieve efficient hemostasis. These composite gauzes are also beneficial for postoperative adhesion prevention.

Hoffmann et al. investigated the peritoneal adhesion after the use of different hemostatic agents in intra-abdominal surgeries (Hoffmann et al. 2009). It was reported that starch (Arista AH) and polyethylene glycol (CoSeal)-based hemostatic agents could effectively reduce the peritoneal adhesion. Arista AH did not leave any residual agent and showed no difference in acute inflammation when compared to other commercially available hemostatic agents such as FloSeal, Bioglue, and Tisseel. Zhu et al. synthesized Ca^{2+}-modified crosslinked porous starch microparticles (CPSM) which were used as a hemostatic agent in the mouse tail amputation model (Zhu et al. 2019). The fast release of Ca^{2+} from porous particles led to enhanced platelet adhesion resulting into faster clot formation. It was reported that incorporation of Ca^{2+} played a pivotal role in blood clot formation via facilitating conversion of prothrombin into thrombin and crosslinking of fibrin (Zhu et al. 2019). Sharma et al. investigated the efficiency and safety of surgicel for the creation of neovagina in 10 patients (Sharma et al. 2007). It was observed that the use of surgicel led to minimum complications, morbidity, and discomfort with 8 out of 10 patients showing satisfactory results. Finley et al. reported the use of fibrin glue–oxidized cellulose sandwich for hemostasis during laparoscopic wedge resection of small renal tumors (Finley et al. 2005). Excellent hemostasis was achieved in all the 15 cases during May 2002–December 2003 omitting the requirement of blood transfusion.

Ono et al. developed chitosan-based photocrosslinked gels to be used as a biological adhesive for soft tissues (Ono et al. 2000). In that work, solubility of chitosan was enhanced by treatment with lactobionic acid which introduced the lactose moieties in the polymer structure. Further, azide moieties were introduced by reacting it with 4-azidobenzoic acid, thus enabling photocrosslinking. The binding efficiency of developed gel was investigated against air leakage from the pinholes on trachea in rabbits showing their potential as tissue adhesive. The efficiency of developed system was compared with fibrin glue and it was observed that chitosan-based adhesives could be applied more easily without any spill (Ono et al. 2000). Similarly, Ishihara et al. reacted chitosan with p-azidebenzoic acid and lactobionic acid to develop sample that could be converted into gel under UV light (Ishihara et al. 2002). These gels could not only stop bleeding from mouse tail within 30s of UV irradiation but also attach two skin parts with each other in mouse model showing their great potential as tissue adhesive and hemostatic agent. Furthermore, Dowling et al. reported the formulation of sprayable foams (Fig. 9a) based on hydrophobically modified chitosan (hmC) for treating noncompressible hemorrhage (Fig. 9b) (Dowling et al. 2015). Here, hemostatic action was achieved by converting the blood into self-supporting gel where hydrophobic chains are inserted into the blood cells bilayer thus creating a network of cell clusters. The developed foam was tested in vivo to treat the liver injury in pig model. It was observed that hemostasis could be sustained for upto 60 min without any compression and total blood loss was 90% lower as compared to the control samples. Development of the sprayable foam able to work without compression paves a new pathway for treating traumatic injury caused to soldiers in combat as well as to mankind in serious accidents (Dowling et al. 2015).

Lih et al. designed tyramine-modified polyethylene glycol which was further grafted on chitosan by reacting with amine groups (Lih et al. 2012). As a next step, tyramine moieties were crosslinked with the help of horseradish peroxidase and H_2O_2 resulting into hydrogels which showed excellent tissue adhesive properties when tested in rat skin model.

Hyaluronic acid has been often reacted with methacrylic anhydride for introducing methacrylic groups which can be further polymerized under the presence of light (Smeds et al. 1999). Photopolymerization of methacrylate functionalized hyaluronic acid results into hydrogel formation. Various mechanical properties (e.g., swelling, compression, and creep resistance) of these hydrogels can be tuned by adjusting the degree of methacrylation which in turn affects the extent of covalent bonding in the hydrogel system (Smeds and Grinstaff 2001). Miki et al. reported that these methacylated hyaluronic acid–based hydrogels can be used for sealing a 3 mm corneal laceration in rabbits (Miki et al. 2002). It was found that the hydrogel patch helped in proper sealing of corneal perforations and normal intraocular pressure was achieved within 7 days. The applied hydrogel showed minimal inflammation, higher proliferation of stromal cells, and caused tight adherence of extracellular matrix. Further, sodium salt of hyaluronic acid (Seprafilm®adhesion barrier) has been used to prevent abdominal adhesion after surgery in hernia treatment and intestinal resection (Kramer et al. 2002; Fazio et al. 2006).

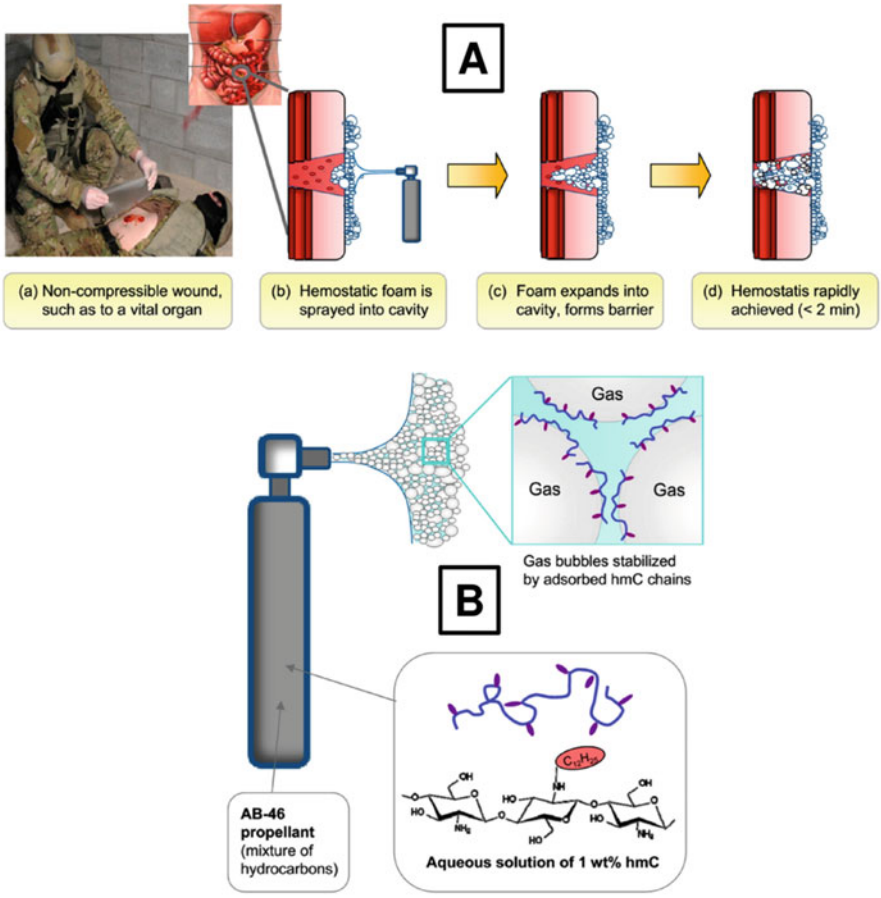


Fig. 9 Schematic representation of hemostatic action of sprayable foam (**a**); hemostatic foam consisting of hydrophobically modified chitosan (hmC) and AB-46 propellent (**b**). (Reproduced with permission from American Chemical Society, Dowling et al. 2015)

Reyes et al. reacted chondroitin sulfate aldehyde with polyvinyl alcohol-co-vinyl amine to develop tissue adhesives for repairing penetrating corneal wounds (Reyes et al. 2005). As the repair of such wounds requires watertight sealing, the efficiency of developed adhesives was compared with sutures. The adhesive was tested ex vivo in enucleated rabbit eyes for treating corneal incision. It was reported that maximum intraocular pressure (104.7 mm Hg) could be achieved by using the adhesive. It showed no leakage of the balanced salt solution that was used to fill the globes and was reported to be much more efficient in avoiding the leakage than sutures. Furthermore, Pirouzmanesh et al. used chondroitin sulfate–based tissue adhesive and sutures for investigating comparative graft stability of donor eyes and astigmatic changes (Pirouzmanesh et al. 2006). Here, less blurred vision was reported in case of

chondroitin sulfate–based tissue adhesive making them an attractive alternative for surgery of corneal endothelial disorders.

Further, dextran is oxidized by reacting with sodium periodate resulting into the formation of dextran aldehyde which in turn can be reacted with amines to get degradable imine bonds. Bhatia et al. developed dextran aldehyde–based surgical glue and investigated the influence of different degree of oxidation (Bhatia et al. 2007a) It was reported that the surgical glues formed by dextran aldehyde having degree of oxidation higher than 60% exhibited very rapid crosslinking leading to inefficient tissue binding. Fifty percent degree of oxidation was reported to be the most optimum with leakage pressure as high as 500 mm Hg. This natural polymer-based biomaterial degraded within 3 days during in vitro study. Here, the faster degradability was credited to the hydrolysis of imine bonds ensuring the clearance of by-products from the body. However, it is to be noted that due to the fast degradation higher amount of material was required for proper healing of corneal incision resulting into foreign body response. To address this issue, Chenault et al. designed a novel tissue adhesive based on dextran aldehyde (MW 10 kDa) which was reacted with PEG 8-arm star polymer (MW 10 kDa) having two amine groups per arm (Chenault et al. 2011). In vitro testing showed no cytotoxicity, higher bonding strength, and the leak pressure was found to be approximately 141 mm Hg. The efficiency of developed tissue adhesive was tested in vivo to seal the corneal incisions in New Zealand White rabbits. It was reported that the corneal incision was healed after 5 days without complete degradation of the adhesive during this time. This tissue adhesive is on its way to be available as commercial material Actamax™ by DSM and DuPont in a joint effort (Bouten et al. 2014). In an effort for increasing its shelf life, aldehyde-functionalized polyglycerol is being used as an alternative for dextran aldehyde (Chenault 2013).

Postsurgical bleeding and undesired tissue adhesion are the two major challenges encountered after endoscopic sinus surgery. In this regard, Athanasiadis et al. investigated the potential of chitosan-/dextran-based hybrid gel in preventing unwanted tissue adhesion and supporting the mucosal wound healing (Athanasiadis et al. 2008). The investigation was performed in sheep model having chronic sinusitis. Severity of adhesion and healing was monitored at regular time intervals exhibiting chitosan/dextran gel as a potential alternative. Aziz et al. further explored the antimicrobial activity of chitosan-/dextran-based hydrogel for surgical application (Aziz et al. 2012). The bacteria killing efficiency of the hydrogel was tested against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, and *Clostridium perfringens*. The surgical concentration of 50,000 mg/L of hydrogel was found to be effective. Here, dextran aldehyde was found to be responsible for antimicrobial activity by disrupting cell walls resulting into loss of cytosolic components.

Balakrishnan et al. fabricated dextran-based injectable tissue adhesives that can stop bleeding, bond the tissue effectively, and can be easily applied while performing a surgery (Balakrishnan et al. 2017). Here, dextran was treated with periodate to generate aldehyde groups which in turn could spontaneously react with chitosan hydrochloride via Schiff's base formation. It was reported that the strength of the developed tissue adhesive is five times higher than that of commercially available

fibrin glue. It was tested to seal the bleeding in rabbit liver injury and was found to be more tissue compliant than BioGlue®. Further, Liu et al. reported dextran aldehyde–based adhesive sponge (pore size ~30–50 μm) with high tissue adhesion properties to control hemorrhage (Liu et al. 2019). The sponge exhibited remarkable reduction in blood loss when tested for ear vein, femoral artery, and liver injuries in rats and rabbits. It was reported that the dextran-based sponge induced RBCs and platelets aggregation promoting blood coagulation along with sealing the wound, thus controlling massive blood loss. These sponges were biocompatible, biodegradable, and showed almost no skin inflammation. In another study, DDA-/PLL-based degradable glue via Schiff base formation prevents the air leakage in lung surgery (Araki et al. 2007). The efficiency of this glue was found to be higher than fibrin glue in preventing air leakage from large pleuroparenchymal defects. It was observed that almost 90% glue degraded within 3 months and the normal lung structure could be achieved within 6 months. Therefore, the product exhibited a strong sealing behavior and good biocompatibility to be used in lung surgery.

Pang et al. synthesized dextran aldehyde (DDA)/carboxymethyl chitosan (CMCS) gels loaded with chitosan nanowhisker (CtNWs) (Pang et al. 2020b) The developed gel exhibited approximately two times higher mechanical strength and tissue adhesive strength. It displayed good hemostatic performance while simultaneously avoiding inflammation and postoperative adhesion when tested for rat liver injury, showing higher efficiency than commercially available 3M™ vetbond™ tissue adhesive. Liu et al. prepared a library of hydrogels with tunable hemostatic and adhesive properties by reacting amine functionalized succinyl chitosan and dextran aldehyde to generate imine linkage (Liu et al. 2009). Succinyl chitosan with enhanced water solubility was synthesized by reacting chitosan with succinic anhydride. The properties of developed gels could be tuned by varying the crosslinking degree showing their great potential for surgical use. Bhatia et al. synthesized DDA which was further reacted with eight-arm amine functional group containing polyethylene glycol crosslinker (Bhatia et al. 2007b) In vitro experimental results with 3T3 fibroblast cells and J774 macrophage cells showed that the developed tissue adhesive was biocompatible and did not cause inflammatory response. Similarly, Nie et al. created CS-/PLL-based gels which showed excellent adhesive and hemostatic properties (Nie et al. 2013). These gels exhibited four times higher adhesive properties than commercially available fibrin glue.

5 Conclusions

Polysaccharides are highly abundant natural polymers and can be obtained from three mainly different renewable resources such as plants, animal, and microorganisms. These polysaccharides provide more beneficial properties in surgical applications as compared to synthetic polymers due to their high availability and renewable nature, easy processing, excellent biocompatibility, and intrinsic immunogenic ability. Among them, microbial polysaccharides or their combination with other plant-/animal-origin polysaccharides, including natural and/or synthetic nanomaterials,

have received a great attention in surgical applications. However, the blending of microbial polysaccharides with other polysaccharides provides better properties as compared to single polysaccharide. Therefore, several modification approaches and processing techniques have been used for getting desired properties similar to that of native tissues. But, their successful use in surgical applications as native-like tissue regeneration is still challenging and further research is needed through a wide range of new processing and modifications. Therefore, in this chapter, a brief review of polysaccharides, especially focusing on microbial polysaccharides and their processing and modifications are presented in their promising application for surgical applications. Recently, these polysaccharides are extensively used in additive manufacturing methods (i.e., 3D printing or bioprinting) to produce tissue hydrogel scaffolds and/or tissue models for future surgical applications (Kumar et al. 2020; Zidarič et al. 2020; McCarthy et al. 2019). Here, the optimization of print-related challenges associated with polysaccharides, cells, printing techniques, and processing parameters is very critical to develop biomaterials with desired properties. Therefore, a comprehensive understanding of polysaccharides, cells, and printing techniques (e.g., 3D, 4D, and 5D printing) is needed to create more complex 3D bioprinted architectures and their clinical translation in surgical applications (Kumar et al. 2019).

Acknowledgments The writing of this chapter was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science, and Technology (Grant No. 2017R1D1A3B03036276). Also, DST Inspire Faculty Award of the Department of Science and Technology, Government of India (DST/INSPIRE/04/2015/003220) is highly acknowledged.

References

Abdel-Mohsen A, Frankova J, Abdel-Rahman RM, Salem A, Sahffie N, Kubena I, Jancar J. Chitosan-glucan complex hollow fibers reinforced collagen wound dressing embedded with aloe vera. II. Multifunctional properties to promote cutaneous wound healing. Int J Pharm. 2020;582:119349.

Agrawal G, Agrawal R, Pich A. Dual responsive poly (N-vinylcaprolactam) based degradable microgels for drug delivery. Part Part Syst Charact. 2017;34:1700132.

Agrawal G, Samal SK, Sethi SK, Manik G, Agrawal R. Microgel/silica hybrid colloids: bioinspired synthesis and controlled release application. Polymer. 2019;178:121599.

Ahmad NH, Mustafa S, Che Man YB. Microbial polysaccharides and their modification approaches: a review. Int J Food Prop. 2015;18:332.

An S, Jeon EJ, Jeon J, Cho S-W. A serotonin-modified hyaluronic acid hydrogel for multifunctional hemostatic adhesives inspired by a platelet coagulation mediator. Mater Horiz. 2019;6:1169.

Annabi N, Yue K, Tamayol A, Khademhosseini A. Elastic sealants for surgical applications. Eur J Pharm Biopharm. 2015;95:27.

Araki M, Tao H, Nakajima N, Sugai H, Sato T, Hyon S-H, Nagayasu T, Nakamura T. Development of new biodegradable hydrogel glue for preventing alveolar air leakage. J Thorac Cardiovasc Surg. 2007;134:1241.

Ates B, Koytepe S, Karaaslan MG, Balcioglu S, Gulgen S. Biodegradable non-aromatic adhesive polyurethanes based on disaccharides for medical applications. Int J Adhes Adhes. 2014;49:90.

Athanasiadis T, Beule AG, Robinson BH, Robinson SR, Shi Z, Wormald PJ. Effects of a novel chitosan gel on mucosal wound healing following endoscopic sinus surgery in a sheep model of chronic rhinosinusitis. Laryngoscope. 2008;118:1088.

Atkins E. Biomolecular structures of naturally occurring carbohydrate polymers. Int J Biol Macromol. 1986;8:323.

Aziz MA, Cabral JD, Brooks HJ, Moratti SC, Hanton LR. Antimicrobial properties of a chitosan dextran-based hydrogel for surgical use. Antimicrob Agents Chemother. 2012;56:280.

Balakrishnan B, Soman D, Payanam U, Laurent A, Labarre D, Jayakrishnan A. A novel injectable tissue adhesive based on oxidized dextran and chitosan. Acta Biomater. 2017;53:343.

Bang S, Lee E, Ko Y-G, Kim WI, Kwon OH. Injectable pullulan hydrogel for the prevention of postoperative tissue adhesion. Int J Biol Macromol. 2016;87:155.

Bang S, Ko Y-G, Kim WI, Cho D, Park WH, Kwon OH. Preventing postoperative tissue adhesion using injectable carboxymethyl cellulose-pullulan hydrogels. Int J Biol Macromol. 2017;105:886.

Bassetto F, Vindigni V, Scarpa C, Botti C, Botti G. Use of oxidized regenerated cellulose to stop bleeding after a facelift procedure. Aesthet Plast Surg. 2008;32:807.

Bedirli A, Gokahmetoglu S, Sakrak O, Ersoz N, Ayangil D, Esin H. Prevention of intraperitoneal adhesion formation using beta-glucan after ileocolic anastomosis in a rat bacterial peritonitis model. Am J Surg. 2003;185:339.

Berger A, Tempfer C, Hartmann B, Kornprat P, Rossmann A, Neuwirth G, Tulusan A, Kubista E. Sealing of postoperative axillary leakage after axillary lymphadenectomy using a fibrin glue coated collagen patch: a prospective randomised study. Breast Cancer Res Treat. 2001;67:9.

Bhatia SK, Arthur SD, Chenault HK, Figuly GD, Kodokian GK. Polysaccharide-based tissue adhesives for sealing corneal incisions. Curr Eye Res. 2007a;32:1045.

Bhatia SK, Arthur SD, Chenault HK, Kodokian GK. Interactions of polysaccharide-based tissue adhesives with clinically relevant fibroblast and macrophage cell lines. Biotechnol Lett. 2007b;29:1645.

Birkholz M-N, Agrawal G, Bergmann C, Schröder R, Lechner SJ, Pich A, Fischer H. Calcium phosphate/microgel composites for 3D powderbed printing of ceramic materials. Biomed Eng/Biomedizinische Technik. 2016;61:267.

Bouten PJ, Zonjee M, Bender J, Yauw ST, van Goor H, van Hest JC, Hoogenboom R. The chemistry of tissue adhesive materials. Prog Polym Sci. 2014;39:1375.

Breech LL, Laufer MR. Surgicel® in the management of labial and clitoral hood adhesions in adolescents with lichen sclerosus. J Pediatr Adolesc Gynecol. 2000;13:21.

Chakoli AN, He J, Cheng W, Huang Y. Enhanced oxidized regenerated cellulose with functionalized multiwalled carbon nanotubes for hemostasis applications. RSC Adv. 2014;4:52372.

Chao HH, Torchiana DF. BioGlue: albumin/glutaraldehyde sealant in cardiac surgery. J Card Surg. 2003;18:500.

Chenault HK. Tissue adhesive and sealant comprising polyglycerol aldehyde. Google Patents: 2013.

Chenault HK, Bhatia SK, DiMaio WG Jr, Vincent GL, Camacho W, Behrens A. Sealing and healing of clear corneal incisions with an improved dextran aldehyde-PEG amine tissue adhesive. Curr Eye Res. 2011;36:997.

Cheng W, He J, Wu Y, Song C, Xie S, Huang Y, Fu B. Preparation and characterization of oxidized regenerated cellulose film for hemostasis and the effect of blood on its surface. Cellulose. 2013;20:2547.

Cheng F, He J, Yan T, Liu C, Wei X, Li J, Huang Y. Antibacterial and hemostatic composite gauze of N, O-carboxymethyl chitosan/oxidized regenerated cellulose. RSC Adv. 2016;6:94429.

Cheng F, Liu C, Li H, Wei X, Yan T, Wang Y, Song Y, He J, Huang Y. Carbon nanotube-modified oxidized regenerated cellulose gauzes for hemostatic applications. Carbohydr Polym. 2018;183:246.

Chou CH, Cheng WT, Kuo TF, Sun JS, Lin FH, Tsai JC. Fibrin glue mixed with gelatin/hyaluronic acid/chondroitin-6-sulfate tri-copolymer for articular cartilage tissue engineering: the results of real-time polymerase chain reaction. J Biomed Mater Res A. 2007;82:757.

Choudhury AR. Synthesis and rheological characterization of a novel thermostable quick setting composite hydrogel of gellan and pullulan. Int J Biol Macromol. 2019;125:979.

Della Giustina G, Gandin A, Brigo L, Panciera T, Giulitti S, Sgarbossa P, D'Alessandro D, Trombi L, Danti S, Brusatin G. Polysaccharide hydrogels for multiscale 3D printing of pullulan scaffolds. Mater Des. 2019;165:107566.

Dev A, Mohanbhai SJ, Kushwaha AC, Sood A, Sardoiwala MN, Choudhury SR, Karmakar S. κ-carrageenan-C-phycocyanin based smart injectable hydrogels for accelerated wound recovery and real-time monitoring. Acta Biomater. 2020;109:121.

Devulapalle KS, de Segura AG, Ferrer M, Alcalde M, Mooser G, Plou F. Effect of carbohydrate fatty acid esters on Streptococcus sobrinus and glucosyltransferase activity. J Carbohydr Res. 2004;339:1029.

Di Lello F, Mullen DC, Flemma RJ. Sutureless fixation of long aortocoronary saphenous vein grafts with oxidized regenerated cellulose. Ann Thorac Surg. 1989;47:473.

Doermbach K, Agrawal G, Servos M, Schipmann S, Thies S, Klemradt U, Pich A. Silica-coating of hematite nanoparticles using reactive water-soluble polyalkoxysiloxanes. Part Part Syst Charact. 2014;31:365.

Dowling MB, MacIntire IC, White JC, Narayan M, Duggan MJ, King DR, Raghavan SR. Sprayable foams based on an amphiphilic biopolymer for control of hemorrhage without compression. ACS Biomater Sci Eng. 2015;1:440.

Du X, Liu Y, Wang X, Yan H, Wang L, Qu L, Kong D, Qiao M, Wang L. Injectable hydrogel composed of hydrophobically modified chitosan/oxidized-dextran for wound healing. Mater Sci Eng C. 2019;104:109930.

Ereth MH, Schaff M, Ericson EF, Wetjen NM, Nuttall GA, Oliver WC Jr. Comparative safety and efficacy of topical hemostatic agents in a rat neurosurgical model. Oper Neurosurg. 2008;63: ONS369.

Fazio VW, Cohen Z, Fleshman JW, Van Goor H, Bauer JJ, Wolff BG, Corman M, Beart RW, Wexner SD, Becker JM. Reduction in adhesive small-bowel obstruction by Seprafilm® adhesion barrier after intestinal resection. Dis Colon Rectum. 2006;49:1.

Finley DS, Lee DI, Eichel L, Uribe CA, McDougall EM, Clayman RV. Fibrin glue-oxidized cellulose sandwich for laparoscopic wedge resection of small renal lesions. J Urol. 2005;173:1477.

Flynn TC, Sarazin D, Bezzola A, Terrani C, Micheels P. Comparative histology of intradermal implantation of mono and biphasic hyaluronic acid fillers. Dermatol Surg. 2011;37:637.

Foster LJR, Thomson K, Marçal H, Butt J, Watson SL, Wakefield D. Chitosan – vancomysin composite biomaterial as a laser activated surgical adhesive with regional antimicrobial activity. Biomacromolecules. 2010;11:3563.

Franceschini G, Visconti G, Terribile D, Fabbri C, Magno S, Di Leone A, Salgarello M, Masetti R. The role of oxidized regenerate cellulose to prevent cosmetic defects in oncoplastic breast surgery. Eur Rev Med Pharmacol Sci. 2012;16:966.

Fricain JC, Schlaubitz S, Le Visage C, Arnault I, Derkaoui SM, Siadous R, Catros S, Lalande C, Bareille R, Renard M. A nano-hydroxyapatite–pullulan/dextran polysaccharide composite macroporous material for bone tissue engineering. Biomaterials. 2013;34:2947.

Garnjanagoonchorn W, Wongekalak L, Engkagul A. Determination of chondroitin sulfate from different sources of cartilage. Chem Eng Process Process Intensif. 2007;46:465.

Gruber-Blum S, Petter-Puchner A, Mika K, Brand J, Redl H, Öhlinger W, Benesch T, Fortelny R. A comparison of a bovine albumin/glutaraldehyde glue versus fibrin sealant for hernia mesh fixation in experimental onlay and IPOM repair in rats. Surg Endosc. 2010;24:3086.

Hamed I, Özogul F, Regenstein JM. Industrial applications of crustacean by-products (chitin, chitosan, and chitooligosaccharides): a review. Trends Food Sci Technol. 2016;48:40.

Hidas G, Kastin A, Mullerad M, Shental J, Moskovitz B, Nativ O. Sutureless nephron-sparing surgery: use of albumin glutaraldehyde tissue adhesive (BioGlue). Urology. 2006;67:697.
Hoffmann NE, Siddiqui SA, Agarwal S, McKellar SH, Kurtz HJ, Gettman MT, Ereth MH. Choice of hemostatic agent influences adhesion formation in a rat cecal adhesion model. J Surg Res. 2009;155:77.
Hyon SH, Nakajima N, Sugai H, Matsumura K. Low cytotoxic tissue adhesive based on oxidized dextran and epsilon-poly-l-lysine. J Biomed Mater Res A. 2014;102:2511.
Ishihara M, Nakanishi K, Ono K, Sato M, Kikuchi M, Saito Y, Yura H, Matsui T, Hattori H, Uenoyama M. Photocrosslinkable chitosan as a dressing for wound occlusion and accelerator in healing process. Biomaterials. 2002;23:833.
Jain R, Wairkar S. Recent developments and clinical applications of surgical glues: an overview. Int J Biol Macromol. 2019;137:95.
Janmey PA, Winer JP, Weisel JW. Fibrin gels and their clinical and bioengineering applications. J R Soc Interface. 2009;6:1.
Jeon EY, Choi B-H, Jung D, Hwang BH, Cha HJ. Natural healing-inspired collagen-targeting surgical protein glue for accelerated scarless skin regeneration. Biomaterials. 2017;134:154.
Jiji S, Udhayakumar S, Maharajan K, Rose C, Muralidharan C, Kadirvelu K. Bacterial cellulose matrix with in situ impregnation of silver nanoparticles via catecholic redox chemistry for third degree burn wound healing. Carbohydr Polym. 2020;245:116573.
Jin R, Teixeira LSM, Dijkstra PJ, van Blitterswijk CA, Karperien M, Feijen J. Chondrogenesis in injectable enzymatically crosslinked heparin/dextran hydrogels. J Control Release. 2011;152:186.
Karaki N, Aljawish A, Humeau C, Muniglia L, Jasniewski J. Enzymatic modification of polysaccharides: mechanisms, properties, and potential applications: a review. Enzym Microb Technol. 2016;90:1.
Khalikova E, Susi P, Korpela T. Microbial dextran-hydrolyzing enzymes: fundamentals and applications. Microbiol Mol Biol Rev. 2005;69:306.
Kramer K, Senninger N, Herbst H, Probst W. Effective prevention of adhesions with hyaluronate. Arch Surg. 2002;137:278.
Kumar A, Rao KM, Han SS. Application of xanthan gum as polysaccharide in tissue engineering: a review. Carbohydr Polym. 2018;180:128.
Kumar A, Kargozar S, Baino F, Han SS. Additive manufacturing methods for producing hydroxyapatite and hydroxyapatite-based composite scaffolds: a review. Front Mater. 2019;6:313. https://doi.org/10.3389/fmats.
Kumar A, Matari IAI, Han SS. 3D printable carboxylated cellulose nanocrystal-reinforced hydrogel inks for tissue engineering. Biofabrication. 2020;12:025029.
Kuo SM, Chang SJ, Wang H-Y, Tang SC, Yang S-W. Evaluation of the ability of xanthan gum/gellan gum/hyaluronan hydrogel membranes to prevent the adhesion of postrepaired tendons. Carbohydr Polym. 2014;114:230.
Learmonth DA, Costa PM, Veloso TR, Cunha CB, Cautela MP, Correia C, Vallejo MC, Sousa RA. Synthesis and biological evaluation of a bioinspired, tissue-adhesive gellan gum-based hydrogel designed for minimally invasive delivery and retention of chondrogenic cells. Biomater Sci. 2020;8:3697.
Lee M-W, Tsai H-F, Wen S-M, Huang C-H. Photocrosslinkable gellan gum film as an anti-adhesion barrier. Carbohydr Polym. 2012;90:1132.
Leggat PA, Smith DR, Kedjarune U. Surgical applications of cyanoacrylate adhesives: a review of toxicity. ANZ J Surg. 2007;77:209.
Lewis KM, Atlee H, Mannone A, Lin L, Goppelt A. Efficacy of hemostatic matrix and microporous polysaccharide hemospheres. J Surg Res. 2015;193:825.
Li Z, Bratlie KM. How cross-linking mechanisms of methacrylated gellan gum hydrogels alter macrophage phenotype. ACS Appl Bio Mater. 2019;2:217.
Li L, Hsieh Y-L. Chitosan bicomponent nanofibers and nanoporous fibers. Carbohydr Res. 2006;341:374.

Li Q, Williams CG, Sun DD, Wang J, Leong K, Elisseeff JH. Photocrosslinkable polysaccharides based on chondroitin sulfate. J Biomed Mater Res A. 2004;68:28.
Li H, Niu R, Yang J, Nie J, Yang D. Photocrosslinkable tissue adhesive based on dextran. Carbohydr Polym. 2011;86:1578.
Li T, Song X, Weng C, Wang X, Sun L, Gong X, Yang L, Chen C. Self-crosslinking and injectable chondroitin sulfate/pullulan hydrogel for cartilage tissue engineering. Appl Mater Today. 2018;10:173.
Lih E, Lee JS, Park KM, Park KD. Rapidly curable chitosan–PEG hydrogels as tissue adhesives for hemostasis and wound healing. Acta Biomater. 2012;8:3261.
Lim SM, Song DK, Oh SH, Lee-Yoon DS, Bae EH, Lee JH. In vitro and in vivo degradation behavior of acetylated chitosan porous beads. J Biomater Sci Polym Ed. 2008;19:453.
Lin C, Zhao P, Li F, Guo F, Li Z, Wen X. Thermosensitive in situ-forming dextran–pluronic hydrogels through Michael addition. Mater Sci Eng C. 2010;30:1236.
Liu G, Shi Z, Kuriger T, Hanton L, Simpson J, Moratti S, Robinson B, Athanasiadis T, Valentine R, Wormald P. Synthesis and characterization of chitosan/dextran-based hydrogels for surgical use. Macromolecular symposia, vol. 279. Wiley Online Library; 2009, p. 151.
Liu J-Y, Li Y, Hu Y, Cheng G, Ye E, Shen C, Xu F-J. Hemostatic porous sponges of cross-linked hyaluronic acid/cationized dextran by one self-foaming process. Mater Sci Eng C. 2018;83:160.
Liu C, Liu X, Liu C, Wang N, Chen H, Yao W, Sun G, Song Q, Qiao W. A highly efficient, in situ wet-adhesive dextran derivative sponge for rapid hemostasis. Biomaterials. 2019;205:23.
Luo J-W, Liu C, Wu J-H, Lin L-X, Fan H-M, Zhao D-H, Zhuang Y-Q, Sun Y-L. In situ injectable hyaluronic acid/gelatin hydrogel for hemorrhage control. Mater Sci Eng C. 2019;98:628.
Mandour YMH, Mohammed S, Menem MA. Bacterial cellulose graft versus fat graft in closure of tympanic membrane perforation. Am J Otolaryngol. 2019;40:168.
Matsumura K, Nakajima N, Sugai H, Hyon S-H. Self-degradation of tissue adhesive based on oxidized dextran and poly-L-lysine. Carbohydr Polym. 2014;113:32.
McCarthy RR, Ullah MW, Booth P, Pei E, Yang G. The use of bacterial polysaccharides in bioprinting. Biotechnol Adv. 2019;37:107448.
Meziane-Tani M, Lagant P, Semmoud A, Vergoten G. The SPASIBA force field for chondroitin sulfate: vibrational analysis of D-glucuronic and N-acetyl-D-galactosamine 4-sulfate sodium salts. Chem A Eur J. 2006;110:11359.
Miki D, Dastgheib K, Kim T, Pfister-Serres A, Smeds KA, Inoue M, Hatchell DL, Grinstaff MW. A photopolymerized sealant for corneal lacerations. Cornea. 2002;21:393.
Min B-M, Lee SW, Lim JN, You Y, Lee TS, Kang PH, Park WH. Chitin and chitosan nanofibers: electrospinning of chitin and deacetylation of chitin nanofibers. Polymer. 2004;45:7137.
Mizrahi B, Stefanescu CF, Yang C, Lawlor MW, Ko D, Langer R, Kohane DS. Elasticity and safety of alkoxyethyl cyanoacrylate tissue adhesives. Acta Biomater. 2011;7:3150.
Mokhtari H, Kharaziha M, Karimzadeh F, Tavakoli S. An injectable mechanically robust hydrogel of Kappa-carrageenan-dopamine functionalized graphene oxide for promoting cell growth. Carbohydr Polym. 2019;214:234.
Mollet B. New technologies in fermented milk. Belg J Brew Biotechnol. 1996;21:63.
Morrison N, Clark R, Chen Y, Talashek T, Sworn G. Physical chemistry and industrial application of gellan gum. Springer; 1999, p. 127.
Murat F-JL, Le CQ, Ereth MH, Piedra MP, Dong Y, Gettman MT. Evaluation of microporous polysaccharide hemospheres for parenchymal hemostasis during laparoscopic partial nephrectomy in the porcine model. JSLS: J Soc Laparoend Surgeons. 2006;10:302.
Ng JY, Obuobi S, Chua ML, Zhang C, Hong S, Kumar Y, Gokhale R, Ee PLR. Biomimicry of microbial polysaccharide hydrogels for tissue engineering and regenerative medicine–a review. Carbohydr Polym. 2020;241:116345.
Nie W, Yuan X, Zhao J, Zhou Y, Bao H. Rapidly in situ forming chitosan/ε-polylysine hydrogels for adhesive sealants and hemostatic materials. Carbohydr Polym. 2013;96:342.
Nishinari K, Takahashi R. Interaction in polysaccharide solutions and gels. Curr Opin Colloid Interface Sci. 2003;8:396.

Okamoto Y, Yano R, Miyatake K, Tomohiro I, Shigemasa Y, Minami S. Effects of chitin and chitosan on blood coagulation. Carbohydr Polym. 2003;53:337.

Ono K, Saito Y, Yura H, Ishikawa K, Kurita A, Akaike T, Ishihara M. Photocrosslinkable chitosan as a biological adhesive. J Biomed Mater Res. 2000;49:289.

Osbun JW, Ellenbogen RG, Chesnut RM, Chin LS, Connolly PJ, Cosgrove GR, Delashaw JB Jr, Golfinos JG, Greenlee JD, Haines SJ. A multicenter, single-blind, prospective randomized trial to evaluate the safety of a polyethylene glycol hydrogel (Duraseal Dural Sealant System) as a dural sealant in cranial surgery. World Neurosurg. 2012;78:498.

Pang M, Huang Y, Meng F, Zhuang Y, Liu H, Du M, Ma Q, Wang Q, Chen Z, Chen L. Application of bacterial cellulose in skin and bone tissue engineering. Eur Polym J. 2020a;122:109365.

Pang J, Bi S, Kong T, Luo X, Zhou Z, Qiu K, Huang L, Chen X, Kong M. Mechanically and functionally strengthened tissue adhesive of chitin whisker complexed chitosan/dextran derivatives based hydrogel. Carbohydr Polym. 2020b;237:116138.

Piasecka-Zelga J, Zelga P, Szulc J, Wietecha J, Ciechańska D. An in vivo biocompatibility study of surgical meshes made from bacterial cellulose modified with chitosan. Int J Biol Macromol. 2018;116:1119.

Pillai CKS, Sharma CP. Absorbable polymeric surgical sutures: chemistry, production, properties, biodegradability, and performance. J Biomater Appl. 2010;25:291.

Pirouzmanesh A, Herretes S, Reyes JM, Suwan-apichon O, Chuck RS, Wang D-A, Elisseeff JH, Stark WJ, Behrens A. Modified microkeratome-assisted posterior lamellar keratoplasty using a tissue adhesive. Arch Ophthalmol. 2006;124:210.

Pozza M, Millner RW. Celox (chitosan) for haemostasis in massive traumatic bleeding: experience in Afghanistan. Eur J Emerg Med. 2011;18:31.

Priya VS, Iyappan K, Gayathri V, William S, Suguna L. Influence of pullulan hydrogel on sutureless wound healing in rats. Wound Med. 2016;14:1.

Rainer G, Menapace R, Schmid KE, Sacu S, Kiss B, Heinze G, Findl O. Natural course of intraocular pressure after cataract surgery with sodium chondroitin sulfate 4%–sodium hyaluronate 3%(Viscoat). Ophthalmology. 2005;112:1714.

Reyes JM, Herretes S, Pirouzmanesh A, Wang D-A, Elisseeff JH, Jun A, McDonnell PJ, Chuck RS, Behrens A. A modified chondroitin sulfate aldehyde adhesive for sealing corneal incisions. Invest Ophthalmol Vis Sci. 2005;46:1247.

Ryšavá J, Dyr J, Homola J, Dostálek J, Kŕížová P, Mášová L, Suttnar J, Briestenský J, Santar I, Myška K. Surface interactions of oxidized cellulose with fibrin (ogen) and blood platelets. Sensors Actuators B Chem. 2003;90:243.

Sarwat F, Qader SAU, Aman A, Ahmed N. Production & characterization of a unique dextran from an indigenous Leuconostoc mesenteroides CMG713. Int J Biol Sci. 2008;4:379.

Sauraj, Vinay K, Kumar B, Priyadarshi R, Deeba F, Kulshreshtha A, Kumar A, Agrawal G, Gopinath P, Negi YS. Redox responsive xylan-SS-curcumin prodrug nanoparticles for dual drug delivery in cancer therapy. Mater Sci Eng C. 2020;107:110356.

Shaffer MP, Belsito DV. Allergic contact dermatitis from glutaraldehyde in health-care workers. Contact Dermatitis. 2000;43:150.

Shantz JAS, Vernon J, Morshed S, Leiter J, Stranges G. Sutures versus staples for wound closure in orthopaedic surgery: a pilot randomized controlled trial. Patient Saf Surg. 2013;7:6.

Sharma J, Gupta N, Mittal S. Creation of neovagina using oxidized cellulose (surgicel) as a surgical treatment of vaginal agenesis. Arch Gynecol Obstet. 2007;275:231.

Shiledar RR, Tagalpallewar AA, Kokare CR. Formulation and in vitro evaluation of xanthan gum-based bilayered mucoadhesive buccal patches of zolmitriptan. Carbohydr Polym. 2014;101:1234.

Shivamurthy D, Singh S, Reddy S. Comparison of octyl-2-cyanoacrylate and conventional sutures in facial skin closure. Nat J Maxillofac Surg. 2010;1:15.

Simson JA, Strehin IA, Allen BW, Elisseeff JH. Bonding and fusion of meniscus fibrocartilage using a novel chondroitin sulfate bone marrow tissue adhesive. Tissue Eng A. 2013;19:1843.

Smeds KA, Grinstaff MW. Photocrosslinkable polysaccharides for in situ hydrogel formation. J Biomed Mater Res. 2001;54:115.
Smeds KA, Pfister-Serres A, Hatchell DL, Grinstaff MW. Synthesis of a novel polysaccharide hydrogel. J Macromol Sci – Pure Appl Chem. 1999;36:981.
Song Z, Zhang Y, Shao H, Ying Y, Mei L, Ma X, Chen L, Ling P, Liu F. Effect of xanthan gum on the prevention of intra-abdominal adhesion in rats. Int J Biol Macromol. 2019;126:531.
Strehin I, Ambrose WM, Schein O, Salahuddin A, Elisseeff J. Synthesis and characterization of a chondroitin sulfate-polyethylene glycol corneal adhesive. J Cataract Refract Surg. 2009;35:567.
Strehin I, Nahas Z, Arora K, Nguyen T, Elisseeff J. A versatile pH sensitive chondroitin sulfate–PEG tissue adhesive and hydrogel. Biomaterials. 2010;31:2788.
Sulaeva I, Hettegger H, Bergen A, Rohrer C, Kostic M, Konnerth J, Rosenau T, Potthast A. Fabrication of bacterial cellulose-based wound dressings with improved performance by impregnation with alginate. Mater Sci Eng C. 2020;110:110619.
Sutherland IW. Structure-function relationships in microbial exopolysaccharides. Biotechnol Adv. 1994;12:393.
Sutherland IW. Microbial polysaccharides from Gram-negative bacteria. Int Dairy J. 2001;11:663.
Taira BR, Singer AJ, Rooney J, Steinhauff NT, Zimmerman T. An in-vivo study of the wound-bursting strengths of octyl-cyanoacrylate, butyl-cyanoacrylate, and surgical tape in rats. J Emerg Med. 2010;38:546.
Tavakoli S, Mokhtari H, Kharaziha M, Kermanpour A, Talebi A, Moshtaghian J. A multifunctional nanocomposite spray dressing of Kappa-carrageenan-polydopamine modified ZnO/L-glutamic acid for diabetic wounds. Mater Sci Eng C. 2020;111:110837.
Thorn J, Sørensen H, Weis-Fogh U, Andersen M. Autologous fibrin glue with growth factors in reconstructive maxillofacial surgery. Int J Oral Maxillofac Surg. 2004;33:95.
Tytgat L, Van Damme L, Arevalo M d PO, Declercq H, Thienpont H, Otteveare H, Blondeel P, Dubruel P, Van Vlierberghe S. Extrusion-based 3D printing of photo-crosslinkable gelatin and κ-carrageenan hydrogel blends for adipose tissue regeneration. Int J Biol Macromol. 2019;140:929.
Valeri CR, Vournakis JN. mRDH bandage for surgery and trauma: data summary and comparative review. J Trauma Acute Care Surg. 2011;71:S162.
Vanhaverbeke C, Heyraud A, Mazeau K. Conformational analysis of the exopolysaccharide from Burkholderia caribensis strain MWAP71: impact on the interaction with soils. Biopolymers: Orig Res Biomol. 2003;69:480.
Wang D-A, Varghese S, Sharma B, Strehin I, Fermanian S, Gorham J, Fairbrother DH, Cascio B, Elisseeff JH. Multifunctional chondroitin sulphate for cartilage tissue–biomaterial integration. Nat Mater. 2007;6:385.
Wang T, Nie J, Yang D. Dextran and gelatin based photocrosslinkable tissue adhesive. Carbohydr Polym. 2012;90:1428.
Weng L, Romanov A, Rooney J, Chen W. Non-cytotoxic, in situ gelable hydrogels composed of N-carboxyethyl chitosan and oxidized dextran. Biomaterials. 2008;29:3905.
Wiemer K, Dörmbach K, Slabu I, Agrawal G, Schrader F, Caumanns T, Bourone S, Mayer J, Steitz J, Simon U. Hydrophobic superparamagnetic FePt nanoparticles in hydrophilic poly (N-vinylcaprolactam) microgels: a new multifunctional hybrid system. J Mater Chem B. 2017;5:1284.
Zhu J, Sun Y, Sun W, Meng Z, Shi Q, Zhu X, Gan H, Gu R, Wu Z, Dou G. Calcium ion–exchange cross-linked porous starch microparticles with improved hemostatic properties. Int J Biol Macromol. 2019;134:435.
Zia T, Usman M, Sabir A, Shafiq M, Khan RU. Development of inter-polymeric complex of anionic polysaccharides, alginate/k-carrageenan bio-platform for burn dressing. Int J Biol Macromol. 2020;157:83.
Zidarič T, Milojević M, Gradišnik L, Stana Kleinschek K, Maver U, Maver T. Polysaccharide-based bioink formulation for 3d bioprinting of an in vitro model of the human dermis. Nano. 2020;10:733.

Kefiran in Tissue Engineering and Regenerative Medicine

41

Susana Correia, Joaquim Miguel Oliveira, Hajer Radhouani, and Rui L. Reis

Contents

Abstract

A wide variety of exopolysaccharides (EPS) are synthesized by microorganisms, and kefiran is an example of EPS produced by the microflora of kefir grains, an ancient culture used to ferment milk to produce the kefir beverage. Kefiran is the main polysaccharide in kefir grains and consists of a water-soluble-branched glucogalactan heteropolysaccharide containing approximately equal amounts of glucose and galactose, mostly produced by *Lactobacillus kefiranofaciens*. Kefiran has attracted a lot of attention due to its unique features and beneficial properties, being explored for numerous applications in diverse areas, mostly in the food industry and biomedical fields. However, kefiran has only recently been more extensively investigated for its potential for tissue engineering and

S. Correia (✉) · J. M. Oliveira · H. Radhouani · R. L. Reis
3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Zona Industrial da Gandra, Barco, Guimarães, Portugal

ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães, Portugal
e-mail: susana.correia@i3bs.uminho.pt; miguel.oliveira@i3bs.uminho.pt; hajer.radhouani@i3bs.uminho.pt; rgreis@i3bs.uminho.pt

J. M. Oliveira et al. (eds.), *Polysaccharides of Microbial Origin*,
https://doi.org/10.1007/978-3-030-42215-8_52

regenerative medicine. Herein, we describe the different kefiran applications and properties that have shown that this exopolysaccharide can indeed represent a promising alternative to already existing treatments and gold standard materials in regenerative medicine and tissue engineering.

Keywords

Biopolymer · Kefiran · Regenerative medicine · Scaffold · Tissue engineering

1 Introduction

Microorganisms synthesize a wide variety of exopolysaccharides (EPS) that are mostly composed of carbohydrates such as glucose, galactose, mannose, and fructose, but also uronic acid and other noncarbohydrate moieties such as acetate, phosphate, pyruvate, and succinate (Cottet et al. 2020). Kefiran is an example of EPS produced by the microflora of kefir grains that are used to ferment milk to produce the kefir beverage. It is the main polysaccharide in kefir grains, which are composed by lactic acid bacteria, acetic acid bacteria, and yeasts, together with casein and complex sugars in a polysaccharide matrix (Arslan 2015; Cottet et al. 2020). Kefiran was first studied and named by La Riviere and Kooiman (1967) and consists of a water-soluble-branched glucogalactan heteropolysaccharide containing approximately equal amounts of glucose and galactose, mainly produced by *Lactobacillus kefiranofaciens* (Moradi and Kalanpour 2019; Radhouani et al. 2018b). This EPS has been receiving increasing interest due to its unique features, such as rheological behavior, biodegradability, biocompatibility, safety, emulsifier effect, stabilizing effect, resistance against hydrolysis, barrier and mechanical properties, and water vapor permeability (Moradi and Kalanpour 2019). Moreover, numerous beneficial properties have been associated to kefiran including antimicrobial, antioxidant, antitumor, and anti-inflammatory properties (Cottet et al. 2020; Moradi and Kalanpour 2019). The unique features and properties of kefiran have been explored for numerous applications in diverse areas, mostly in the food industry and in biomedical fields. It is one of the biopolymers whose film making capabilities have been studied in detail and has also attracted considerable attention as a new biological and medical material, due to its biocompatibility and water sensitivity (Ahmed and Ahmad 2017; Moradi and Kalanpour 2019). Kefiran was found to be effective in decreasing blood pressure, in cancer prevention, and in inhibiting and healing allergic disorders and inflammatory diseases. It has also showed prebiotic behavior and antitumor, hypocholesterolemic, hypotensive, atherosclerosis, and immunomodulatory effects by oral administration. These and other healing effects and health benefits reveal its great potential to be used in medical applications (Moradi and Kalanpour 2019). However, the unique features and properties of kefiran have only recently been more extensively explored for tissue engineering and regenerative medicine (TERM) applications.

2 Kefiran Potential for TERM Applications

The field of regenerative medicine and tissue engineering has emerged due to the promising possibility to promote tissue repair and regeneration, being regenerative biology focused on using degradable biomaterials to either release bioactive factors to promote healing and/or provide scaffolds to seed therapeutic cells that can repair and regenerate tissues (Radhouani et al. 2018b).

Natural biomaterials have received an increasing interest for tissue-engineering scaffolding due to their unique structure and composition similarities with the natural extracellular matrix, having the additional advantage of interacting with cells and cellular enzymes, being remodeled and/or degraded as space for growing tissue is needed (Moradi and Kalanpour 2019; Radhouani et al. 2018b).

Kefiran has already shown great potential to form proper scaffolds for TERM applications. Montesanto et al. (2016) obtained dense films and porous scaffolds from 2% (w/v) kefiran-aqueous solutions and characterized them to evaluate their potential application for tissue engineering. Freeze-dried scaffolds with high porosity and interconnected pore structure were obtained (Fig. 1a) allowing good cellular penetration and the diffusion of both waste products out of the scaffold and supply of nutrients into the tissue (Montesanto et al. 2016). The dense films obtained (Fig. 1b) were proposed to have potential as support for submerged culture; the authors propose that the obtained results provide new insights into the foaming methods and fabrication parameters for kefiran scaffolding that could address new applications of bioabsorbable scaffolds in TERM (Montesanto et al. 2016).

Later, Radhouani and collaborators also developed kefiran cryogels from 2% (w/v) kefiran-aqueous solutions to be evaluated as proper scaffolds for TERM and controlled drug delivery (Fig. 2). The developed scaffoldds were more extensively characterized showing stability, elastic behavior, and high porosity, being capable of controlled release of diclofenac for 2 weeks; the cryogels also showed to be biocompatible, sustaining human adipose-derived Stem Cells (hASCs) metabolic activity for 72 h, which is a fundamental feature for TERM (Radhouani et al. 2019; Radhouani et al. 2017).

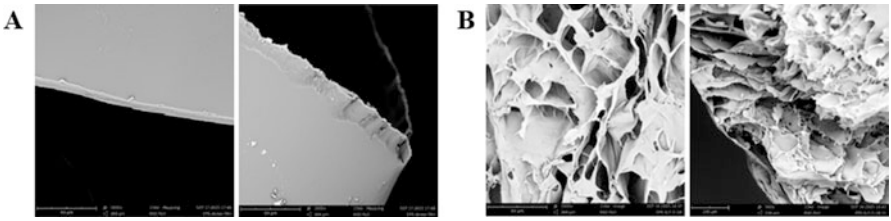


Fig. 1 Morphology of kefiran cryogels obtained by Montesanto et al. (2016). SEM microstructure of (**a**) dense films obtained via solvent casting (left, outer surface; right, cross-section) and (**b**) porous scaffolds obtained via direct quenching (left, outer surface; right, cross-section). (Adapted with permission from Montesanto et al. (2016). Copyright (2016) AIDIC Servizi S.r.l.)

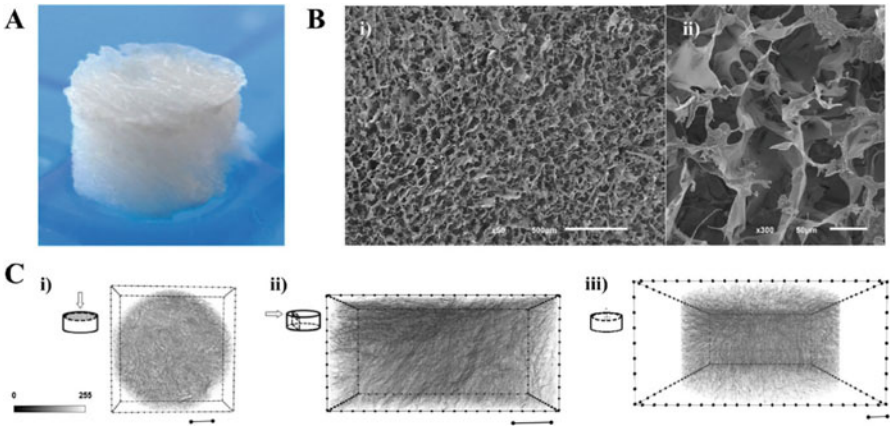


Fig. 2 Morphology of kefiran cryogels obtained by Radhouani et al. (2019): (**a**) macroscopic image; (**b**) SEM surface microstructure at different magnifications: (**i**) x50 and (**ii**) x300; and (**c**) 3D reconstructed kefiran scaffolds by microcomputed tomography: (**i**) cross-sectional, (**ii**) longitudinal, and (**iii**) fully reconstructed views. Scale bar: 250 μm. (Reprinted with permission from Radhouani et al. (2019). Copyright (2019) Elsevier)

More recently, Sabatino et al. (2020) developed injectable, in situ forming kefiran/propylene glycol gels to vary their potential as scaffolds for tissue regeneration or implantable drug delivery devices, showing prospects of enabling the diffusion of small molecules (e.g., nutrients) and the entrapment of large biomolecules (e.g., growth factors).

Kefiran-electrospun nanofibers with distilled water as solvent were first reported by Esnaashari et al. (2014) showing promising properties for further application as a biomaterial for cell-growth scaffolds, wound dressing, and also as drug carriers. The authors reported an increase in the diameter of the developed kefiran nanofibers with the increase of applied voltage, kefiran concentration, and biopolymer feeding rate (Esnaashari et al. 2014).

Jenab and collaborators encapsulated platelets in kefiran and tested the system for bioavailability as a new drug for surface bleeding, showing it may have potential as a model in a range of biomedical applications, including local and sustained drug delivery, versatile artificial blood, and immune protection of artificial tissues (Jenab et al. 2015a). Afterward, the same team developed electrospun kefiran/polyethylene oxide (PEO) nanofibers to evaluate their antimicrobial activity as a biocontrol agent for food packaging and food preservation (Jenab et al. 2017) and very recently developed and characterized different electrospun pure poly-acrylonitrile (PAN)-kefiran nanofibrous scaffolds (Fig. 3) with suggested superior properties for neural stem cell culture (especially for spinal cord injury repair) and tissue engineering (Jenab et al. 2020). This last study also showed that pure kefiran could be used for the enhancement of peripheral blood mononuclear cells (PBMC) growth and for reducing the growth of MCF7 cancerous cells, highlighting again the promising use of kefiran for regenerative medicine (Jenab et al. 2020).

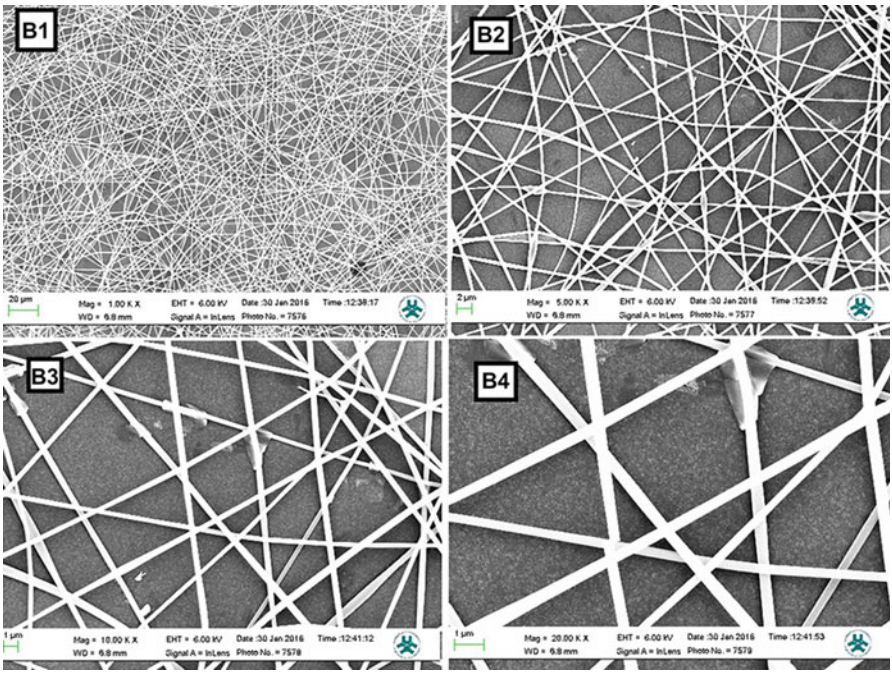


Fig. 3 SEM images of kefiran nanofibers obtained by Jenab et al. (2020) at different magnifications (B1: x1000; B2: x5000; B3: x10,000; B4: x20,000). (Re-used with permission from Jenab et al. (2020). Copyright (2020) Dove Medical Press)

Hyaluronic acid (HA) and HA-based materials are extensively used for the preparation of gels, hydrogels, and scaffolds for a variety of tissues, cartilage being the most intensively studied (Chircov et al. 2018). HA is among the most widely used biomaterials in the biomedical field, being considered the gold standard treatment for viscosupplementation and currently used as a commercial injectable biomaterial for osteoarthritis (OA) treatment (Radhouani et al. 2018a). Hence, Radhouani et al. (2018a) performed a comparative study regarding the biological performance of kefiran and its potential for regenerative medicine applications when compared with HA. The antioxidant activity of kefiran and HA was assessed through their reducing power activity (ascorbic acid equivalent reducing capacity), metal chelating activity (ferrous ion-chelating capacity), and through their hydroxyl and superoxide radical scavenging activity. The high antioxidant potential of kefiran against reactive oxygen species was demonstrated, highlighting its ability to protect cells in oxidative stress environments, having in fact outperformed HA regarding reducing power performance. Kefiran and HA anti-inflammatory activity was evaluated through their capacity to scavenge nitric oxide radicals generated in a cell-free system where again kefiran outperformed HA. Kefiran also proved to be an interesting immunostimulant by considerably reducing the concentration of NO, which is

a significant mediator of various physiologic and pathologic processes and a powerful inflammatory mediator by strongly reacting with oxygen, superoxide, and iron-containing compounds. Additionally, the incorporation of kefiran in the culture media of human adipose derived stem cells (hASC) improved their viability and metabolic activity, representing a promising finding as these stem cells are able to differentiate into several lineages, displaying great potential for TERM applications. The results obtained by Radhouani et al. (2018a) encourage the use of kefiran as an alternative or adjunct treatment to promote tissue repair and regeneration while reducing inflammation, particularly in OA contexts.

3 Kefiran Physicochemical and Biological Properties of Interest for TERM Applications

The development of biodegradable polymeric materials for biomedical applications has advanced significantly in the last half century. Such materials are preferred for the development of therapeutic devices, including gels and three-dimensional scaffolds for tissue engineering, and the potential role of polysaccharide-based materials in clinical applications of tissue-engineered medical products has been increasing (Oliveira and Reis 2011; Song et al. 2018). Due to their physicochemical behavior and interesting structural similarities with biological molecules, great potential has been envisioned for the future application of polysaccharide-based materials in the biomedical field, especially in cartilage regeneration (Oliveira and Reis 2011). Analyzing the physico-chemical properties of polysaccharide-based scaffolds and how they influence their suitability for the desired biological setting is critical in defining the appropriate design needed to achieve the necessary bioactivity for a particular TERM application (Tchobanian et al. 2019). Moreover, evaluation of the biocompatibility and biomimetic efficiency of a material is essential when evaluating its potential for biomedical applications (Pradhan et al. 2017; Sousa et al. 2017).

Characterization of the physicochemical and biological properties of polysaccharide-based TE products is often overlooked but is essential to improve the design of these materials, and crucial in improving their ability to attain the intended biological response and activity (Tchobanian et al. 2019). The physicochemical and biological properties of kefiran have been vastly studied for applications in the food industry; however, few studies reported these kefiran properties envisioning TERM applications. Radhouani and collaborators specifically investigated kefiran's physicochemical and biological properties in order to demonstrate its competence and clarify its high application potential in TERM. Kefiran properties were assessed for (i) articular cartilage or bone defect applications by modulating its structure (Radhouani et al. 2018b); (ii) as a promising viscosupplementation alternative or adjunct treatment to promote tissue repair and regeneration while reducing the inflammation in OA (Radhouani et al. 2018a); and (iii) as scaffolds for TE and controlled drug delivery (Radhouani et al. 2019).

3.1 Kefiran Structural Properties

The interaction of a polymeric biomaterial with the surrounding biological environment and the respective biological response determines its biocompatibility; hence, before using a polymeric biomaterial in therapeutic applications, it is critical to acquire detailed structural and chemical information to attain an accurate characterization and to evaluate its biomimetic efficiency (Pradhan et al. 2017). Physical properties are also governed by the molecular structure of polymers, being instrumental in the study of polysaccharides. The applicability of a polysaccharide for tissue engineering is greatly dependent on its physical behavior, and the presence of reactive functional groups makes a polysaccharide easily flexible to modifications, which are of high importance for medical applications. The molecular structure and component dynamics of materials are commonly examined by nuclear magnetic resonance (NMR) spectroscopy and Fourier-transform infrared spectroscopy (FTIR).

Quantitative NMR has become a well-recognized and widely applied analytical tool for the quantification of diversified classes of compounds in a great variety of samples, representing an essential tool when studying the chemistry of polysaccharides, being used over decades for the structural characterization of polysaccharides from different origins (de Souza 2017; Radhouani et al. 2018b). ^{1}H-NMR spectroscopy is characterized by some interesting advantages upon chromatography, such as easy sample preparation, easy equipment calibration, and fast obtained results (de Souza 2017; Radhouani et al. 2018b). The ^{1}H-NMR spectrum of kefiran extracted from kefir grains in D_2O at 60 °C was obtained by Radhouani et al. (2018b) and allowed the identification of the molecule structure of the extracted kefiran polysaccharide, revealing a peak at 5.15 ppm for an anomeric β hydrogen and six signals at the chemical shifts of 4.85, 4.83, 4.78, 4.76, 4.67, and 4.62 ppm for several anomeric α hydrogens, assigned to a sugar on a lateral branch (Fig. 4a). Similar results were previously obtained by Micheli et al. (1999) in kefiran polysaccharides obtained from both a ropy *Lactobacillus* strain and kefir grains which were also analyzed by ^{1}H-NMR in D_2O but at 80 °C; peaks at 5.1 ppm for an anomeric β hydrogen and at 4.82, 4.77, 4.52, 4.50, 4.45, and 4.65 ppm for several anomeric α hydrogens were described. The ^{1}H-NMR spectrum of kefiran produced *by L. kefiranofaciens* has also been obtained by Maeda et al. (2004) in D_2O at 80 °C (Fig. 4b), where the anomeric region (δ 4.4–5.5) contained seven signals corresponding to a minor peak at δ 4.61, assigned to (1→6)-β-D-Gal*p* (corresponding to a small proportion of 2,3,4-tri-*O*-methyl D-galactose), and other six peaks containing three well-resolved signals and three overlapping signals corresponding to the hexasaccharide repeating unit; the residue at 5.14 ppm (c1, Fig. 4b) was assigned to an α-hexapyranosyl residue, and the residues at 4.82, 4.68, 4.53, 4.53, and 4.49 ppm were assigned to a pyranose ring formed in a β anomeric configuration (respectively f1, b1, e1, d1, and a1, Fig. 4b). The heteropolysaccharide structure of kefiran isolated from kefir grains grown in cheese whey has also been analyzed by Ghasemlou et al. (2012) who described in the anomeric

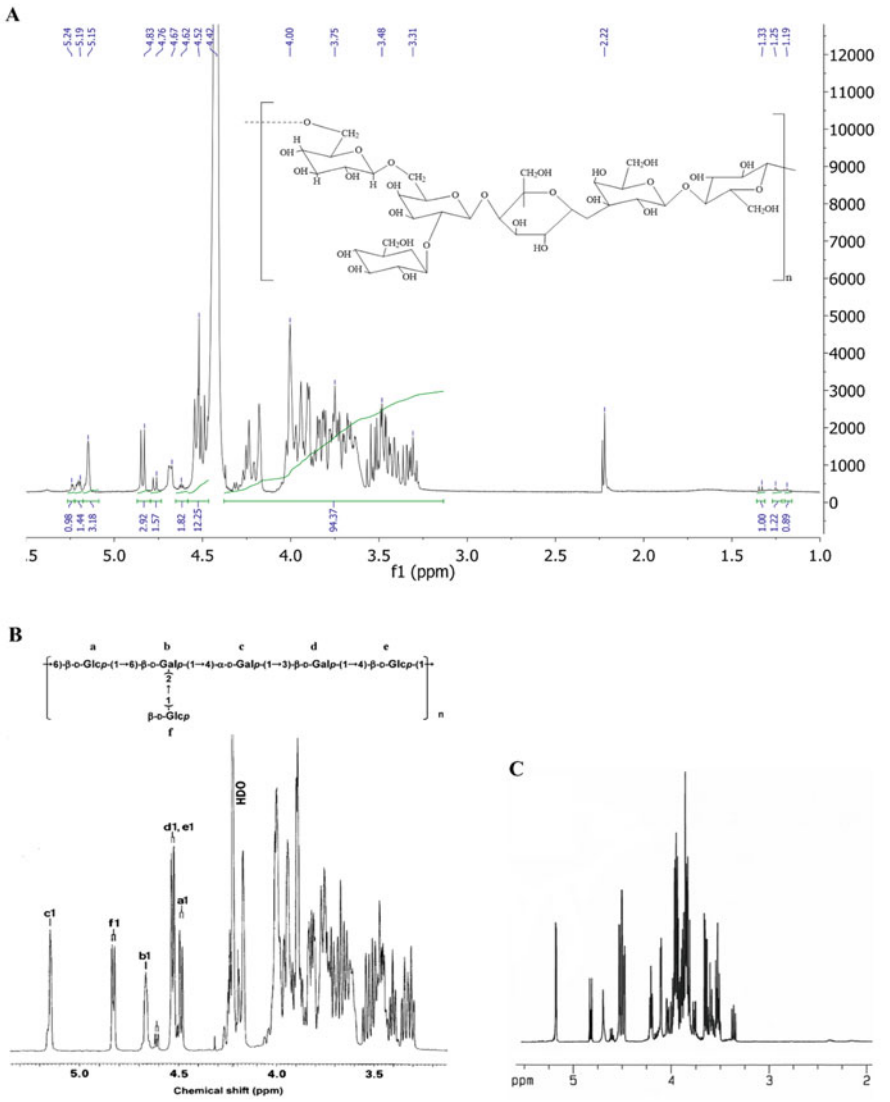


Fig. 4 ^{1}H-NMR spectrum of the kefiran polysaccharide obtained by (**a**) Radhouani et al. (2018b) (D_2O, 60 °C); (**b**) Maeda et al. (2004) (D_2O, 80 °C); and (**c**) Ghasemlou et al. (2012) (D_2O, 27 °C). (Adapted/reprinted with permission from the referenced sources. Copyright (2018) SAGE Publications. Copyright (2004) American Chemical Society. Copyright (2012) Elsevier)

region of the ^{1}H-NMR spectrum obtained in D_2O at 27 °C six signals at 4.80, 4.69, 4.62, 4.53, 4.52, and 4.48 ppm, assigned to anomeric protons of β-D-Glc*p*-(1→, →2,6)-β-D-Gal*p* (1→, →6)-β-D-Gal*p*-(1→, →4)-β-D-Glc*p*-(1→, →3)-β-D-Gal*p*-(1→, and →6)- β-D-Glc*p*-(1→, respectively, and a signal at 5.16 ppm that was assigned to anomeric protons of →4)-α-D-Gal*p*-(1→ (Fig. 4c). Later, Jenab et al.

(2017) obtained the ^{1}H-NMR spectrum of the kefiran used to produce the abovementioned kefiran/PEO nanofibers for food packaging; deproteinized kefiran was assayed in D_2O at 25 °C revealing signals on the anomeric region at 4.31, 4.12, 3.79, 3.63, 3.60, 3.52, 3.15, 2.50, 2.12, 1.24, and 1.56 ppm.

FTIR analysis allows functional group determination in polymeric biomaterials, which is necessary to evaluate their biological response, biocompatibility, and biodegradability; moreover, drug-polymer interaction studies can be confirmed by observing the loss or appearance of FTIR peaks from the drug and polymer conjugate (Pradhan et al. 2017). FTIR has been performed to identify the fundamental groups present in the kefiran structure, representing a useful tool to investigate structural changes in biopolymers (Jenab et al. 2015b). IR analysis is also very important to identify the reactive functional groups in kefiran which make it more flexible to many modifications (Moradi and Kalanpour 2019).

Envisioning TERM applications, Radhouani et al. (2018b) studied the FTIR spectrum of kefiran extracted from kefir grains (Fig. 5a) reporting a band at 3430 cm^{-1} corresponding to the hydroxyl groups (region attributed to the stretching vibration O–H in the constituent sugar residues), a band at 2930 cm^{-1} linked with the stretching vibration of C–H in the sugar ring which was assigned to methyl and methylene groups, a band at 1700 cm^{-1} associated to the stretching vibration of O–H, and a band around 1400 cm^{-1} ascribed to CH_2 and OH groups. Moreover, the region of 1100–1150 cm^{-1} showed intense absorptions, characteristic of C–O–C stretches and alcohol groups in carbohydrates, and the presence of a band at 900 cm^{-1} indicated a β-configuration and vibration modes of glucose and galactose (Radhouani et al. 2018b).

Wang et al. (2008) reported the FTIR spectra of kefiran produced by *L. kefiranofaciens* ZW3 isolated from Tibet kefir (Fig. 5b) revealing the presence of carboxyl, hydroxyl, and amide groups, which correspond to a typical heteropolymeric polysaccharide (broad-stretching hydroxyl group at 3405 cm^{-1}; weak C–H stretching peak of methyl group at 2924 cm^{-1}; broad stretch of C–O–C, C–O at 1000–1200 cm^{-1} related to carbohydrates; absorption at 1643 cm^{-1} attributed to amide I > C = O str and C–N bending of protein and peptide amines; and peak at 1378 cm^{-1} assigned to C = O str of the COO− and C–O bond from COO−). The FTIR spectra of the EPS accumulated during milk fermentation by Tibetan kefir was also obtained by Chen et al. (2015) (Fig. 5c), allowing the identification of carboxyl, hydroxyl, and carbonyl functional groups, and also from the EPS obtained from fermented kefir grains of liquid soymilk (Fig. 5d) by Botelho et al. (2014).

The FTIR spectra of kefiran isolated from kefir grains by different extraction conditions were obtained by Pop et al. (2016), identifying four major absorption zones (Fig. 5e): 3700–3310 cm^{-1} (water and hydroxyl groups); 2720–2900 cm^{-1} (C–H stretching vibration zone; bands at 2850 and 2933 cm^{-1} were attributed to C–H, and peak intensity reduction was related to disruption of the kefiran solution structure due to absorbed water molecules whose presence masked the C–H bond of the carbohydrate rings, thus reducing the contribution of C–H absorbance bands); 1650–1413 cm^{-1} (specific for water molecules, assigned to the bending mode of O–H; provides relevant information for industrial applications, since the relative

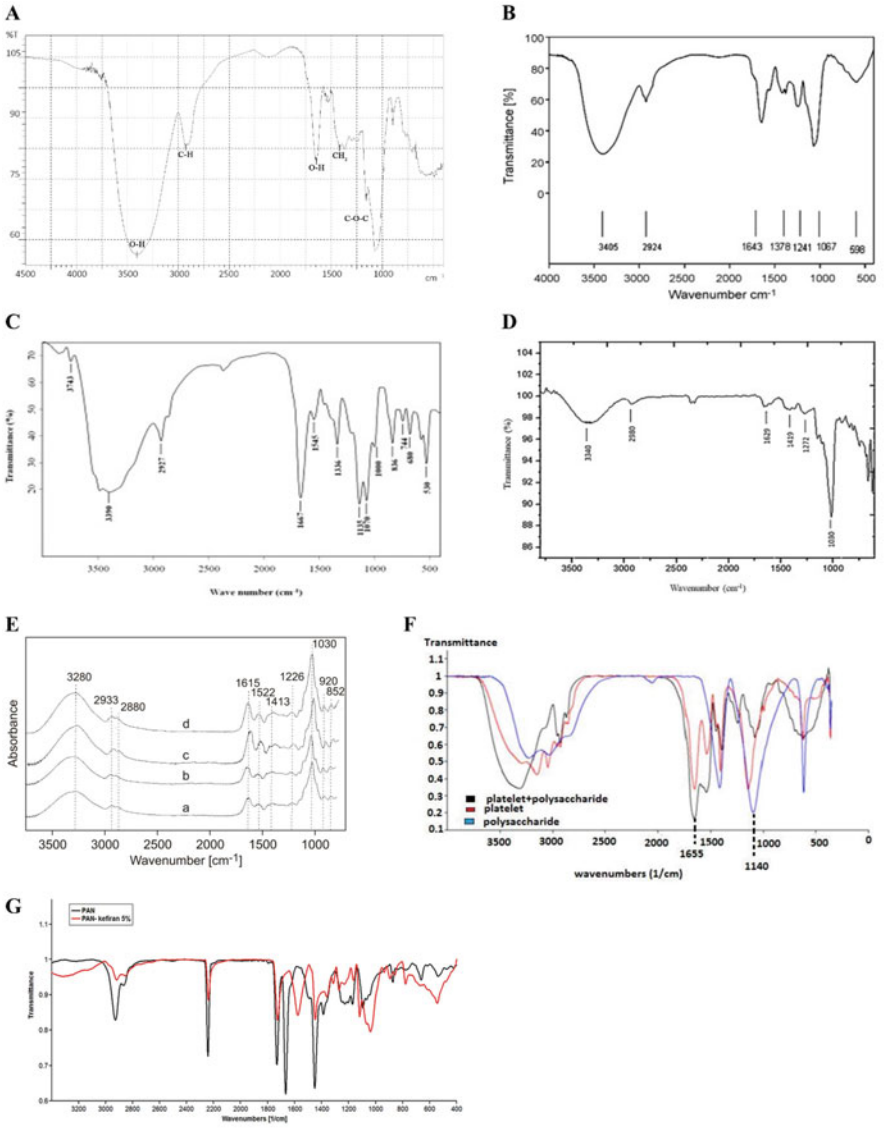


Fig. 5 Infrared spectrum of kefiran obtained by (**a**) Radhouani et al. (2018b); (**b**) Wang et al. (2008); (**c**) Chen et al. (2015); (**d**) Botelho et al. (2014); (**e**) Pop et al. (2016); (**f**) Jenab et al. (2015b); and (**g**) Jenab et al. (2020). (Adapted/reprinted with permission from the referenced sources. Copyright (2018) SAGE Publications. Copyright (2008) Elsevier. Copyright (2015) Elsevier. Copyright (2014) Elsevier. Copyright (2016) National Agricultural and Food Centre, Slovakia. Copyright (2020) Dove Medical Press)

absorption intensities of bands assigned to water molecules depend on the kefiran solution quality); and 1200–852 cm^{-1} (distinct for each polysaccharide, being this region dominated by ring vibrations overlapped with stretching vibrations of C–OH side groups and C–O–C glycosidic band vibrations; absorptions at 1035, 1080, and 1153 cm^{-1} indicated a pyranose form of carbohydrates, associated with the presence of the glucose and galactose components of the purified kefiran structure). The results obtained by Pop et al. (2016) suggest that kefiran was composed of α- and β-configurations of saccharides in pyranose form, and the disruption of the polymer chain was related to the high temperature of extraction.

Jenab and collaborators have also investigated the platelet-kefiran interaction by FTIR (Fig. 5f) to assess platelet encapsulation in kefiran and to detect the bioavailability of immobilized platelets in kefiran as a possible new probiotic drug for surface bleeding (Jenab et al. 2015b). FTIR of kefiran revealed different peaks including 3224 cm^{-1} (O–H group, C–H, N–H), 3036 cm^{-1} (O–H, =CH), 2846 cm^{-1} (C–H, aldehyde, O–H), 2053 cm^{-1} (triple), 1414 cm^{-1} (O–H, COC), 1092 cm^{-1} (C–C, COC, CO stretch), and 615 cm^{-1} (C–CL), with peaks 1092 and 1414 cm^{-1} containing bands of carbohydrates and peaks 3224, 3036, 2846, and 2053 cm^{-1} associated to the kefiran chemical structure. The mixture of encapsulated platelet-polysaccharide and platelets was shown as the amide I and II did not change, but peak 1140 cm^{-1} in the polysaccharide region changed; the fingerprint region changes showed to be less than in the polysaccharide region, all changes being almost in the polysaccharide region (900–1200 cm^{-1}) of the encapsulated platelet-polysaccharide. Although envisioning applications in the food industry, the same authors (Jenab et al. 2017) have also investigated the composition and in vitro biodegradation of kefiran and electrospun kefiran/PEO nanofibers through FTIR. The specific wavenumbers of 3201, 3367, and 3435 cm^{-1} identified O–H bands in the oxidizing functional groups in kefiran/PEO nanofibers, but only one peak at 3415 cm^{-1} belonging to this group was observed in kefiran, these groups being more obviously presented in the electrospun kefiran/PEO nanofibers than in the neat kefiran. Biodegradability was shown by the lower number of peaks detected in the spectrum of the nanofibers after the biodegradation period and higher functional group concentration (Jenab et al. 2017). More recently, Jenab and collaborators characterized electrospun kefiran/PAN nanofibers manufactured to be used for regenerative medicine through attenuated total reflectance FTIR spectroscopy (Jenab et al. 2020), identifying in the FTIR spectra of PAN-kefiran scaffolds two peaks (1038 and 1118 cm^{-1}) related to the polysaccharide region and one peak (1576 cm^{-1}) related to NH_2 (Fig. 5g).

While FTIR allows analysis of the overall availability of different functional groups in the kefiran chemical structure, X-ray photoelectron spectroscopy (XPS) supports the identification of surface functional groups, FTIR and XPS results being complementary (Radhouani et al. 2018b). XPS allows verification of surface viability, meaning that it can, among other aspects, demonstrate the suitability of a polymer to be functionalized and also the study of a range of properties that include

polymer biocompatibility (Yahia and Mireles 2017). The XPS spectra of kefiran extracted from kefir grains were obtained by Radhouani et al. (2018b); the C1s XPS spectra revealed the presence of the C–OH bond at 286.6 eV and O–C–O bond at 287.8 eV, and the oxygen O1s peak was decomposed in two components attributed mainly to the O–C bond at 533 eV and to the oxygen bond to hydrogen [H–O–C] at 534.6 eV. The XPS results obtained by Radhouani et al. (2018b) also revealed a C/O atomic ratio of 1.46.

3.2 Kefiran Molecular Weight

Molecular weight and molecular weight distribution are important parameters which define the behavior and physicochemical properties of polymers such as degradation, solubility, mechanical strength and performance (e.g., viscosity, rheological behavior), flexibility, and resistance to deformation, among others, and the particular applications of a specific polymer are closely related to these properties (Dragostin and Profire 2017; Radhouani et al. 2018b). Analysis and characterization of polymeric structures is usually accomplished through spectroscopic techniques (e.g., IR spectroscopy, NMR spectroscopy), which contribute to common functional group identification; however, these traditional methods were subsequently complemented with specific techniques for the investigation of molecular weight and molecular weight distribution such as gel permeation chromatography (GPC) and size exclusion chromatography (SEC) (Dragostin and Profire 2017). To determine the suitability of the kefiran polysaccharide for TERM applications, Radhouani et al. (2018b) obtained through SEC a number-average molecular weight (Mn) of 357 kDa and weight-average molecular weight (Mw) of 534 kDa (polydispersity index (PDI) of 1.49) for kefiran extracted from kefir grains, highlighting the interest and desirability of low-molecular weight biomaterials in modern medicine due to shorter degradation rates. Maeda et al. (2004) studied the molecular weight of kefiran produced *by L. kefiranofaciens* through GPC, reporting a Mw of 7.6×10^5 Da and an estimated z-average radius of gyration (R_{gz}) of 39.9 nm. Ahmed et al. (2013) reported a Mw of 5.5×10^4 Da after GPC analysis of kefiran produced by *L. kefiranofaciens* ZW3 isolated from Tibet kefir. Exarhopoulos et al. (2018a) reported a Mw of 6.144×10^5 Da, a PDI of 1.978 and a R_{gz} of 35.84 ± 0.1 nm after SEC analysis of kefiran produced from kefir grains. Ghasemlou et al. (2012) obtained kefiran with a Mw of 1.35×10^6 Da from kefir grains grown in cheese whey, and Piermaria et al. (2008) reported a Mw of 10^7 Da in kefiran from kefir grains. Pop et al. (2016) investigated the molecular weight of kefiran extracted from kefir grains under different conditions reporting values ranging between 2.4×10^6 Da and 1.5×10^7 Da. More recently, Sabatino et al. (2020) reported a Mw of 4.32 ± 0.44 MDa ($R_{gz} = 88.5 \pm 8.9$ nm) through static light scattering (SLS) and a Mn of 2.17 MDa and Mw of 4.38 MDa (PDI = 2) through gel filtration chromatography (GFC) for kefiran extracted from commercial kefir grains for the development of injectable in situ forming gels for TERM and drug delivery applications.

3.3 Kefiran Thermal Properties

The applicability of a polysaccharide is greatly determined by its thermal performance (Chen et al. 2015). Thermal analysis delivers fundamental information for research and development and quality control of new materials/products such as transition temperatures, crystallinity, heat, and thermal expansion; a variety of techniques in which the physical property of a material is continuously measured as a function of temperature are used in polymer research (Tanzi 2017). Differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) are among the most widely used techniques to obtain quantitative calorimetric measurements, being effective analytical tools to characterize the thermal properties and transitions of a polymer (Radhouani et al. 2018b; Tanzi 2017). In order to characterize kefiran for TERM applications, Radhouani et al. (2018b) subjected samples obtained from kefir grains to DSC and TGA (Fig. 6a, b) obtaining agreeable results. The kefiran thermogram obtained through DSC (endothermic heat flow) revealed a marked endothermic peak at 98.7 ± 0.2 °C, being the transition (approx. 99 °C) discussed to be linked to the polysaccharide melting point, justified by the hydrophilic nature of kefiran functional groups and the existence of a water bound. TGA analysis revealed two events, one with approximately 9% mass loss during temperature increase from 40 to 106 °C, which could be associated with the loss of moisture, and a second one with most weight loss (12–65%) during increase from 264 to 350 °C, being related to major degradation of the kefiran structure and associated with the extensive enthalpy change observed in the DSC curve (Radhouani et al. 2018b). The thermal stability of kefiran (degradation at 352 °C) was also reported by Botelho et al. (2014) in the EPS obtained from the fermented kefir grains of liquid soymilk (Fig. 6c). Montesanto et al. (2016) analyzed kefiran dense films and porous scaffolds by DSC (Fig. 6d) in order to evaluate their potential application for tissue engineering, reporting melting peaks of 102.4 and 107.3 °C and melting enthalpies of about 355.2 and 294.8 and J/g, respectively. Thermal analysis has also been performed by DSC for kefiran produced by *L. kefiranofaciens* ZW3 from Tibet kefir by Wang et al. (2008) (melting at about 93.38 °C, endothermic enthalpy change of 249.7 J/g) and by Ahmed et al. (2013) through TGA (melting at 93.38 °C and degradation at 299.62 °C, Fig. 6e). A melting point of 131.46 °C was reported by Chen et al. (2015) for kefiran obtained from Tibetan kefir grains being the enthalpy change 209.6 J/g (Fig. 6f). During DSC, heat absorption and emission are associated with physical changes in the polymeric material, such as structure deformations or melting of crystals (Wang et al. 2008). Sabatino et al. (2020) also used TGA to evaluate the purity and molecular structure of kefiran obtained from commercial kefir grains for the development of injectable, in situ forming kefiran gels to use as potential scaffolds for tissue regeneration or implantable drug delivery devices. The thermogravimetric curve revealed an initial polymer weight loss (55–150 °C) that was attributed to moisture (free and bound water representing approx. 10%w), and a second inflection point at 280 °C (weight loss of about 3%w) was proposed to reflect residual proteins in the system; thermal degradation of the biopolymer was observed at 300 °C, as reported by other authors, where the main inflection point reflected the most prominent mass loss (Sabatino et al. 2020).

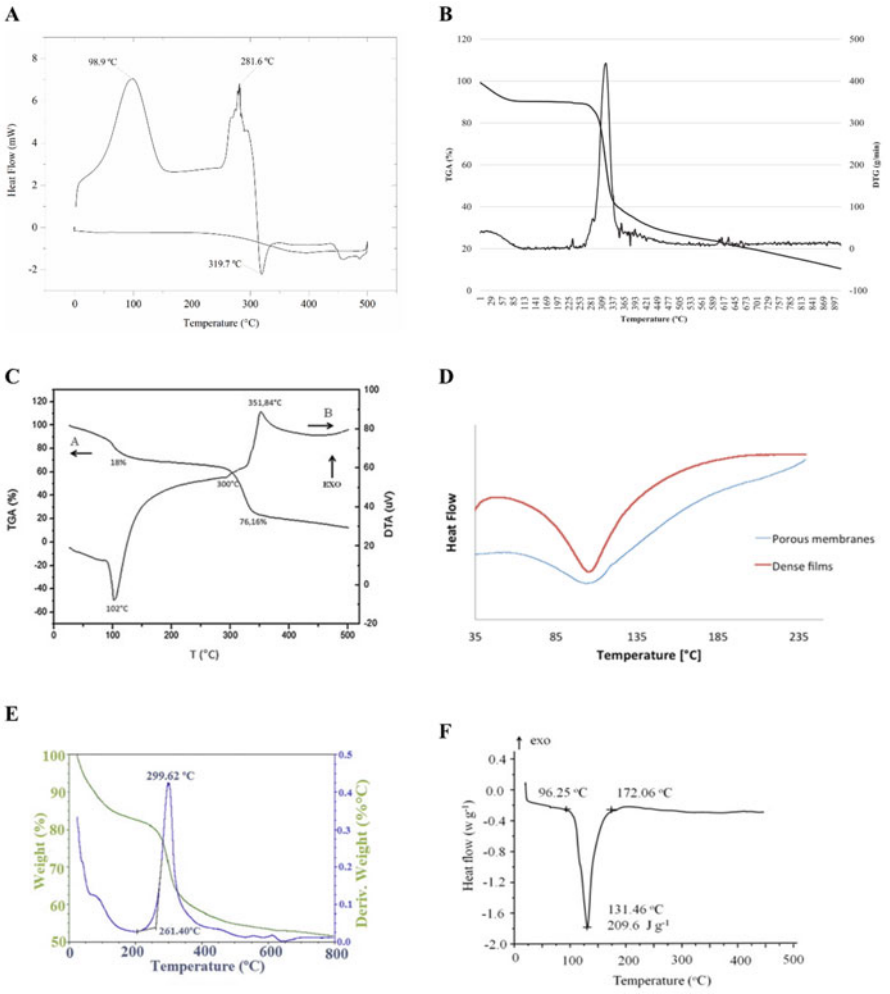


Fig. 6 Thermal properties of kefiran obtained by Radhouani et al. (2018b) through (**a**) DSC and (**b**) TGA, (**c**) Botelho et al. (2014) by TGA, (**d**) Montesanto et al. (2016) by DSC, (**e**) Ahmed et al. (2013) by TGA, and (**f**) Chen et al. (2015) by DSC. (Adapted/reprinted with permission from the referenced sources. Copyright (2018) SAGE Publications. Copyright (2014) Elsevier. Copyright (2016) AIDIC Servizi S.r.l. Copyright (2013) Elsevier. Copyright (2015) Elsevier)

3.4 Kefiran Mechanical Properties

Rheological characterization plays a major role in the evaluation and modeling of the macroscopic behavior exhibited by complex systems under flow and deformation conditions; polymeric and disperse systems are complex systems composed of both viscous and elastic component and therefore show intermediate mechanical responses between those of solids and liquids. Among polymeric biomaterials,

hydrogels and polymer solutions or suspensions represent complex materials being rheological characterization of major importance to investigate their structural features that are essential to evaluate their potential use and performance in different applications (Borzacchiello et al. 2017).

The rheological behavior of kefiran has been investigated in detail for a range of applications, mostly in the food industry (Exarhopoulos et al. 2018b; Moradi and Kalanpour 2019).

Radhouani and collaborators investigated the rheological properties of both kefiran aqueous solutions (Radhouani et al. 2018b) and cryogels (Radhouani et al. 2019) in order to evaluate their potential for drug delivery and TERM applications. Flow behavior of kefiran 1% and 10% w/v solutions was analyzed through rotational experiments where shear viscosity was shown to decrease with the increment of shear rate at 37 °C (Fig. 7a). The pseudoplastic behavior and gelation ability reported by Radhouani et al. (2018b) emphasize that kefiran may be exploited as matrix environments of various therapeutic agents such as stem cells, genetically engineered cells, or proteins. From curves of shear stress in function of shear rate, kefiran solutions revealed an infinite viscosity until a sufficiently high stress is applied to initiate flow (Fig. 7b) (Radhouani et al. 2018b). The viscoelastic properties demonstrated through frequency sweep curves obtained from oscillatory

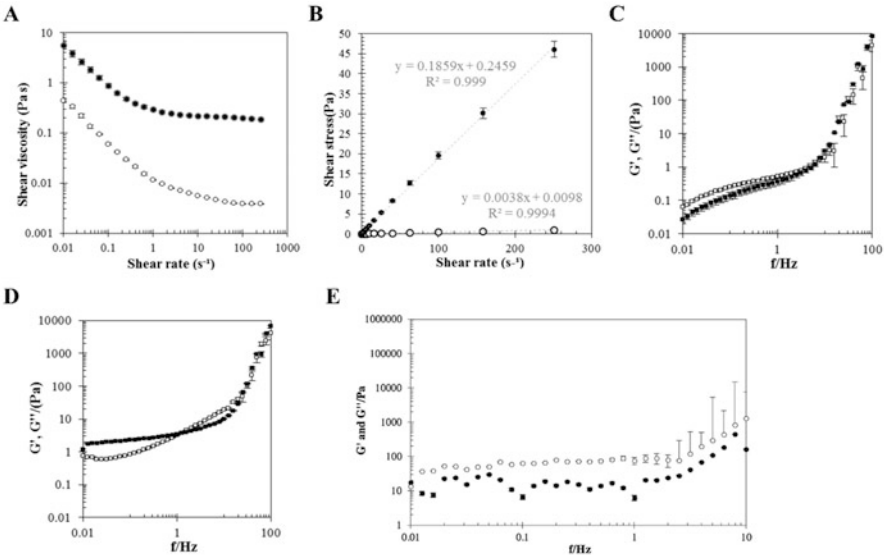


Fig. 7 Kefiran rheological properties. Shear viscosity (**a**) and shear stress (**b**) versus shear rate of 1% (w/v) (open symbol) and 10% (w/v) (filled symbol) samples; frequency dependence of G″ (open symbol) and G′ (filled symbol) of 1% (w/v) (**c**) and 10% (w/v) (**d**) samples at 37 °C (Radhouani et al. 2018b). G″ and G′ (open and filled symbols) versus frequency of 2% (w/v) kefiran scaffolds (**e**) at 37 °C (Radhouani et al. 2019). G′, storage modulus; G″, loss modulus. (Adapted/reprinted with permission from the referenced sources. Copyright (2018) SAGE Publications. Copyright (2019) Elsevier)

experiments (Fig. 7c, d) highlighted the applicability of the studied kefiran solutions in TERM applications, namely as viscosupplement in OA to restore the joint synovial fluid viscoelastic properties; their applicability as an economically and environmentally improved alternative to the gold-standard hyaluronic acid is also proposed (Radhouani et al. 2018b). The adhesive performance of kefiran was shown through preliminary pull-away experiments, this effect being much higher for 10% solutions (1.159 ± 0.018 N/s) than for 1% solutions which presented adhesion values similar to water (0.135 ± 0.049 N/s) (Radhouani et al. 2018b).

The elastic/gel character, which is an important feature for TERM, was also observed in kefiran scaffolds obtained through freeze-drying (Fig. 7e) where the elastic behavior was quantified by a constant average phase angle of 16 ± 0.7° (Radhouani et al. 2019). The mechanical properties of scaffolds are of major importance in many tissue-engineering applications. For regeneration, the energy stimulus (stored and dissipated energy) depends on scaffold viscoelasticity, and promising mechanical properties were obtained with only 2% (w/v) kefiran concentrations, revealing that this type of information can be used to improve design strategies to create kefiran scaffolds with specific properties for biomedical applications (Radhouani et al. 2019).

From an application point of view for injectable biomaterials, the knowledge of the dependence of viscosity upon shear rate is essential as it is highly desirable that these materials show fast decreasing viscosity when subjected to increasing shear rate in order to have low resistance during injection (Borzacchiello et al. 2017). The injectability of kefiran has been investigated by Radhouani et al. (2018b) by analyzing the pressure or force required for injection, evenness of flow, and freedom from clogging (no blockage of the syringe needle). When measured at room temperature and a rate of 1 mL/min by an injectable measurement device (syringe pump with a 1 mL plastic syringe and a needle gauge of 21), kefiran showed an extrusion force of 1 N, a much lower value than HA (11.3 N). This highlighted the interest of using kefiran formulations as viscosupplementation products, for instance over HA intra-articular injections, as they allow smoother injectability by low extrusion-forces (Radhouani et al. 2018b). Injectable, in situ forming kefiran gels have very recently been developed by Sabatino et al. (2020) for potential applications as implantable drug delivery devices or scaffolds for tissue regeneration. Kefiran aqueous solutions at 4, 5, and 6% (w/v) were assessed for their capacity to undergo gelation when mixed with different alcohols, and for viscosity and injectability through G26 syringe needles (Sabatino et al. 2020). The injection time to extrude 1 mL of solution at 25 °C ranged from approximately 10 to 20 s/mL, being within the range of commonly recommended injections rates. The viscoelastic properties of the different kefiran solutions at 25 °C were presented as the apparent viscosity as a function of shear rate, and as storage (G') and loss (G'') moduli as a function of frequency. At higher concentrations, kefiran exhibited decreasing viscosity typical of pseudoplastic behavior in the whole shear rate range; however, at the lower concentration, a Newtonian plateau was observed at low shear rates. In the mechanical spectra, obtained in small amplitude oscillation mode, all solutions showed typical behavior of viscoelastic liquids (G'' higher than G') at low frequencies; however, at

higher frequencies, there was not enough time for polymer chain disentanglement (G′ curve approached G″ curve). Sabatino et al. (2020) also investigated the gelation of aqueous kefiran with propylene glycol (PG) by rheological analysis where oscillatory tests were performed to measure time to gel and gel strength (varying frequency with constant deformation amplitude). The viscoelastic properties of all kefiran-PG gels were plotted as G′, G″ versus time (values extracted at 1 Hz from consecutive frequency sweep runs at 25 °C) and versus frequency (48 h room temperature-stored gels). Kefiran at 4% (w/v) with PG required a longer time to set into gel and formed a relatively weak gel, leading to the rejection of this system rejected. G′, G″ versus frequency plots were then obtained for the highest kefiran/PG gel formulation incubated for 1, 2, 6, 12, 24, 36, and 48 h at 25 and 37 °C. An increased gel time and reduced gel strength at 37 °C when compared to 25 °C were observed and discussed to be due to weakened hydrogen bonding interactions at higher temperatures, with reduced driving force for gelation (Sabatino et al. 2020).

3.5 Kefiran Biological Characterization

Evaluation of the biocompatibility of a material is essential when evaluating its potential for biomedical applications and the use of cell culture models represents the gold standard for the in vitro testing of biomaterials. Biocompatibility has been defined in many different ways in the past decades; however, following from its numerous definitions, determining the biocompatibility of a biomaterial must rely not only on the potential damaging effects associated with biosafety but also on the desirable properties it may present in the context of its intended application and function (Sousa et al. 2017). For instance, regarding TERM applications, the biocompatibility of a scaffold also denotes its performance as an adequate substrate to support and/or promote appropriate cellular activities (cell growth, extracellular matrix deposition, and induction of desired gene expression patterns) for optimal tissue repair and/or regeneration, being in fact currently designed to be cell instructive (i.e., capable of guiding cell behavior and of prompting specific cell responses) rather than bioinert (Sousa et al. 2017). Hence, after assessing the cytotoxicity of a biomaterial, other traits regarding cellular behavior and cell-material interactions are usually evaluated including metabolic activity, adhesion, proliferation, motility and migration, morphology, and protein/gene expression, among others (Sousa et al. 2017).

Envisioning the applicability of kefiran for TERM, Radhouani and collaborators have assessed the cytotoxicity of kefiran extracts and scaffolds over the mouse L929 fibroblastic cell line and hASCs (Radhouani et al. 2018a, b, 2019). Kefiran extracts showed a lack of cytotoxic response over L929 cells by supporting cell-metabolic activity and cell proliferation, having no effect on cell morphology (Radhouani et al. 2018b), and were shown to improve both the viability and metabolic activity of hASCs (Radhouani et al. 2018a). Also, hASCs seeded on kefiran scaffolds were metabolically active during 72 h, showing their biocompatibility (Radhouani et al. 2019); considering that this an essential feature in TERM and the fact that hASCs are

able to differentiate into several lineages, kefiran revealed great potential for TERM applications.

The biocompatibility of the before-mentioned kefiran/PG scaffolds has also been tested over preosteoblastic MC3T3-E1 cells; no toxic effects were observed on cells placed in direct contact with the kefiran gel, encouraging further biological evaluation of kefiran/PG-water formulations as injectable, in situ forming scaffolds or implantable delivery devices (Sabatino et al. 2020).

Kefiran cytotoxicity over PC12 and MCF7 cells (neural stem cell culture and breast cancer cell line, respectively), and morphological changes of PC12 cells, has also been evaluated for the already mentioned electrospun PAN/Kefiran nanofibers revealing their potential as an antimicrobial, antitumor, biocompatible, and cost-effective system that is exploitable for regenerative medicine and promising for neural stem cell culture (Jenab et al. 2017, 2020).

Kefiran has also exhibited a better resistance to hyaluronidase degradation as compared to HA, highlighting its potential as a promising alternative visco-supplement to the gold standard HA (Radhouani et al. 2018b). Moreover, in a comparative study with HA, kefiran has demonstrated great antioxidant performance against reactive oxygen species, showing stronger reducing power activity and superoxide and nitric oxide radical scavenging capacity over HA. This study highlighted not only the immunostimulatory potential of kefiran but also its potential to protect cells in oxidative stress environments; this substantiated the knowledge regarding its biological performance, such as antioxidant and anti-inflammatory properties, encouraging its application as an alternative or adjunct treatment to promote tissue repair and regeneration while reducing inflammation, for instance, in the osteoarthritis context (Radhouani et al. 2018a).

Given the major medical burden that antimicrobial infections represent worldwide, and the rising concerns regarding antimicrobial resistance, the antimicrobial properties of kefiran represent one of its most interesting biological characteristics. Anti-infective biodegradable materials are highly demanded for clinical applications as they could represent a solution to tackle both problematics by preventing possible infections and reducing the use of antimicrobial agents. Kefiran antimicrobial properties have been vastly reported for a variety of applications (Moradi and Kalanpour 2019). However, studies investigating how these antimicrobial properties prove beneficial in specific contexts of TERM are scarce and of utmost importance.

4 Conclusions and Future Perspectives

Kefiran has been explored for numerous applications in food, medicine, health, and film making. It has been reported to act as an anticancer, antioxidant, and antimicrobial agent, decrease cholesterol levels, reduce bleeding, heal wounds, and support drug delivery. Kefiran has also been used for encapsulation of either bionutrients or immobilized platelets that can be applied to treat surface bleeding. However, only recently, kefiran has been more explored specifically for TERM applications, and the recent studies described herein have shown that this exopolysaccharide can indeed

represent a promising alternative to already existing treatments and gold standard materials. Nonetheless, despite the remaining drawback of the low-kefiran production yields reported so far, the therapeutic attributes and technological applications of kefiran, together with its generally recognized as safe status and numerous health benefits, make this an ideal candidate for solutions in the tissue engineering and regenerative medicine fields.

Acknowledgments S. Correia is supported by the Portuguese Foundation for Science and Technology (FCT) though the R&D Project KOAT – Kefiran exopolysaccharide: Promising biopolymer for use in regenerative medicine and tissue engineering, with reference PTDC/BTMMAT/29760/2017 (POCI-01-0145-FEDER-029760), financed by FCT and cofinanced by European Regional Development Fund (FEDER) and Operational Programme for Competitiveness and Internationalisation (POCI). H. Radhouani is supported by the FCT (CEECIND/00111/2017), and J. M. Oliveira would like to thank the FCT for funding provided under the program Investigador FCT 2015 (IF/01285/2015).

References

Ahmed Z, Ahmad A. Chapter 8: Biopolymer produced by the lactic acid Bacteria: production and practical application. In: Holban AM, Grumezescu AM, editors. Microbial production of food ingredients and additives. Academic; 2017. p. 217–57. https://doi.org/10.1016/B978-0-12-811520-6.00008-8.

Ahmed Z, Wang Y, Anjum N, Ahmad A, Khan ST. Characterization of exopolysaccharide produced by Lactobacillus kefiranofaciens ZW3 isolated from Tibet kefir – part II. Food Hydrocoll. 2013;30:343–50. https://doi.org/10.1016/j.foodhyd.2012.06.009.

Arslan S. A review: chemical, microbiological and nutritional characteristics of kefir. Cyta-J Food. 2015;13:340–5.

Borzacchiello A, Sala FD, Ambrosio LA. 10 – Rheometry of polymeric biomaterials. In: Tanzi MC, Farè S, editors. Characterization of polymeric biomaterials. Woodhead Publishing; 2017. p. 233–53. https://doi.org/10.1016/B978-0-08-100737-2.00010-8.

Botelho PS, Maciel MI, Bueno LA, Marques Mde F, Marques DN, Sarmento Silva TM. Characterisation of a new exopolysaccharide obtained from of fermented kefir grains in soymilk. Carbohydr Polym. 2014;107:1–6. https://doi.org/10.1016/j.carbpol.2014.02.036.

Chen Z, Shi J, Yang X, Nan B, Liu Y, Wang Z. Chemical and physical characteristics and antioxidant activities of the exopolysaccharide produced by Tibetan kefir grains during milk fermentation. Int Dairy J. 2015;43:15–21. https://doi.org/10.1016/j.idairyj.2014.10.004.

Chircov C, Grumezescu AM, Bejenaru LE. Hyaluronic acid-based scaffolds for tissue engineering. Romanian J Morphol Embryol. 2018;59:71–6.

Cottet C, Ramirez-Tapias YA, Delgado JF, de la Osa O, Salvay AG, Peltzer MA. Biobased materials from microbial biomass and its derivatives. Materials (Basel). 2020;13(6):1263.

de Souza AC. Quantification of food polysaccharides by means of NMR. In: Webb GA, editor. Modern magnetic resonance. Cham: Springer International Publishing; 2017. p. 1–19. https://doi.org/10.1007/978-3-319-28275-6_5-1.

Dragostin O, Profire L. 5 – Molecular weight of polymers used in biomedical applications. In: Tanzi MC, Farè S, editors. Characterization of polymeric biomaterials. Woodhead Publishing; 2017. p. 101–21. https://doi.org/10.1016/B978-0-08-100737-2.00005-4.

Esnaashari SS, Rezaei S, Mirzaei E, Afshari H, Rezayat SM, Faridi-Majidi R. Preparation and characterization of kefiran electrospun nanofibers. Int J Biol Macromol. 2014;70:50–6. https://doi.org/10.1016/j.ijbiomac.2014.06.014.

Exarhopoulos S, Raphaelides SN, Kontominas MG. Conformational studies and molecular characterization of the polysaccharide kefiran. Food Hydrocoll. 2018a;77:347–56. https://doi.org/10.1016/j.foodhyd.2017.10.011.

Exarhopoulos S, Raphaelides SN, Kontominas MG. Flow behavior studies of kefiran systems. Food Hydrocoll. 2018b;79:282–90. https://doi.org/10.1016/j.foodhyd.2017.12.030.

Ghasemlou M, Khodaiyan F, Jahanbin K, Gharibzahedi SM, Taheri S. Structural investigation and response surface optimisation for improvement of kefiran production yield from a low-cost culture medium. Food Chem. 2012;133:383–9. https://doi.org/10.1016/j.foodchem.2012.01.046.

Jenab A, Roghanian R, Emtiazi G. Bioavailability of immobilized platelet in probiotic kefiran as a new drug for surface bleeding. J Med Bacteriol. 2015a;4:45–55.

Jenab A, Roghanian R, Emtiazi G. Encapsulation of platelet in kefiran polymer and detection of bioavailability of immobilized platelet in probiotic kefiran as a new drug for surface bleeding. J Med Bacteriol. 2015b;4:55–66.

Jenab A, Roghanian R, Emtiazi G, Ghaedi K. Manufacturing and structural analysis of antimicrobial kefiran/polyethylene oxide nanofibers for food packaging. Iran Polym J. 2017;26:31–9. https://doi.org/10.1007/s13726-016-0496-7.

Jenab A, Roghanian R, Ghorbani N, Ghaedi K, Emtiazi G. The efficacy of electrospun PAN/kefiran nanofiber and kefir in mammalian cell culture: promotion of PC12 cell growth, anti-MCF7 breast cancer cells activities, and cytokine production of PBMC. Int J Nanomedicine. 2020;15:717–28. https://doi.org/10.2147/IJN.S232264.

La Riviere JW, Kooiman P. Kefiran, a novel polysaccharide produced in the kefir grain by Lactobacillus brevis. Arch Mikrobiol. 1967;59:269–78. https://doi.org/10.1007/BF00406340.

Maeda H, Zhu X, Suzuki S, Suzuki K, Kitamura S. Structural characterization and biological activities of an exopolysaccharide kefiran produced by Lactobacillus kefiranofaciens WT-2B (T). J Agric Food Chem. 2004;52:5533–8. https://doi.org/10.1021/jf049617g.

Micheli L, Uccelletti D, Palleschi C, Crescenzi V. Isolation and characterisation of a ropy Lactobacillus strain producing the exopolysaccharide kefiran. Appl Microbiol Biotechnol. 1999;53:69–74. https://doi.org/10.1007/s002530051616.

Montesanto S, Calo G, Cruciata M, Settanni L, Brucato VB, La Carrubba V. Optimization of environmental conditions for kefiran production by kefir grain as scaffold for tissue engineering. Chem Engineer Trans. 2016;49:607–12. https://doi.org/10.3303/Cet1649102.

Moradi Z, Kalanpour N. Kefiran, a branched polysaccharide: preparation, properties and applications: a review. Carbohydr Polym. 2019;223:115100. https://doi.org/10.1016/j.carbpol.2019.115100.

Oliveira JT, Reis RL. Polysaccharide-based materials for cartilage tissue engineering applications. J Tissue Eng Regen Med. 2011;5:421–36. https://doi.org/10.1002/term.335.

Piermaria JA, de la Canal ML, Abraham AG. Gelling properties of kefiran, a food-grade polysaccharide obtained from kefir grain. Food Hydrocoll. 2008;22:1520–7. https://doi.org/10.1016/j.foodhyd.2007.10.005.

Pop CR, Salanţă L, Rotar AM, Semeniuc CA, Socaciu C, Sindic M. Influence of extraction conditions on characteristics of microbial polysaccharide kefiran isolated from kefir grains biomass. J Food Nutr Res. 2016;55:121–30.

Pradhan S, Rajamani S, Agrawal G, Dash M, Samal SK. 7 – NMR, FT-IR and raman characterization of biomaterials. In: Tanzi MC, Farè S, editors. Characterization of polymeric biomaterials. Woodhead Publishing; 2017. p. 147–73. https://doi.org/10.1016/B978-0-08-100737-2.00007-8.

Radhouani H, Goncalves C, Reis RL, Oliveira JM. Kefiran for use in regenerative medicine and/or tissue engineering. WO2018042405A1. 2017.

Radhouani H, Goncalves C, Maia FR, Oliveira JM, Reis RL. Biological performance of a promising kefiran-biopolymer with potential in regenerative medicine applications: a comparative study with hyaluronic acid. J Mater Sci Mater Med. 2018a;29:124. https://doi.org/10.1007/s10856-018-6132-7.

Radhouani H, Goncalves C, Maia FR, Oliveira JM, Reis RL. Kefiran biopolymer: evaluation of its physicochemical and biological properties. J Bioact Compat Pol. 2018b;33:461–78.

Radhouani H, Bicho D, Goncalves C, Maia FR, Reis RL, Oliveira JM. Kefiran cryogels as potential scaffolds for drug delivery and tissue engineering applications. Mater Today Commun. 2019;20:100554. https://doi.org/10.1016/j.mtcomm.2019.100554.

Sabatino MA, Carfi Pavia F, Rigogliuso S, Giacomazza D, Ghersi G, La Carrubba V, Dispenza C. Development of injectable and durable kefiran hydro-alcoholic gels. Int J Biol Macromol. 2020;149:309–19. https://doi.org/10.1016/j.ijbiomac.2020.01.244.

Song R, Murphy M, Li C, Ting K, Soo C, Zheng Z. Current development of biodegradable polymeric materials for biomedical applications. Drug Des Devel Ther. 2018;12:3117–45. https://doi.org/10.2147/DDDT.S165440.

Sousa A, Neves SC, Gonçalves IC, Barrias CC. 12 – In vitro interaction of polymeric biomaterials with cells. In: Tanzi MC, Farè S, editors. Characterization of polymeric biomaterials. Woodhead Publishing; 2017. p. 285–315. https://doi.org/10.1016/B978-0-08-100737-2.00012-1.

Tanzi MC. 6 – Characterization of thermal properties and crystallinity of polymer biomaterials. In: Tanzi MC, Farè S, editors. Characterization of polymeric biomaterials. Woodhead Publishing; 2017. p. 123–46. https://doi.org/10.1016/B978-0-08-100737-2.00006-6.

Tchobanian A, Van Oosterwyck H, Fardim P. Polysaccharides for tissue engineering: current landscape and future prospects. Carbohydr Polym. 2019;205:601–25. https://doi.org/10.1016/j.carbpol.2018.10.039.

Wang Y, Ahmed Z, Feng W, Li C, Song S. Physicochemical properties of exopolysaccharide produced by Lactobacillus kefiranofaciens ZW3 isolated from Tibet kefir. Int J Biol Macromol. 2008;43:283–8. https://doi.org/10.1016/j.ijbiomac.2008.06.011.

Yahia LH, Mireles LK. 4 – X-ray photoelectron spectroscopy (XPS) and time-of-flight secondary ion mass spectrometry (ToF SIMS). In: Tanzi MC, Farè S, editors. Characterization of polymeric biomaterials. Woodhead Publishing; 2017. p. 83–97. https://doi.org/10.1016/B978-0-08-100737-2.00004-2.

Gums for Tissue Engineering Applications 42

Pritisha S. Khillar and Amit Kumar Jaiswal

Contents

Abstract

Tissue engineering involves the replacement and renewal of desired cells, tissues, and organs to restore the normal biological functions of specific cells or tissue types. Recently, the field is growing actively at both the laboratory and clinical levels contributing to research and medicines. The increase in population, changes in lifestyle, and better life expectancy have increased the chances of disease and trauma causing tissue damage which in turn leads to the need for advancement in the field of tissue engineering. The regeneration of the damaged tissues requires a supporting material known as a scaffold. The scaffolds are used for cell adhesion, proliferation, and differentiation for proper tissue regeneration and also help *in vitro* and *in vivo* drug and gene delivery to the biological system. The fabrication of the scaffolds is achieved by combining some biologically active materials known as biomaterials with some specific cell types that are needed for the desired tissue repair process. The natural polymers used as the biomaterials in the fabrication of scaffolds are especially gums that show excellent biological response and mimic the nature of the native extracellular matrix. This chapter briefly explains the gums used in tissue engineering, from where they can be derived, their classifications, properties, and applications.

P. S. Khillar · A. K. Jaiswal (✉)
Centre for Biomaterials, Cellular and Molecular Theranostics, Vellore Institute of Technology, Vellore, Tamil Nadu, India
e-mail: pritisha.subha@vit.ac.in; amitj@vit.ac.in

J. M. Oliveira et al. (eds.), *Polysaccharides of Microbial Origin*,
https://doi.org/10.1007/978-3-030-42215-8_53

Keywords

Tissue engineering · Biopolymers · Gums · Polysaccharides

1 Introduction

Every year millions of patients are facing tissue damage, organ failure issues, and problems associated with the same. Conventionally it can be solved by transplantation of the organ from a donor either as an autograft or allograft. But sometimes it leads to donor shortage along with immunological rejection to the donated body part. Tissue engineering and regenerative medicine have the potential to overcome the shortage of tissues and organs, and it can mimic the nature of the specific tissue and organ with some promising results (Dzobo et al. 2018). Tissue engineering combines bioactive molecules, cells, and scaffolds with biomaterials (Fig. 1) that encourage tissue formation and integration in the host environment. Biomaterials

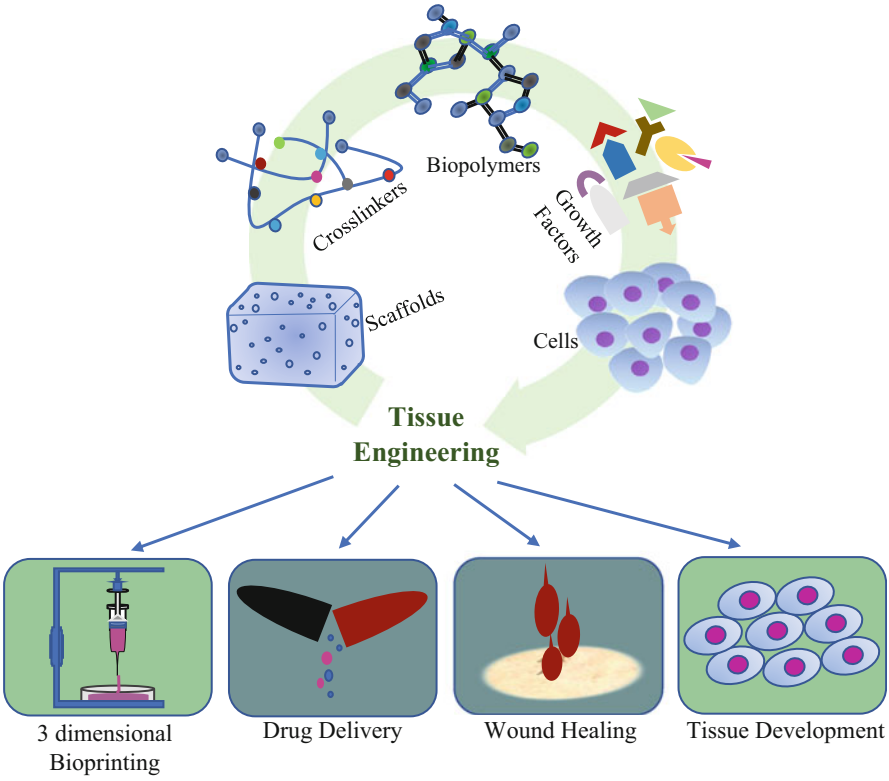


Fig. 1 The design and applications of tissue engineering

provide structural support implicative of the native extracellular matrix (ECM) to stimulate cell growth and serve as an integral component ensuing tissue regeneration. Biomaterials offer several choices in regulating the frame, morphology, and chemistry that help in mimicking the ECM functions. The biomaterials are biodegradable, biocompatible, and should be clinically compliant. They need to be produced, purified, and processed for the delivery of the cells and growth factors to the biological system. They help to provide a specific niche for the cells and tissues for better growth, development, and differentiation. The materials are two types such as natural and synthetic. Both natural and synthetic polymers are extensively used in tissue engineering and regenerative medicine field applications. Natural polymers are typically obtained from nature. They are especially proteins, polysaccharides, and polypeptides, including collagen, alginate, gelatin, actin, albumin, chitosan, keratin, chitin, cellulose, silk, gellan, and hyaluronic acid. Biological systems easily accept these polymers, support cell adhesion, and function, but sometimes there is a lack of desirable immunogenicity, structural complexity, and biomechanical properties (Tang et al. 2014). Synthetic polymers are biodegradable polymers used for tissue engineering and drug delivery applications. It can mimic the nature of macromolecular components of the biological system. It has a well-defined structure with fewer immunological concerns. The synthetic polymers which are actively employed in tissue engineering include polyesters, polyphosphazene, poly(glycerol sebacate), polyanhydrides, and polyurethane (BaoLin and Ma 2014). Natural gums are polysaccharides, hydrophilic compounds with a high molecular weight, and possess numerous biological applications. These gums are used in several industries such as food, environment, medical, and biotechnology, including research areas and tissue engineering (Ahmed et al. 2019). Besides, compared to synthetic polymers, natural polymers are easily available, low in cost, biodegradable, biocompatible, nontoxic, and eco-friendly. These gums have achieved lots of curiosity in the field of tissue engineering and three-dimensional (3D) cell culture because of their effectiveness in tissue regeneration and development in several areas like bone tissue engineering, cartilage repair, skin and wound repair, as well as retinal, neural, and also in three-dimensional bioprinting studies. These are used as binders, crosslinkers, thickening agents, and gelling agents, and also used as hydrogels because of their hydrophilic nature (Mohammadinejad et al. 2020). Besides, the gums can be manipulated to act in several forms such as scaffolds, hydrogels, emulsion, film-forming, and encapsulating agents (Fig. 2). Natural gums are also known as hydrocolloids. These hydrocolloids are divided into several groups depending upon their structure, function, applications, and also according to the charge, viz. ionic and nonionic, according to the source where they are derived from, viz. marine, plant, and animal, according to their shape, viz. linear and branched, and according to monomeric units present in it, viz. homoglycans, di-, tri-, tetra-, and penta-heteroglycans. So, here in this chapter, we are going to briefly understand some natural gums used in tissue engineering, their application, advantages, and disadvantages along with the source and the class where they are derived from.

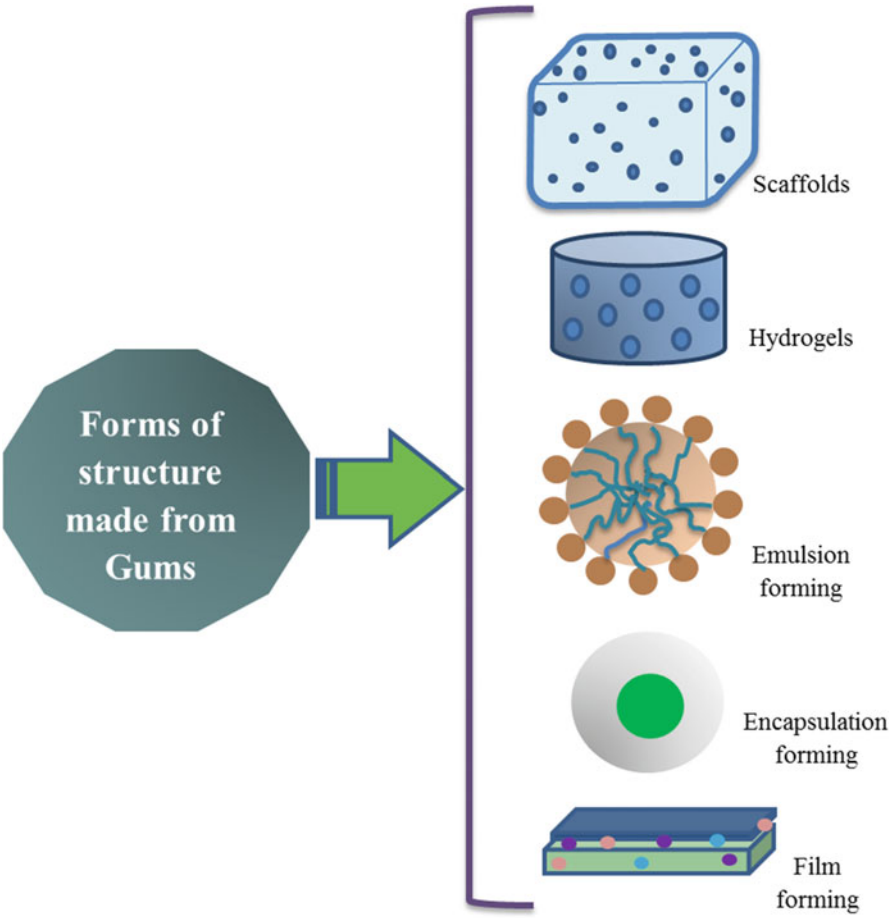


Fig. 2 Different forms that can be made out of gums

2 Natural Gums

Natural gums are polysaccharides usually obtained from natural sources like plant origin, marine origin, and microbial origin, and they are highly effective, biodegradable, biocompatible, nontoxic, chemically inert, easily available, environment friendly, and less expensive along with a high molecular weight. They are commonly used as a binder, stabilizer, thickening agent, gelling agent, disintegrant in tablets, protective colloids in suspensions and diluents, emulsifier, etc. in several fields like pharmaceutical industries, biotech industries, food industry, agriculture, cosmetics, textiles, paper, personal care products, paint industries, biomedical and tissue engineering, etc. (Ahmed et al. 2019; Bhosale et al. 2014; Mohammadinejad et al. 2020).

2.1 Classification of Natural Gums

As directed before the natural gums are usually polysaccharides derived from natural sources; the classification of these gums depends upon the source from which they are derived. They are further classified into several other groups depending upon their charge, shape, structure, and functional groups present in them. As described below (Fig. 3):

Generally, the gums are derived from plants, animals, marine sources, and bacteria (Fig. 3). The plants that produce gums are mainly herbaceous and woody and usually belong to the families of Fabaceae, Sterculiaceae, and Combretaceae. The gums are generally secreted by some physical injury or pathological attack on the plant surface or due to some adverse circumstances such as cell wall breakdown or drought forming gummosis, an extracellular formation. The gums are extruded from seeds, tree exudate, tubers, and roots. Most of the gums are water soluble and highly viscous (Mano et al. 2007; Mohammadinejad et al. 2020; Ahmed et al. 2019; Bhosale et al. 2014; Silva et al. 2017) (Table 1).

Here we are going to briefly discuss some of the gums that are widely used in tissue engineering applications.

2.1.1 Gum Arabica

Gum arabica is a heteropolysaccharide consisting of complex polysaccharides, oligosaccharides, and glycoproteins with the main chain that has galactan with lateral multibranched chains that consist of galactose/arabinose (Rad et al. 2018).

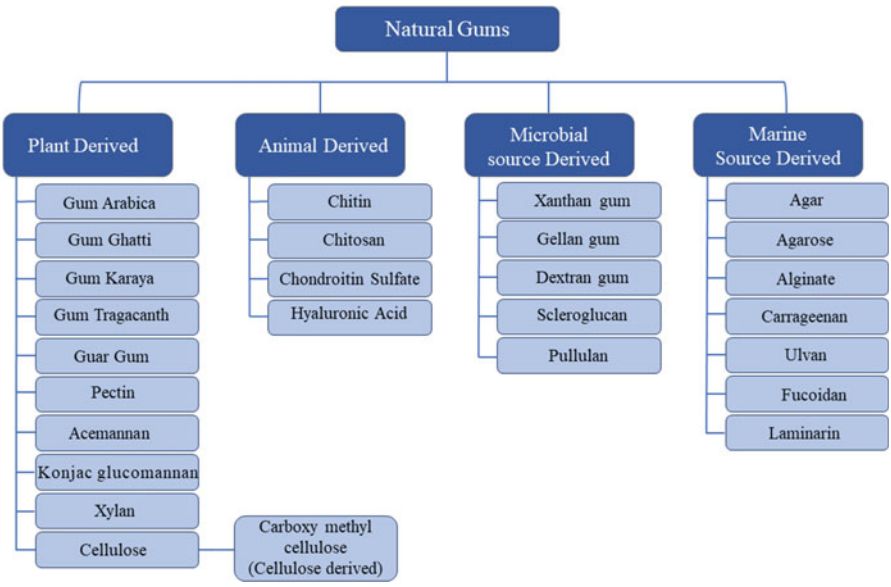


Fig. 3 Classification of the gums based on derived sources

Table 1 Classification of the gums

Gums		Charge	Shape	Units
Plant derived	Arabica gum	Anionic	Branched	Heterogeneous
	Ghatti gum	Anionic	Branched	Heterogeneous
	Karaya gum	Anionic	Branched	Heterogeneous
	Tragacanth gum	Anionic	Branched	Heterogeneous
	Guar gum	Nonionic	Branched	Heterogeneous
	Pectin	Anionic	Linear	Heterogeneous
	Acemannan	Anionic	Linear	Homogeneous
	Konjac glucomannan	Anionic	Branched	Heterogeneous
	Xylan	Nonionic	Branched	Heterogeneous
	Cellulose	Anionic	Linear	Homogeneous
Animal derived	Chitin	Cationic	Linear	Homogeneous
	Chitosan	Cationic	Linear	Heterogeneous
	Chondroitin sulfate	Anionic	Linear	Heterogeneous
	Hyaluronic acid	Anionic	Linear	Heterogeneous
Microbial source derived	Xanthan gum	Anionic	Branched	Heterogeneous
	Gellan gum	Anionic	Linear	Heterogeneous
	Dextran gum	Nonionic	Branched	Homogeneous
	Scleroglucan	Nonionic	Branched	Homogeneous
	Pullulan	Nonionic	Linear	Homogeneous
Marine source derived	Alginate	Anionic	Linear	Heterogeneous
	Agar	Anionic	Linear	Heterogeneous
	Agarose	Anionic	Linear	Heterogeneous
	Carrageenan	Anionic	Linear	Heterogeneous
	Ulvan	Anionic	Branched	Heterogeneous
	Fucoidan	Anionic	Branched	Heterogeneous
	Laminarin	Anionic	Branched	Homogeneous
Others (cellulose derived)	Carboxy methyl cellulose	Anionic	Linear	Homogeneous

It is a naturally existing biomaterial and derived from the exudate of *Acacia senegal* and *Acacia seyal* tree that belongs to the family Leguminosae (Silva et al. 2010). It has β-D-glucopyranuronic acid, α-L-rhamnopyranose, β-D-galactopyranose, β-D-4-glucopyranuronic acid, α-L-arabinose furanose, and α-D-galactopyranose constituent units (Ahmed et al. 2019). The main chain of the polysaccharide contains β-(1→3) and (1→6)-linked D-galactose units in addition to β-(1→6)-linked D-glucopyranosyl uronic acid units (Mano et al. 2007). This gum is highly branched. It is highly water soluble and has a lower viscosity and is used as a suspending agent, binding agent, demulcent, emulsifier, thickener, stabilizer, and crosslinker (Izydorczyk et al. 2005; Bhosale et al. 2014). In tissue engineering, it is used in the fabrication of several scaffolds, and it has bone healing properties. The gum possesses biocompatibility and antioxidant and antibacterial properties. It is nontoxic and because of its wound healing and hydrophilic properties, it can be used as a convenient material

in skin tissue engineering (Ahmed 2018). The addition of gum arabica in a scaffold with other biomaterials increases the water uptake activity of the composite scaffold. This gum also results in the formation of flexible films (Silva et al. 2010). Gum arabica–based scaffold was taken in order to produce a porous scaffold that would mimic the ECM of the skin. The final findings showed that the scaffold containing gum arabica increased the hydrophilicity of the scaffold; the scaffold showed antibacterial property, cell viability, and excellent cell proliferation *in vitro* proving its potential toward the skin tissue engineering field (Rad et al. 2018).

2.1.2 Gum Ghatti

Gum ghatti is one of the effective polysaccharides among the natural plant-based gums that is actively used in the research field because of its biodegradability, sustainability, and safe nature (Lett et al. 2020). It is a complex nonstarch polysaccharide obtained from the bark of *Anogeissus latifolia* trees. It is a promising biomaterial having the chemical structure of polysaccharides and has β-D-glucopyranuronic acid, β-D-galactopyranose, α-L-arabinosefuranose, α-L-arabinopyranose, and β-D-mannopyranose constituent units in addition to several functional groups present, viz. OH, -NH_2, -$CONH_2$, -COOH, and -SO_3 (Sharma et al. 2014; Izydorczyk et al. 2005). It is used as an emulsifier, binder, stabilizer, thickener, flocculating agent, suspending agent, macromolecular surfactants, drug delivery carriers, and superabsorbent materials. It is biodegradable, biocompatible, bioabsorbable, nontoxic, and renewable. It has a controlled swelling property, permeability, mechanical strength, and pH stability along with a higher solubility. In tissue engineering, this gum is used in the fabrication of scaffolds and hydrogels (Sharma et al. 2015; Mohammadinejad et al. 2020). In bone tissue engineering gum ghatti has been used as a binder for the preparation of porous scaffolds along with other biomaterials like hydroxyapatite (HAp). The concentration of gum ghatti affects the rheology and helps to bring down a favorable rheological property for the fabrication of scaffold. The presence of gum ghatti improves the porosity of the scaffold. Moreover, the gum ghatti–based scaffolds support cell growth and proliferation, thus it can be said that this gum serves as an excellent binder for the production of more useful scaffolds that would contribute to bone tissue regeneration applications (Lett et al. 2020).

2.1.3 Karaya Gum

Karaya gum is a polysaccharide extracted from *Sterculia urens* plant. It is a complex, highly branched heteropolysaccharide having α-L-rhamnopyranose, β-D-galactopyranose, α-D-galacturonic acid, and β-D-glucopyranuronic acid constituents units. It is anionic, hydrophilic, and acidic (Ahmed et al. 2019). The gum is made up of rhamnogalacturonan, has α-1,4-linked D-galacturonic acid and α-1,2-linked-L-rhamnosyl residues as the backbone, and the side chain contains 1,3-linked β-D-glucuronic acid or 1,2-linked β-D-galactose on a galacturonic acid unit where half of its unit is partly substituted by 1,4-linked β-D-galactose (Reddy et al. 2019; Izydorczyk et al. 2005). It is biocompatible with high gel forming and adhesion abilities. It has a high viscosity, pH-dependent solubility, good swelling, and water

retention properties. It is nontoxic and used as suspending agent, emulsifying agent, sustaining agent, binder, crosslinker and dental adhesive (Bhosale et al. 2014). In the biomedical field, it is used in controlled drug release in drug delivery systems. The gums are also used in soft and hard tissue engineering in hydrogels and scaffold fabrication along with other biopolymers (Mohammadinejad et al. 2020; Patra et al. 2013). In tissue engineering, a scaffold made of gum karaya and silk fibroin was used for a study and the scaffold was found to exhibit remarkable cytocompatibility, cell adhesion, and proliferation property showing its potential toward skin tissue regeneration (Patra et al. 2013).

2.1.4 Gum Tragacanth

Gum tragacanth is a branched exudate polysaccharide found in several plants belonging to the genus *Astragalus*. It comprises several constituent units such as D-galacturonic acid, D-galactose, D-glucose, D-xylose, L-fucose, L-arabinose, and L-rhamnose (Ahmed et al. 2019). It is biocompatible and biodegradable with excellent rheological properties (Ghani et al. 2016). It acts as an emulsifier, demulcent, adhesive, and suspending agent (Bhosale et al. 2014). Thus, it is involved in several applications in biomedicals, tissue engineering, and controlled drug delivery (Ahmed et al. 2019). Its functionality depends upon its molecular weight and viscosity. It has low toxicity, better cell adhesion ability, and is also shown to have greater cell viability and osteogenic differentiation. Besides gum tragacanth is reported as one of the novel materials used for osteogenic purposes. So, this polymer is likely to be actively used in bone tissue engineering. Gum tragacanth can be said as an appropriate agent for skin tissue engineering as the scaffolds containing tragacanth gum may produce an ultrathin highly porous scaffold with high stability and flexibility with wound healing properties. That can enhance attachment and proliferation of skin cells and leads to skin tissue regeneration (Ghani et al. 2016).

2.1.5 Guar Gum

Guar gum is a natural polysaccharide procured from *Cyamompsis tetraganoloba* seeds. This is nonionic and water soluble with a high molecular weight (Bhosale et al. 2014). It can form gels by changing its chemical and physical properties. The gum is comprised of β-D-mannopyranose and α-D-galactopyranose with a backbone consisting of a long linear chain of β-(1,4)-linked mannose residues with α-(1,6)-D-galactose (Ahmed et al. 2019; Lett et al. 2018). It is highly hydrophilic, biodegradable, nontoxic, viscous, and used as an emulsifier, binder, stabilizer, crosslinker, disintegrating, and thickening agent (Bhosale et al. 2014). Therefore, they are tremendously used in several fields like pharmaceuticals, food industries, cosmetics, and biomedical genetics. The gum has good water blocking, swelling, and gelling properties. In the tissue engineering field, it is considered to have a high potential to develop artificial bone scaffolds as well. These are used as gelling agents in hydrogels (Bhosale et al. 2014; Thankam et al. 2018). It can be used as wound care material. In a bone tissue engineering study guar gum was used as a binder for the preparation of porous scaffolds of HAp. The use of guar gum helped in a

successful scaffold preparation with the desired density, porosity, and mechanical property (Lett et al. 2018).

2.1.6 Pectin

Pectin is a linear anionic plant polysaccharide used as a stabilizer (Bhosale et al. 2014). Pectin can be found in most of the plant cell walls. It is a biodegradable, biocompatible, and water-soluble polysaccharide that promotes cell adhesion and proliferation results to show a better osteogenic activity. It also possesses antiulcer, anti-inflammatory, and antitumor properties. So, the pectin-based scaffolds are applied in wound dressing, bone, and skin tissue engineering. It is comprised of (1,4)-linked α-D-galacturonic acid residues, containing L-rhamnopyranose units (Zhao et al. 2016). Pectin can be used as a stabilizer and crosslinking agent (Bhosale et al. 2014). As crosslinker pectin can improve the resistance and compressibility capability of a scaffold. Pectin can also be believed to overcome low mechanical strength to lead the material to show better characteristics with no toxic effect. Pectin is used in skin tissue engineering as it is hydrophilic and can promote wound healing and cellular interaction. Pectin-based scaffolds have been reported to promote skin regeneration in a promising time limit. Apart from that pectins are believed to manage chemical modifications. This polymer is applicable in both conventional freeze-drying and modern 3D bioprinting technologies (Turkkan et al. 2017).

2.1.7 Acemannan

Acemannan is a plant polysaccharide extracted from *Aloe vera*. It consists of β-(1,4)-acetylated polymannose (Silva et al. 2017; Liu et al. 2019). Being a medicinal plant, it has numerous biological properties. It is proven to have antiviral, antibacterial, anticancerous, anti-inflammatory, and antioxidant activity. It shows immunoregulation and immunomodulatory properties (Liu et al. 2019). This has wound healing properties and is used in pharmaceutical, cardiovascular disease, and dental applications. Acemannan stimulates the immune system, vitalizes tissue regeneration, promotes wound healing, reduces skin tissue damage, and helps in increasing the proliferation of dermal tissues (Liu et al. 2019). In bone tissue engineering acemannan can increase dental pulp tissue proliferation and bone morphogenic protein activity. It is also reported to be biocompatible and promotes bone mineralization, wound healing, and tissue organization. Acemannan scaffolds were studied in periodontal tissue applications and it has been suggested as a useful biomaterial in the case of periodontal tissue regeneration. As periodontium is the soft and hard tissue construct, acemannan can be considered valuable to both soft and hard tissue engineering. Acemannan is also reported to show osteogenesis, bone tissue regeneration, and periodontal tissue regeneration (Liu et al. 2019).

2.1.8 Konjac Glucomannan

Konjac glucomannan is a plant polysaccharide derived from *Amorphophallus konjac* that belongs to the family Araceae. It is composed of β-(1-4)-linked D-glucose and D-mannose with branched of β-1, 6-glucosyl units (Kondo et al. 2009). It is highly viscous (Kondo et al. 2009). It is biodegradable, biocompatible, and behaves as a

gelling and thickening agent. It has excellent viscosity and water retention properties. It has several health-related applications such as it can reduce cholesterol level, promote wound dressing, immune function, and be involved in heart disease treatment, diabetes, hyperthyroidism, and wound dressing. It forms thermally stable gels and applied in several fields such as drug delivery, forming biodegradable materials and materials for enzyme encapsulation (Behera and Ray 2016). Konjac glucomannan is used in several tissue engineering applications such as bone, skin, cartilage, and vascular tissue engineering. In bone tissue engineering, konjac glucomannan results in producing a mechanically strong porous scaffold with extensive interconnectivity. The scaffolds are nontoxic and exhibit osteointegration properties (Kanniyappan et al. 2018). Konjac glucomannan–based scaffolds are also involved in cartilage tissue engineering and have been reportedly provide a promising platform for the viability and culture of chondrocytes that can result in cartilage tissue regeneration (Kondo et al. 2009). Besides, as previously said, it helps in wound healing and wound dressing, so konjac glucomannan composites may further be applied in skin injury treatments.

2.1.9 Xylan

Xylan is a plant polysaccharide, generally produced during the xylem differentiation mechanism. It consists of β-(1-4) glycosidic bonds linked to D-xylopyranose residues as its backbone. It is a heterogeneous polymer having glucuronic acid, arabinose, and acetate as its substitute units. Xylan-based materials are nontoxic, biodegradable, and biocompatible and can be employed in the biomedical field and tissue engineering (Venugopal et al. 2013). Xylan affects the immune system and has been shown to have immunomodulatory properties. In bone tissue engineering the xylan-based composites are reported to show bone repair mechanism. A study revealed that xylan-based composite helps in healing and remodeling a fractured bone along with the regeneration of bone tissues (Bush et al. 2016). Besides, xylan-based scaffolds are also used in cardiac tissue engineering. In a study, an electrospun xylan-based scaffold was investigated for its property toward myocardial infarction. Xylan was taken for the study for its functional properties toward myocardial infarction and the outcomes proved that the scaffold had a suitable mechanical strength and Young's modulus. The scaffolds were found to provide a satisfactory platform for cell culture and proliferation and the cultured cells performed normal cell morphology (Venugopal et al. 2013). These functional properties of the polymer xylan prove the potential of the polysaccharide toward its application in tissue engineering.

2.1.10 Cellulose

Cellulose is a natural gum derived from plants. Those are sustainable, low cost, renewable, biocompatible, bioactive, and nontoxic with high mechanical and thermal stability (Latour et al. 2020; Hickey and Pelling 2019). It is one of the major polysaccharides naturally available on earth that consists of a linear chain of D-glucose units linked to β-(1,4) glycosidic bonds (Silva et al. 2017; Mano et al. 2007). Cellulose is considered as one of the ideal biomaterials because of its tunable

physical, chemical, and mechanical properties; apart from this, it has a highly porous structure with adjustable stability (Hickey and Pelling 2019). Due to these satisfactory properties, cellulose has vital applications in several areas like bone tissue engineering in hydrogel and scaffold fabrications, bone regeneration, skin tissue engineering, artificial skin making, wound healing purposes, drug delivery system, promotes neuron regeneration, scaffolds for regenerative medicine, 3D cell culture, etc. (Hickey and Pelling 2019; Silva et al. 2017). Cellulose is also used in cardiac tissue engineering. The scaffold made of cellulose is uniformly fibrous porous composite with high molecular strength and it supports cell growth and proliferation. Besides, as stated before it takes part in bone tissue engineering; a study was accomplished by selecting apple-derived cellulose scaffolds for bone tissue engineering. The prepared scaffold showed satisfying porosity, osteoblast cell adhesion, proliferation, and also promoted mineralization. These properties confirm the capabilities of cellulose toward bone tissue engineering (Latour et al. 2020).

2.1.11 Chitin

Chitin is a linear polysaccharide generally extracted from exoskeletons of crabs, shrimps, insects, and fungi (Silva et al. 2017; Mano et al. 2007). Chitin has been reported as the second largest available natural polysaccharide after cellulose. It comprises a repeated oxygen-containing hexose ring. Besides it has an acetamido group tied unitedly by β-glycosidic bonds at the second carbon (C2) position and referred to (1,4)-2-acetamido-2-deoxy-β-D-glucan (Wan and Tai 2013). Chitin is a nitrogenous polysaccharide with several excellent properties such as biocompatibility, biodegradability, antibacterial activity, mechanical integrity, and nontoxicity for which it is highly useful in tissue engineering (Jayakumar et al. 2011). Also, it has been shown to enhance some biological activities like immunogenicity, better drug delivery, and wound healing properties with greater compatibility in humans (Jayakumar et al. 2011). Chitin-based materials are broadly used in biomedical fields. Chitin is applied as fibrous scaffolds, porous sponges, and hydrogels in tissue engineering (Venkatesan et al. 2014a). Chitin-based materials stimulate wound healing, skin tissue repair, bone regeneration, and construction of engineered scaffolds for skin tissue engineering (Silva et al. 2017). Chitin can maintain the morphology of chondrocytes; scaffolds composed of pure β-chitin showed the ability to support chondrocytes and can be used in cartilage tissue engineering as spongy scaffolds (Jayakumar et al. 2011). Apart from this chitin-based scaffolds are used in nerve tissue engineering.

2.1.12 Chitosan

Chitosan is an unbranched amino polysaccharide consisting of D-glucosamine and *N*-acetyl D-glucosamine bonds with β-(1,4) bonds and repeating units of glucosamine. The polysaccharide is nontoxic, cationic, soluble in dilute acid solution, and biocompatible (Venkatesan et al. 2015a; Mano et al. 2007). It is obtained from the deacetylation of chitin, from the insect, fungi, and yeast (Pandey et al. 2017). Chitosan is considered an essential biomaterial because of its cationic and polyelectrolyte nature, biodegradability, mucoadhesion, film-forming ability, antimicrobial

action, hemostatic action, and bioactive nature (Silva et al. 2017). It is widely used in the biomedical and tissue engineering field in bone skin and cartilage tissue engineering (Silva et al. 2017). It plays a vital role in wound repair and wound healing for burn injuries and surgeries. Due to its biodegradability, biocompatibility, low cost, and wound healing properties it gained a lot of interest in the skin tissue engineering field and used in scaffold fabrication for skin graft applications. The pure chitosan was found to show cell adhesion, proliferation, and tissue regeneration. But chitosan-based scaffolds fabricated along with other natural polymers showed better stability, mechanical property, and biological activity and were recognized as a favorable material for skin grafting and skin tissue engineering applications (Pandey et al. 2017). Chitosan in bone tissue engineering serves as an ideal biomaterial. Chitosan in scaffolds performs surface modification making the scaffold eligible for bone regeneration and believed to promote bone bioactivity (Venkatesan et al. 2015a). Chitosan helps improve neovascularization, thus supports heart function. In a study chitosan-based scaffold was modified to a conductive scaffold when doped with carbon nanofibers. The resulted scaffold showed to have better elasticity showing satisfying viable cardiac cells with greater metabolic activity than the pure chitosan scaffold producing a better tissue construct for cardiac tissue engineering (Martins et al. 2014). In cartilage tissue engineering chitosan-based scaffolds combined with other biomaterials are being used in an articular cartilage repair application. The involvement of chitosan was notable as it showed an effect on the deacetylation degree and molecular weight of the scaffold. The scaffold was porous like the articular cartilage tissue and it was effective for proliferation and differentiation of chondrocyte showing an adequate capacity toward cartilage tissue repair (Li et al. 2018).

2.1.13 Chondroitin Sulfate

Chondroitin sulfate (CS) is a linear unbranched polysaccharide generally as part of a proteoglycan that remains attached to proteins. It is generally sulfated glycosaminoglycan with proteoglycan attachments composed of variable monosaccharide chains such as D-glucuronic and *N*-acetyl-D-galactosamine (Pal and Saha 2019; Kwon and Han 2016). It is biodegradable, biocompatible, easily available, and highly versatile. Chondroitin sulfate is used in cartilage tissue-specific applications and cartilage tissue engineering (Kwon and Han 2016). It supports osteogenic differentiation with the increase of bone anabolic growth factors effectiveness, bone formation, and also involved in the fabrication of hydrogels in bone tissue engineering. CS-based materials are also involved in skin tissue engineering and dermal tissue engineering; it promotes wound healing and stimulates regeneration in skin defects. CS is also been involved in nerve tissue engineering by supporting nerve roots growth *in vitro* and resulting in nerve tissue damage repair (Kwon and Han 2016).

2.1.14 Hyaluronic Acid

Hyaluronic acid (HA) is a linear, anionic polysaccharide. It is comprised of repeated β-(1,4)-D-glucuronic acid and β-(1,3)-*N*-acetyl-D-glucosamide disaccharides units (Silva et al. 2017; Mohammadinejad et al. 2020). It is usually present in the body

at specific matrices, in specialized tissues like vitreous humor of eyes, cartilage, and several bodily fluids (Falcone et al. 2016). It has numerous biological properties, viz. biodegradability, biocompatibility, and nontoxicity. It often lacks mechanical strength and immunogenicity, but when combined with crosslinkers and other polymers the mechanical stability can be improved (Zhu et al. 2017). It is hydrophilic and used in the tissue engineering field in processing techniques, scaffolds, hydrogels, cryogels, sponges, and injectable hydrogels. In bone tissue engineering HA is used in the craniofacial as well in dental fields; HA-incorporated scaffolds help in bone regeneration. These scaffolds also have great potential for the improvement of the formation and mineralization of bones. Thus, it is considered a favorable material in bone regeneration (Zhai et al. 2020). It promotes cell migration, proliferation, wound healing and tissue regeneration. HA hydrogels are also used in cartilage regeneration in cartilage tissue engineering (Zhu et al. 2017).

2.1.15 Xanthan Gum

Xanthan gum is a branched heteropolysaccharide extracted from the bacteria *Xanthomonas campestris* (Mano et al. 2007). It is comprised of repeating units of (1,4)-linked β-D-glucose residues, with a side chain of trisaccharide β-D-mannose β-D-glucuronic acid α-D-mannose attached to D-glucose alternate units (Mano et al. 2007; Ahmed et al. 2019). It is anionic, soluble in water, highly biocompatible, immunogenic, thermally stable with modified plasticity, and high molecular weight (Kumar et al. 2017). This gum is nontoxic, bioadhesive, and biodegradable. As it possesses all these properties, it has several applications in tissue engineering. It is usually applied as an emulsifier, stabilizer, and suspending agent (Bhosale et al. 2014). Xanthan gum in combination with other polymers is used in hydrogel and scaffolds preparation for skin tissue engineering and it also has wound healing properties. Xanthan gum reportedly can protect cartilage at joints and helps reduce papain-induced osteoarthritis (Kumar et al. 2017). Xanthan gum–based scaffolds exhibit improved mechanical properties, swelling behavior, and cell proliferation showing promising distribution toward bone tissue engineering (Kumar et al. 2017).

2.1.16 Gellan Gum

Gellan gum, an unbranched anionic high molecular exopolysaccharide achieved by the fermentation of *Sphingomonas paucimobilis* bacteria. This has repeating (1→3)-β-D-glucose, (1→4)-β-D-glucuronic acid, (1→4)-β-D-glucose, and (1→4)-α-L-rhamnose units with a COOH side group (Muthukumar et al. 2019; Mano et al. 2007). It is used as a disintegrating, gelling, stabilizing, suspending, and thickening agent. It is gel forming, elastic, thermally stable, nonangiogenic, nonimmunogenic, and low cost with excellent rheological property. The polymer shows promising applications toward cell adhesion, dental care, bone regeneration, and wound healing (Muthukumar et al. 2019). Gellan gum–based materials are biocompatible, they are involved in the fabrication of injectable hydrogels in tissue engineering applications (Spera et al. 2018). Gellan gum can assist support in cartilage regeneration applications (Muthukumar et al. 2019). In bone tissue engineering gellan gum is considered a promising agent to provide support to

cartilaginous and promoting bone regeneration; also the improved gellan gum–based scaffolds with some other biomaterials can result in highly porous scaffolds with high mechanical strength and better swelling properties (Spera et al. 2018). The gum has been studied for soft tissue engineering and angiogenesis enhancement by Gorustovich et al. (2010) A novel skin graft was proposed with the gellan gum and studied *in vitro*. The result showed that this gum has the potential for applications in skin regeneration.

2.1.17 Dextran

Dextran is a branched exopolysaccharide derived from bacterial sources synthesized by the activity of an enzyme called dextransucrase (Mano et al. 2007). These are the homopolysaccharides that contain repeated glucose units (Silva et al. 2017). It has a linear backbone of α-(1,6)-linked D-glucopyranose residues linked with α-(1,2), α-(1,3), and α-(1,4)-linked small side chains. It is a nontoxic, biodegradable, and biocompatible polysaccharide with a less inflammatory response (Mohammadinejad et al. 2020). This polymer is directed to form scaffolds, hydrogels, gelling agents, and encapsulation/coating material. Based on these properties and applications, the polymer is considered a promising material in the areas of biomedical and tissue engineering. In cardiac tissue engineering, a study showed that dextran was used as a protective agent to maintain the bioactivity of the encapsulated material in the scaffold resulting in more sustainable growth factor release helping in cardiovascular tissue regeneration (Tian et al. 2012). A dextran-based hydrogel was examined and found to be suitable for cell viability and cartilaginous tissue generation, proving its potential for cartilage tissue engineering strategies. Dextran hydrogel was used as a wound dressing layer and the result showed that it can also promote wound healing with the complete regeneration of dermal tissue and skin appendages. In another study, a dextran-based scaffold was found to show a high swelling property, good compressive strength, and supported cell proliferation showing its promising applications toward the field (Pan et al. 2014).

2.1.18 Scleroglucan

Scleroglucan is a microbial source–derived polysaccharide consisting of β-1,3-linked glucopyranosyl residue as the backbone substituted by the β-1,6-glucose at every third unit (Corrente et al. 2013). This is a natural polymer used in pharmaceuticals and tissue engineering applications. They are used as hydrogels in tissue engineering as well in drug delivery systems (Corrente et al. 2013; Coviello et al. 2005). It is also known as sclerogum. It could be used as a crosslinker, laxative, stabilizer, and thickening agent with an amazing rheological property. It possesses antimicrobial and antitumor properties and is able to stimulate the immune system (Coviello et al. 2005). Scleroglucan along with other biomaterials results in the formation of stronger hydrogels and can be modified to improve the mechanical properties of the hydrogels. The concentration of scleroglucan in the mixture affects the hardness and cohesiveness of the hydrogel. Scleroglucan-based materials are more viscous and in tissue engineering applications they show natural

tissue-like behavior by showing swelling properties when associated with biological fluids (Corrente et al. 2013).

2.1.19 Pullulan

Pullulan is a microbial polysaccharide found in the exoskeleton of *Aureobasidium pullulans*. It is linear having α-(1,4) and α-(1,6)-linked maltotriose with glycoside linkages (Singh et al. 2016). It is biocompatible and nontoxic with extensive mechanical strength. The polymer is soluble in water and has no taste or smell. Pullulan is thermally stable, biodegradable, nontoxic, nonhygroscopic, non-carcinogenic, and elastic with structural flexibility (Rekha and Sharma 2007). Due to all these properties, it is involved in several biomedical applications. It encourages cell adhesion, proliferation, and also bone tissue formation in bone tissue engineering. The polymer is involved in vascular and dermal tissue engineering and regeneration. Pullulan scaffolds are yet a suggesting material in skin tissue engineering as involved in full-thickness skin graft and wound healing with better water uptake properties (Singh et al. 2016). Pullulan is nonimmunogenic and can act as a cytoadhesive material for cell-mediated cartilage repair if applied in cartilage tissue engineering. These biomaterials also result in cartilage tissue regeneration (Singh et al. 2016). It can be modified to improve several properties like degradability, solubility, colloidal and structural stability, and can also be applied in drug and gene delivery for liver targeting therapies. Heparin-pullulan-based materials used in scaffolds inhibit smooth muscle cell generation, hence can be used for vascular endothelial cell regeneration (Rekha and Sharma 2007). In tissue engineering, pullulan can be used as hydrogels, scaffolding material, nanocomposites, and injectable beads. Pullulan-based scaffolds can result in advanced calcification, bone tissue formation, differentiation, regeneration, and mineralization. These scaffolds are also included in trabecular tissue-related applications. Pullulan provides a proper environment for wound healing and dressing, so it might be a satisfactory material in skin tissue engineering (Singh et al. 2016).

2.1.20 Alginate

Alginate is one of the linear natural polysaccharides made of β(1,4)-linked D-mannuronic acid and α(1,4)-linked L-guluronic acid; the blocks of this polymer consist of consecutive and alternative G and M residue as (GGGGGG), (MMMMMM) in consecutive residue, and (GMGMGM) in alternative residue, respectively (Mano et al. 2007; Venkatesan et al. 2014b). It is procured from brown algae. It has anti-inflammatory and antioxidant activities. It has tunable chemical and physical properties, including biodegradability, biocompatibility, mechanical strength, and gelation property (Sun and Tan 2013). It is used as a suspending and sustained release agent (Bhosale et al. 2014). It is easily available, low cost, and nontoxic. It has several biomedical applications such as drug delivery, skin, bone, cartilage repair, and wound healing. In tissue engineering, it can be used as hydrogels, microcapsules, and 3D scaffolding materials (Sun and Tan 2013; Venkatesan et al. 2015b). Gelatin is also used in cardiovascular tissue engineering and hepatic tissue engineering.

2.1.21 Agar

Agar is obtained from specific marine red seaweeds and algae. The polysaccharide is unbranched, composed of agarose and agaropectin units (Mano et al. 2007). It behaves like a gel, thickener, and stabilizer. It has the property that it can be prepared easily and can be given any kind of shape. Due to its porous structure, it behaves like a sponge. The gel of a particular shape can be dried as can regain its shape upon rehydration, which can be a promising technique in tissue engineering (Verma et al. 2006). It is biocompatible, biodegradable, and antimicrobial in nature. Because of its favorable compatibility, elasticity, controllable size, and adjustable mechanical properties, it can be regarded as a promising biological agent in tissue engineering applications (Thu-Hien et al. 2018). Agar-based scaffolds exhibit excellent cytocompatibility and swelling properties, so they can be also used as a suitable polymer in skin tissue engineering (Verma et al. 2006). Agar-based materials are also used in bone tissue engineering and regeneration.

2.1.22 Agarose

Agarose is a natural linear polysaccharide extracted from red algae (Zarrintaj et al. 2018). Agarose is comprised of a repeated (1→3)-linked β-D-galactose and (1→4)-linked 3,6-anhydro-α-L-galactose units (Mano et al. 2007). It resembles ECM, behaves as a gel, exquisitely biocompatible, thermoreversible, and has physicochemical properties that support cell growth and drug delivery in biological systems with a low rejection rate to the immune system (Zarrintaj et al. 2018). It behaves as a gel and used in the formation of hydrogels and sponges (Mano et al. 2007). It is involved in cartilage tissue engineering. In neural tissue engineering it has been used in an electroactive scaffold with some other conductive polymers; because of its cell adhesion potential, biodegradability, and charged behavior it can also be a favorable material in neural tissue engineering (Zarrintaj et al. 2018). Agarose is used in bone tissue engineering as it shows better porosity distribution over the scaffold, supports biomineralization, and plays a promising role. Apart from these, the agarose-based scaffolds are also used in skin tissue engineering as they show noncytotoxic and anti-inflammatory effects and reportedly capable of helping in the regeneration of the histological structure of human skin (Zarrintaj et al. 2018).

2.1.23 Carrageenan

Carrageenan is an unbranched polysaccharide derived from red algae and consists of 1,3-linked β-D-galactose and 1,4-linked α-D-galactose chains (Mano et al. 2007). The polymer possesses sulfate groups, depending upon the sulfated groups present carrageenans are divided into three separate classes named κ-carrageenan, ι-carrageenan, and λ-carrageenan. κ-Carrageenan contains one sulfate group, ι-carrageenan has two, and λ-carrageenan has three sulfate groups. κ-carrageenans are generally involved in bone tissue engineering and regeneration of bone tissues (Silva et al. 2017). They are nontoxic, biocompatible, and low cost (Mohammadinejad et al. 2020). κ-carrageenan and ι-carrageenan form gels in the presence of some specific ions such as potassium ion (K^+) and calcium ion (Ca^{2+}) and λ-carrageenan is viscous (Mano et al. 2007).

Carrageenan supports both conventional freeze-drying and 3D scaffolding technology. Carrageenans can produce scaffolds with excellent mechanical behavior. They provide a niche for cell proliferation proving its ability toward tissue engineering applications (Sharma et al. 2013). k-carrageenan hydrogels are also said to be as an active material with satisfying properties toward the application in cartilage tissue engineering.

2.1.24 Ulvan

Ulvan is a marine-based polysaccharide found in green algae. Rhamnose, uronic acid, xylose, and sulfate are the major constituent units of this polymer (Silva et al. 2017). It is hydrophilic and anionic. It shows antiviral, antibacterial, and anticoagulant activity (Kogelenberg 2017; Tziveleka et al. 2019). The polymer shows osteoconductivity and ulvan-based scaffolds are biocompatible and applicable for tissue regeneration applications with improved stability and mechanical strength. The addition of this material to the scaffold can result in better water uptake capacity and a highly porous scaffold. Besides, the scaffolds are shown to possess satisfying cell adhesion, proliferation, and viability (Tziveleka et al. 2019). Because of all these satisfying properties, the polymer has gathered a lot of recognition in biomedical fields for the involvement in the preparation of hydrogels, nanofibres, 3D porous scaffolds, wound healing, cells, and drug delivery (Silva et al. 2017). Ulvan is utilized in the fabrication of scaffolds for skin tissue engineering as it affects wound repair and wound healing mechanism. Apart from all these ulvan is a satisfying material for 3D bioprinting and can be used as a bioink (Kogelenberg 2017).

2.1.25 Fucoidan

Fucoidan is a marine-based polysaccharide derived from brown algae. It is heterogeneous comprising several constituent units, viz. D-galactose, D-mannose, D-fucose, D-glucose, and D-xylose (Pajovich and Banerjee 2017). It is biodegradable, biocompatible, and noncytotoxic (Venkatesan et al. 2014c). The polymer is soluble in water. It is anti-inflammatory, antitumor, antithrombin, and anticoagulant in nature, thus applicable in wound healing, biomineralization, tissue formation, and regeneration in bone and skin tissue engineering (Silva et al. 2017; Pajovich and Banerjee 2017). The polymer also has potential toward skin tissue engineering, and composites having fucoidan help wound healing, skin tissue formation, and regeneration by replacing damaged tissue. Scaffolds having other biomaterials combined with fucoidan are shown to have better cytocompatibility and helps in bone tissue regeneration (Pajovich and Banerjee 2017). Fucoidan-based scaffolds are observed to exhibit higher water uptake ability. The presence of fucoidan in the scaffold results in an increment of the free functional group, thus resulting in a higher swelling degree (Venkatesan et al. 2014c). Fucoidan exhibits calcium ion binding properties that help in calcium deposition and biomineralization. The polymer enhances alkaline phosphatase activity and osteocalcin production promoting bone tissue formation and differentiation (Pajovich and Banerjee 2017; Venkatesan et al. 2014c). It has

been also experimentally proved that the addition of fucoidan to the composite scaffold can enhance bioactivity and porosity of the scaffold leading to bone regeneration and mineralization. Thus, it can act as a suitable agent in areas of bone tissue engineering (Venkatesan et al. 2014c).

2.1.26 Laminarin

Laminarin is a marine-based natural polysaccharide derived from brown algae. It behaves like a rich source of carbon in the marine ecosystem. It is comprised of repeating units of β-(1-3)-linked glucose with irregular β-(1-6) branches (Custódio et al. 2016). It is less viscous having a lower molecular weight that supports the material to be involved in microfabrication techniques (Martins et al. 2018). The polymer has immune-stimulatory and antibacterial properties (Custódio et al. 2016). Laminarin assists in cell adhesion and proliferation (Martins et al. 2018; Larguech et al. 2017). Laminarin has an abundant hydroxyl group that helps its molecules to bind to the bioactive agent, thus making the polymer a bioactive material. Modified laminarin-based materials such as methacrylate laminarin particles provide a suitable platform for cell culture and cell growth and can enhance cell proliferation, differentiation, and migration. With these properties, laminarin has been suggested as a useful material for tissue engineering applications (Martins et al. 2018). Though there is no complete evidence of the utilization of laminarin in cartilage tissue engineering, still this polymer has shown some impact on chondrogenic differentiation that can be applicable in mesenchymal stem cells therapies. As mesenchymal stem cells are considered as one of the relevant agents in cartilage tissue repair and regeneration process, this role of laminarin can allow itself to be involved in cartilage tissue engineering application (Larguech et al. 2017).

2.1.27 Carboxymethyl Cellulose

Carboxymethyl cellulose (CMC) is cellulose-derived unbranched polysaccharide. It is achieved by the alkalization of cellulose with sodium monochloroacetate. It is used in pharmaceutical, food, and adhesive industries as a gum. It is white in colur with no taste and odor (Alizadeh et al. 2017). It is a low cost, biodegradable, biocompatible, and nontoxic gum that is actively used in tissue engineering applications and supports cell growth. In coordination with metal ions, carboxymethyl cellulose can produce hydrogels of improved mechanical strength. CMC-based composite in combination with other biomaterials like HAp in bone tissue engineering promotes osteoconductivity, swelling degree, stability, compressive strength, and mechanical properties of the scaffold and supports bone tissue regeneration (He et al. 2019). Carboxymethyl cellulose is also used in cartilage tissue engineering that results in cartilage tissue regeneration and healing.

In Table 2 we have given a concise overview of all the gums described in this chapter in order to provide some instantaneous information.

Table 2 Overview of the gums

Gums	Structural constituents	Properties	Applications	References
Gum arabica	β-D-glucopyranuronic acid, α-L-rhamnopyranose, β-D-galactopyranose, β-D-4-glucopyranuronic acid, α-L-arabinose furanose, α-D-galactopyranose	Water soluble, lower viscosity, biocompatible, antioxidant, antibacterial, nontoxic, and hydrophilic	Suspending agent, binding agent, demulcent, emulsifier, thickener, stabilizer, and crosslinker	Izydorczyk et al. (2005), Bhosale et al. (2014), Ahmed (2018)
Gum ghatti	β-D-glucopyranuronic acid, β-D-galactopyranose, α-L-arabinosefuranose, α-L-arabinopyranose, β-D-mannopyranose	Biodegradable, bioabsorbable, nontoxic, renewable, controlled swelling property, permeability, mechanical strength, and pH stability	Emulsifier, binder, stabilizer, thickener, flocculating agent, suspending agent, macromolecular surfactants, drug delivery carriers, and superabsorbent materials	Sharma et al. (2015), Mohammadinejad et al. (2020), Sharma et al. (2014), Izydorczyk et al. (2005)
Gum karaya	α-L-rhamnopyranose, β-D-galactopyranose, α-D-galacturonic acid, β-D-glucopyranuronic acid	Biocompatibile, gel forming, adhesive, highly viscous, pH-dependent solubility, nontoxic, good swelling, and water retention properties	Suspending agent, emulsifying agent, sustaining agent, binder, crosslinker, and dental adhesive	Reddy et al. (2019), Izydorczyk et al. (2005), Ahmed et al. 2019, Bhosale et al. (2014)
Gum tragacanth	D-galacturonic acid, D-galactose, D-glucose, D-xylose, L-fucose, L-arabinose, and L-rhamnose	Biocompatible biodegradable, excellent rheological properties, low toxicity, and better cell adhesion ability	Emulsifier, demulcent, adhesive, and suspending agent	Ahmed et al. (2019), Ghani et al. (2016), Bhosale et al. (2014)
Guar gum	β-D-mannopyranose, α-D-galactopyranose	Nonionic, water soluble, high molecular weight, highly hydrophilic, biodegradable, nontoxic, viscous, good water blocking, swelling, and gel forming	Emulsifier, binder, stabilizer, crosslinker, disintegrating, and thickening agent	Ahmed et al. (2019), Lett et al. (2018), Bhosale et al. (2014), Thankam et al. (2018)

(continued)

Table 2 (continued)

Gums	Structural constituents	Properties	Applications	References
Pectin	α-D-galacturonic acid and L-rhamnopyranose	Biodegradable, biocompatible, water soluble, nontoxic, and hydrophilic	Stabilizer, crosslinking agent, promotes wound healing, and manages chemical modifications	Zhao et al. (2016), Bhosale et al. (2014)
Acemannan	B-acetylated polymannose	Antiviral, antibacterial, anticancerous, anti-inflammatory, antioxidant, immunoregulatory, and immunomodulatory	Stimulates the immune system, tissue regeneration, and tissue proliferation	Silva et al. (2017), Liu et al. (2019)
Konjac glucomannan	Glucose, mannose and glucosyl units	Highly viscous, biodegradable, biocompatible, and water retention ability	Gelling, thickening agent, promotes osteointegration, and tissue regeneration	Kondo et al. (2009), Behera and Ray (2016), Kanniyappan et al. (2018)
Xylan	Xylopyranose, glucuronic acid, arabinose, and acetate	Nontoxic, biodegradable, biocompatible, and immunomodulatory	Tissue healing, repair, and regeneration mechanism	Venugopal et al. (2013), Bush et al. (2016)
Cellulose	D-glucose units linked to β-(1,4) glycosidic bonds	Low cost, renewable, biocompatible, bioactive, and nontoxic, mechanically and thermally stable, tunable physical, chemical, and mechanical properties	Tissue regeneration, wound healing, and 3D cell culture	Latour et al. (2020), Hickey and Pelling (2019), Silva et al. (2017), Mano et al. (2007)
Chitin	Oxygen-containing hexose ring, acetamido group, β-D-glucan	Biocompatibile, biodegradabile, nontoxic, antibacterial, mechanical integrity, and nonantigenic	Promotes immunogenicity, drug delivery, wound healing, supports tissue growth, and regeneration	Wan and Tai (2013), Jayakumar et al. (2011)
Chitosan	Glucosamine with and *N*-acetyl D-glucosamine		Wound healing, wound repair, tissue regeneration and	

		Biocompatible, biodegradable, antimicrobial, better stability, and mechanical property	development, and promotes osteogenesis	Venkatesan et al. (2015a), Silva et al. (2017), Pandey et al. (2017), Mano et al. (2007)
Chondroitin sulfate	Glycosaminoglycan, proteoglycan, D-glucuronic, and *N*-acetyl-D-galactosamine	Biodegradable, biocompatible, easily available, and highly versatile	Tissue-specific application, osteogenic differentiation, wound healing, and stimulates tissue regeneration	Pal and Saha (2019), Kwon and Han (2016)
Hyaluronic acid	β-(1,4)-D-glucuronic acid and β-(1,3)-*N*-acetyl-D-glucosamide disaccharides units	Biodegradable, biocompatible, nontoxic, and hydrophilic	Bone tissue development and regeneration, promotes cell adhesion, proliferation, and wound healing	Silva et al. (2017), Mohammadinejad et al. (2020), Zhu et al. (2017)
Xanthan gum	β-D-glucose, β-D-mannose β-D-glucuronic acid-α-D-mannose	Soluble in water, highly biocompatible, thermally stable with modified plasticity, and high molecular weight	Emulsifier, stabilizer, suspending agent, wound healing, and enhances the mechanical properties and cell proliferation	Ahmed et al. (2019), Kumar et al. (2017), Bhosale et al. (2014)
Gellan gum	β-D-glucose, β-D-glucuronic acid and α-L-rhamnose units	Gel forming, elastic, thermally stable, biocompatible nonangiogenic, nonimmunogenic, low cost, and excellent rheological property	Disintegrating agent, multifunctional, gelling agent, stabilizing agent, suspending agent, thickening agent, promotes cell adhesion and wound healing	Muthukumar et al. (2019), Mano et al. (2007), Bhosale et al. (2014)
Dextran gum	D-glucopyranose with α-(1,2), α-(1,3) and α-(1,4)-linked small side chains	Nontoxic, biodegradable, biocompatible, and less inflammatory response	Encapsulation material, drug delivery, cell therapy, cell viability, promotes wound healing, dermal, and cardiovascular tissue regeneration	Mohammadinejad et al. (2020), Tian et al. (2012), Pan et al. (2014)

(continued)

Table 2 (continued)

Gums	Structural constituents	Properties	Applications	References
Scleroglucan	B- glucopyranosyl residue substituted with β-1,6-glucose	Rheological property, antimicrobial, antitumor, and viscous	Crosslinker, laxative, stabilizer, thickener, stimulates the immune system, and develops mechanical properties	Corrente et al. (2013), Coviello et al. (2005)
Pullulan	α-(1,4) and α-(1,6)- maltotriose with glycoside linkages	Biocompatible, nontoxic, thermally stable, biodegradable, nontoxic, nonhygroscopic, noncarcinogenic, elastic with structural flexibility, and water uptake properties	Cell adhesion, proliferation, tissue formation, and cell-mediated tissue repair applications	Singh et al. (2016), Rekha and Sharma (2007)
Alginate	D-mannuronic acid and -guluronic acid	Anti-inflammatory, antioxidant, tunable chemical and physical properties, biodegradable, biocompatibile, high mechanical strength, gel forming and nontoxic properties	Suspending and sustained release agent, drug delivery, 3D fabrication, tissue repair, and encapsulating agent	Mano et al. (2007), Venkatesan et al. (2014b), Sun and Tan (2013)
Agar	Agarose and agaropectin	Gel forming, water holding capacity, biocompatible, biodegradable, and antimicrobial	Thickener, stabilizer, promotes excellent cytocompatibility and bone tissue regeneration	Mano et al. (2007), Verma et al. (2006)
Agarose	β-D-galactose and 3,6-anhydro-α-L-galactose	Gel forming, biocompatible, thermo-reversible, biodegradable, noncytotoxic, and anti-inflammatory	Support cell growth and tissue repair	Mano et al. (2007), Zarrintaj et al. (2018)

Carrageenan	α-D-galactose, β-D-galactose	Nontoxic, biocompatible, low cost, gel forming, viscous, excellent mechanical behavior, thickener, and emulsifier	2D and 3D culture, promotes cell proliferation and tissue regeneration	Mano et al. (2007), Silva et al. (2017), Mohammadinejad et al. (2020)
Ulvan	Rhamnose, uronic acid, xylose, and sulfate	Hydrophilic, antiviral, antibacterial, anticoagulant, and better water uptake capacity	Promotes osteoconductivity, tissue regeneration, improves stability, mechanical strength, cell adhesion, cell viability, wound healing, and drug delivery	Silva et al. (2017), Kogelenberg (2017), Tziveleka et al. (2019)
Fucoidan	D-galactose, D-mannose, D-fucose, D-glucose, and D-xylose	Water soluble, biodegradable, biocompatible, and noncytotoxic, anti-inflammatory, antitumor, antithrombin, anticoagulant, higher water uptake ability, higher swelling degree, and calcium ion binding properties	Wound healing, tissue formation and regeneration, and replaces damaged tissue, and promotes cytocompatibility	Pajovich and Banerjee (2017), Venkatesan et al. (2014c)
Laminarin	Glucose residues with β-(1–3) and β-(1–6) branches	Less viscous, lower molecular weight, immune stimulatory, and antibacterial	Supports microfabrication techniques, assists cell adhesion, proliferation, and migration	Custódio et al. (2016), Larguech et al. (2017), Martins et al. (2018)
Carboxymethyl cellulose	D-glucose unit with sodium monochloroacetate	Low cost, biodegradable, biocompatible, and nontoxic	Supports cell growth, improves mechanical strength, promotes osteoconductive and tissue regeneration	Alizadeh et al. (2017), He et al. (2019)

3 Conclusion

In this chapter, we have reviewed some of the natural gums that are biodegradable, biocompatible, nontoxic, easily available, and low cost with tuneable mechanical properties and mostly used in soft and hard tissue engineering and tissue regeneration applications. The gums are commonly derived from some natural sources like plants, animals, marine, and bacteria. They are further distinguished according to their charge (ionic and nonionic), shape (linear and branched), and unit (homogenous and heterogeneous). The natural gums are likely to provide a suitable environment to mimic the native ECM, support cell attachment, and new tissue formation. It displays improved biological activity compared to other synthetic biomaterials. This chapter outlines the properties and application of the natural gums or gum-based materials in the fabrication of scaffolds and hydrogels as well as their use in the form of crosslinkers, binders gelling, thickening, and emulsifying agents in bone, skin, cartilage, cardiac, retinal, and neural tissue engineering applications. These gums sometimes show superior performance when combined with other biomaterials, some of their properties can be modified and improved with the addition of other natural and synthetic polymers and biomolecules (e.g., growth factors) to perform toward specific tissue engineering applications. Either in pure form or as a modified polysaccharide, these gums can be used as bioinks in 3D bioprinting that appears to be a promising approach in tissue engineering applications. Apart from tissue engineering, these natural polysaccharides have numerous applications in drug delivery, nanomedicines, and therapeutic research as shown in Fig. 4. Due to these valuable contributions, these gums are being considered promising materials in biomedical research.

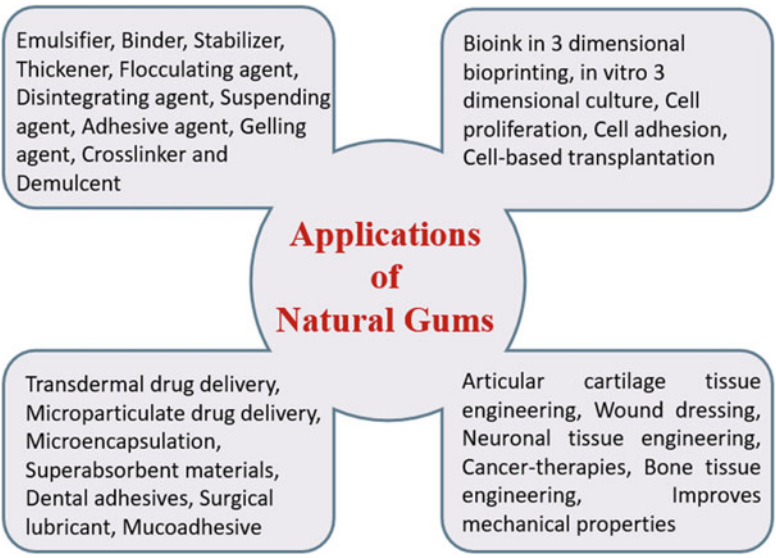


Fig. 4 Different applications of natural gums

References

Ahmed AA. Health benefits of gum Arabic and medical use. Gum Arabic. 2018. https://doi.org/10.1016/b978-0-12-812002-6.00016-6.

Ahmed S, Ahmed M, Manzoor K, Purwar R, Ikram S. A review on latest innovations in natural gums based hydrogels: preparation and applications. Int J Biol Macromol. 2019;136:870–90.

Alizadeh AS, Mousavi M, Labbafi M. Synthesis and characterization of carboxymethyl cellulose from sugarcane bagasse. J Food Process Technol. 2017. https://doi.org/10.4172/2157-7110.1000687.

BaoLin G, Ma PX. Synthetic biodegradable functional polymers for tissue engineering: a brief review. Science China Chem. 2014;57(4):490–500. https://doi.org/10.1007/s11426-014-5086-y.

Behera SS, Ray RC. Konjac Glucomannan, a promising polysaccharide of *Amorphophallus konjac* K. Koch in health care. Int J Biol Macromol. 2016. https://doi.org/10.1016/j.ijbiomac.2016.07.098.

Bhosale RR, Osmani RA, Moin A. Natural gums and mucilages: a review on multi-faceted excipients in pharmaceutical science and research. Int J Pharma Phytochem. 2014;6(4):901–12.

Bush JR, Liang H, Dickinson M, Botchwey EA. Xylan hemicellulose improves chitosan hydrogel for bone tissue regeneration. Polym Adv Technol. 2016;27(8):1050–5. https://doi.org/10.1002/pat.3767.

Corrente F, Amara AHM, Pacelli S, Paolicelli P, Casadei MA. Novel injectable and in situ cross-linkable hydrogels of dextran methacrylate and scleroglucan derivatives: preparation and characterization. Carbohydr Polym. 2013;92(2):1033–9. https://doi.org/10.1016/j.carbpol.2012.10.018.

Coviello T, Palleschi A, Grassi M, Matricardi P, Bocchinfuso G, Alhaiqu F. Scleroglucan: a versatile polysaccharide for modified drug delivery. Molecules. 2005;10(1):6–33.

Custódio CA, Reis RL, Mano JF. Photocrosslinked Laminarin based hydrogels for biomedical applications. Biomacromolecules. 2016. https://doi.org/10.1021/acs.biomac.5b01736.

Dzobo K, Thomford NE, Senthebane DA, Shipanga H, Rowe A, Dandara C, Pillay M, Motaung KSCM. Advances in regenerative medicine and tissue engineering: innovation and transformation of medicine. Stem Cells Int. 2018. https://doi.org/10.1155/2018/2495848.

Falcone SJ, Palmeri D, Berg RA. Biomedical applications of hyaluronic acid. In: Zhu XX, Ravenelle F, editors. Polysaccharides for drug delivery and pharmaceutical applications. San Luis Obispo: FzioMed Inc., Bonetti Drive; 2016. p. 155–74.

Ghani M, Mak WC, Cheung KY, Montazer M, Rezaei B, Griffith M. Cross-linked superfine electrospun tragacanth-based biomaterial as scaffolds for tissue engineering. Eur Cells Mater. 2016;21(1):204.

Gorustovich AA, Roether JA, Boccaccini AR. Effect of bioactive glasses on angiogenesis: a review of *in vitro* and *in vivo* evidences. Tissue Eng Part B. 2010;16(2):199–207. https://doi.org/10.1089/ten.TEB.2009.0416.

He X, Tang K, Li X, Wang F, Liu J, Zou F, Yang M, Li M. A porous collagen-carboxymethyl cellulose/hydroxyapatite composite for bone tissue engineering by bi-molecular template method. Int J Biol Macromol. 2019;137:45–53.

Hickey RJ, Pelling AE. Cellulose biomaterials for tissue engineering. Front Bioeng Biotechnol. 2019;7:2296–4185. https://doi.org/10.3389/fbioe.2019.00045.

Izydorczyk M, Cui SW, Wang Q. Polysaccharide gums: structures, functional properties, and applications. In: Cui SW, editor. Food carbohydrates: chemistry, physical properties, and applications. Boca Raton: CRC Press; 2005. p. 43.

Jayakumar R, Chennazhi KP, Srinivasan S, Nair SV, Furuike T, Tamura H. Chitin scaffolds in tissue engineering. Int J Mol Sci. 2011;12(3):1876–87. https://doi.org/10.3390/ijms12031876.

Kanniyappan H, Thangavel P, Chakraborty S, Arige V, Muthuvijayan V. Design and evaluation of Konjac glucomannan-based bioactive interpenetrating network (IPN) scaffolds for engineering vascularized bone tissues. Int J Biol Macromol. 2018. https://doi.org/10.1016/j.ijbiomac.2019.12.012.

Kogelenberg SV. Fabrication of ulvan based structures for cell culture in wound Fabrication of ulvan based structures for cell culture in wound healing. Thesis collections, University of Wollongong; 2017.

Kondo T, Shinozaki T, Oku H, Takigami S, Takagishi K. Konjac glucomannan-based hydrogel with hyaluronic acid as a candidate for a novel scaffold for chondrocyte culture. J Tissue Eng Regen Med. 2009;3:361–7. https://doi.org/10.1002/term.170.

Kumar A, Rao KM, Han SS. Application of xanthan gum as polysaccharide in tissue engineering: a review. Carbohydr Polym. 2017;180:128–44. https://doi.org/10.1016/j.carbpol.2017.10.009.

Kwon HJ, Han Y. Chondroitin sulfate-based biomaterials for tissue engineering. Turk J Biol. 2016;40:290–9. https://doi.org/10.3906/biy-1507-16.

Larguech G, Cesaro A, Toumi H, Martinić I, Petoud S, Daniellou R, Lespessailles E. Growth inhibition of mesenchymal stem cells by laminarin: impact on chondrocyte differentiation. Anat Physiol. 2017. https://doi.org/10.4172/2161-0940.1000263.

Latour ML, Tarar M, Hickey RJ, Cuerrier CM, Catelas I, Pelling AE. Plant-derived cellulose scaffolds for bone tissue engineering. BioRxiv. 2020. https://doi.org/10.1101/2020.01.15.906677.

Lett JA, Sundareswari M, Ravichandran K, Sagadeva S. The fabrication of porous hydroxyapatite scaffold using gaur gum as a natural binder. Dig J Nanomater Biostruct. 2018;13(1):235–43.

Lett AJ, Sagadevan S, Shahnavaz S, Muthiah LB, Alagarswamy K, Hossain MA, Mohammad F, Johan MR. Exploration of gum ghatti-modified porous scaffolds for bone tissue engineering applications. R Soc Chem. 2020. https://doi.org/10.1039/c9nj05575d.

Li H, Hu C, Yu H, Chen C. Chitosan composite scaffolds for articular cartilage defect repair: a review. RSC Adv. 2018;8(7):3736–49. https://doi.org/10.1039/c7ra11593h.

Liu C, Cui Y, Fuwei P, Cheng Y, Yahui G, Qian H. Extraction, purification, structural characteristics, biological activities and pharmacological applications of acemannan, a polysaccharide from *Aloe vera*: a review. Molecules. 2019. https://doi.org/10.3390/molecules24081554.

Mano JF, Silva GA, Azevedo HS, Malafaya PV. Natural origin biodegradable systems in tissue engineering and regenerative medicine: present status and some moving trends. J R Soc. 2007;4(17):999–1030. https://doi.org/10.1098/rsif.2007.0220.

Martins AM, George E, Caridade SG, Mano JF, Reis RL, Vunjak-Novakovi G. Electrically conductive chitosan/carbon scaffolds for cardiac tissue engineering. Biomacromolecules. 2014;15(2):635–43. https://doi.org/10.1021/bm401679q.

Martins CR, Custodio CA, Mano JF. Multifunctional laminarin microparticles for cell adhesion and expansion. Carbohydr Polym. 2018. https://doi.org/10.1016/j.carbpol.2018.08.029.

Mohammadinejad R, Kumar A, Ranjbar-Mohammadi M, Ashrafizadeh M, Han SS, Khang G, Roveimia Z. Recent advances in natural gum based biomaterials for tissue engineering and regenerative medicine: a review. Polymers. 2020;12(1):176. https://doi.org/10.3390/polym12010176.

Muthukumar T, Song JE, Khang G. Biological role of gellan gum in improving scaffold drug delivery, cell adhesion properties for tissue engineering applications. Molecules. 2019;24(24):4514. https://doi.org/10.3390/molecules24244514.

Pajovich HT, Banerjee IA. Biomineralization of fucoidan-peptide blends and their potential applications in bone tissue regeneration. JFB. 2017;8(3):41. https://doi.org/10.3390/jfb8030041.

Pal D, Saha S. Chondroitin: a natural biomarker with immense biomedical applications. RSC Adv. 2019;9:28061–77. https://doi.org/10.1039/c9ra05546k.

Pan J-F, Liu N-H, Sun H, Xu F. Preparation and characterization of electrospun PLCL/poloxamer nanofibers and dextran/gelatin hydrogels for skin tissue engineering. PLoS One. 2014;9(11):e112885. https://doi.org/10.1371/journal.pone.0112885.

Pandey AR, Singh US, Momin M, Bhavsar C. Chitosan: application in tissue engineering and skin grafting. J Polym Res. 2017;24(8):125. https://doi.org/10.1007/s10965-017-1286-4.

Patra N, Vojtová L, Lenka M. Biomimetic electrospun nanofibrous scaffold from gum karaya (sterculia urens)/silk fibroin blend for tissue regeneration. In: Nanocon, Brno, Czech Republic, EU;16–18 Oct 2013.

Rad ZP, Mokhtari J, Abbasi M. Fabrication and characterization of PCL/zein/gum arabic electrospun nanocomposite scaffold for skin tissue engineering. Mater Sci Eng C. 2018;93:356–66. https://doi.org/10.1016/j.msec.2018.08.010.

Reddy SO, Subha MCS, Jithendra T, Madhavi C, Rao KC. Fabrication of Gelatin/Karaya gum blend microspheres for the controlled release of distigmine bromide. J Drug Deliv Ther. 2019;9(3):1–11. https://doi.org/10.22270/jddt.v9i3-s.2720.

Rekha MR, Sharma CP. Pullulan as a promising biomaterial for biomedical applications: a perspective. Trends Biomater Artif Organs. 2007;20(2):111–6.

Sharma A, Bhat S, Vishnoi T, Nayak V, Kumar A. Three-dimensional supermacroporous carrageenan-gelatin cryogel matrix for tissue engineering applications. Biomed Res Int. 2013. https://doi.org/10.1155/2013/478279.

Sharma K, Kaith BS, Kumar V, Kalia S, Kumar V, Som S, Swart HC. Gum ghatti based novel electrically conductive biomaterials: a study of conductivity and surface morphology. eXPRESS Polym Lett. 2014;8(4):267–81.

Sharma K, Kumar V, Kaith BS, Som S, Kumar V, Pandey A, Kalia S, Swart HC. Synthesis of biodegradable gum ghatti based poly (methacrylic acid-aniline) conducting IPN hydrogel for controlled release of amoxicillin trihydrate. Ind Eng Chem Res. 2015;54(7):1982–91. https://doi.org/10.1021/ie5044743.

Silva RA, Mehl P, Wilson OC. Gum Arabic-chitosan composite biopolymer scaffolds for bone tissue engineering. In: 26th Southern biomedical engineering conference SBEC, College Park, Maryland, USA;30 April–2 May 2010.

Silva SS, Fernandes EM, Silva-Correia J, Pina S, Vieira S, Oliveira JM, Reis RL. Natural-origin materials for tissue engineering and regenerative medicine. Natural polymers. In: Duchevne P, Healy KE, Hutmacher DE, Grainger DW, Kirkpatrick CJ, editors. Comprehensive biomaterials II. 2nd ed. Amsterdam: Elsevier; 2017. p. 228–52.

Singh RS, Kaur N, Rana V, Kennedy JF. Recent insights on applications of pullulan in tissue engineering. Carbohydr Polym. 2016;153:455–62. https://doi.org/10.1016/j.carbpol.2016.07.118.

Spera R, Nobile S, Trapani LD. Gellan gum for tissue engineering applications: a mini review. Biomed J Sci Tech Res. 2018;7(2):2574–1241. https://doi.org/10.26717/BJSTR.2018.07.001474.

Sun J, Tan H. Alginate-based biomaterials for regenerative medicine applications. Materials. 2013;6(4):1285–309. https://doi.org/10.3390/ma6041285.

Tang X, Thankappan SK, Lee P, Fard SE, Matthew DH, Tran K, Yu X. Polymeric biomaterials in tissue engineering and regenerative medicine. Nat Synthetic Biomed Polym. 2014. https://doi.org/10.1016/b978-0-12-396983-5.00022-3.

Thankam A, Al-Anbaky Q, Al-karakooly Z, Magar ABR, Chhetri BP, Ali N, Ghosh A. Fabrication and characterization of hydroxypropyl guar-Poly (vinylalcohol)-Nano hydroxyapatite composite hydrogels for bone tissue engineering. J Biomater Sci Polym Ed. 2018;29(17):2083. https://doi.org/10.1080/09205063.2018.1494437.

Thu-Hien L, Thanh-Truc N, Toi VV, Khon HC, Bao BC, Niem VVT, Anh MNT, Hai ND, Chuong PD, Hiep NT. Evaluation of the morphology and biocompatibility of natural silk fibers/agar blend scaffolds for tissue regeneration. Int J Polym Sci. 2018. https://doi.org/10.1155/2018/5049728.

Tian L, Prabhakaran MP, Ding X, Kai D, Ramakrishna S. Emulsion electrospun vascular endothelial growth factor encapsulated poly (L-lactic acid-co-e-caprolactone) nanofibers for sustained release in cardiac tissue engineering. J Mater Sci. 2012;47:3272–81.

Turkkan S, Atila D, Akdag A, Tezcaner A. Fabrication of functionalized citrus pectin/silk fibroin scaffolds for skin tissue engineering. J Biomed Mater Res B Appl Biomater. 2017. https://doi.org/10.1002/jbm.b.34079.

Tziveleka L-A, Ioannou E, Roussis V. Ulvan, a bioactive marine sulphated polysaccharide as a key constituent of hybrid biomaterials: a review. Carbohydr Polym. 2019. https://doi.org/10.1016/j.carbpol.2019.04.074.

Venkatesan J, Vinodhini PA, Sudha PN, Kim S-K. Chitin and chitosan composites for bone tissue regeneration. Adv Food Nutr Res. 2014a. https://doi.org/10.1016/B978-0-12-800268-1.00005-6.

Venkatesan J, Nithya R, Sudha PN, Kim S-K. Role of alginate in bone tissue engineering. Adv Food Nutri Res. 2014b. https://doi.org/10.1016/b978-0-12-800268-1.00004-4.

Venkatesan J, Bhatnagar I, Kim S-K. Chitosan-alginate biocomposite containing fucoidan for bone tissue engineering. Mar Drugs. 2014c;12(1):300–16. https://doi.org/10.3390/md12010300.

Venkatesan J, Kim S-K, Wong TW. Chitosan and its application as tissue engineering scaffolds. Nanotechnol Appl Tissue Eng. 2015a. https://doi.org/10.1016/b978-0-323-32889-0.00009-1.

Venkatesan J, Bhatnagar I, Manivasagan P, Kang K-H, Kim S-K. Alginate composites for bone tissue engineering: a review. Int J Biol Macromol. 2015b;72:269–81. https://doi.org/10.1016/j.ijbiomac.2014.07.008.

Venugopal J, Rajeswari R, Shayanti M, Sridhar R, Sundarrajan S, Balamurugan R, Ramakrishna S. Xylan polysaccharides fabricated into nanofibrous substrate for myocardial infarction. Mater Sci Eng C. 2013;33:1325–31.

Verma V, Verma P, Kar S, Ray P, Ray AR. Fabrication of agar-gelatin hybrid scaffolds using a novel entrapment method for *in vitro* tissue engineering applications. Biotechnol Bioeng. 2006;96(2):392–400. https://doi.org/10.1002/bit.21111.

Wan ACA, Tai BCU. CHITIN – a promising biomaterial for tissue engineering and stem cell technologies. Biotechnol Adv. 2013;31(8):1776–85. https://doi.org/10.1016/j.biotechadv.2013.09.007.

Zarrintaj P, Manouchehri S, Ahmadi Z, Saeb MR, Urbanska AM, Kaplan DL, Mozafari M. Agarose-based biomaterials for tissue engineering. Carbohydr Polym. 2018;187:66–84. https://doi.org/10.1016/j.carbpol.2018.01.060.

Zhai P, Peng X, Li B, Liu Y, Sun H, Li X. The application of hyaluronic acid in bone regeneration. Int J Biol Macromol. 2020;151:1224–39.

Zhao L, Li J, Zhang L, Wang Y, Wang J, Gu B, Chen J, Hao T, Wang C, Wen N. Preparation and characterization of calcium phosphate/pectin scaffolds for bone tissue engineering. RSC Adv. 2016;6(67):62071–82. https://doi.org/10.1039/c6ra07800a.

Zhu Z, Wang YM, Yang J, Luo XS. Hyaluronic acid: a versatile biomaterial in tissue engineering. Plast Aesthet Res. 2017;4:219–27.

Microbial Polysaccharides as Cell/Drug Delivery Systems

43

M. Ramesh, K. Sakthishobana, and S. B. Suriya

Contents

Abstract

Polysaccharides are considered to be excellent materials when compared with other polymers, due to their ease in tailoring, biocompatibility, bioactivity, homogeneity, bioadhesive nature, and ability to mimic the natural extracellular matrix microenvironment. Polysaccharides show great potentials for drug delivery and

M. Ramesh (✉)
Department of Mechanical Engineering, KIT-Kalaignarkarunanidhi Institute of Technology, Coimbatore, Tamil Nadu, India

K. Sakthishobana · S. B. Suriya
Department of Biotechnology, KIT-Kalaignarkarunanidhi Institute of Technology, Coimbatore, Tamil Nadu, India

J. M. Oliveira et al. (eds.), *Polysaccharides of Microbial Origin*,
https://doi.org/10.1007/978-3-030-42215-8_54

tissue engineering applications. The main aim of this chapter is to spotlight the new advancements and challenges concerned with microbial polysaccharides used for cell/drug delivery applications. In this chapter, the essential microbial polysaccharides including cellulose, Dextran, Pullulan, Xanthan, Salecan, Gellan, etc., which are sourced from plants, microbes, and animals, respectively, are reviewed. From the chapter it is concluded that the series of biocompatible microbial polysaccharides were promising candidates as smart drug delivery systems in future biomedical field.

Keywords

Microbial · Polysaccharides · Biocompatible · Drug delivery systems · Dextran · Cellulose

1 Introduction

From the middle of twentieth century, microbial polysaccharides contributed to applications in various fields including biomedical field where dextran solution is used as plasma expanders for first clinical trial. Many other microbial polysaccharides are utilized as pharmaceutical excipients like xanthan gum as suspension stabilizer or pullulan in capsules and enteral products. They originate from plant or animal sources but are mostly produced from microbial fermentation process. In current modern biomedical field, bacterial cellulose is used in wound dressings and as scaffolds. Control release drug systems of both micro- and nano-particulate materials were emerging as a new application for biopolymers in biomedical field. Polysaccharide based nano-particulate systems plays major role as drug-targeting and carriers since they display biocompatibility and nontoxicity (Moscovici 2015).

Treatments for many noncommunicative diseases include tablets, capsules, and syrups. They cause adverse effects like microbial resistance, cytotoxicity, and hypersensitivity reactions. In search for the novel methods of therapy without producing adverse effects, researchers designed novel drug delivery system, microbe-based delivery systems, and gene delivery systems. Microbes, especially whole bacteria, and its toxin, viruses or fungi express some desired results in drug delivery system. Drug delivery systems that use microbes are considered safe, nontoxic, with minimal side effects and site-specific targeted action. They serve as a drug carrier and as a therapeutic agent in current research. From the results gathered, microbe-based drug delivery system has become one of the most hopeful drug delivery systems for many diseases like cancer, inflammatory bowel diseases, and also in cancer therapy. Viruses and bacteria can aid in anticancer therapy by accumulating in the cancer cells, which will be helpful in tumor and tumor imaging and it will be the futuristic advanced system for dynamic improvement in patient's health (Shende and Basarkar 2019).

2 Polysaccharides

2.1 Microbial Polysaccharides

Polysaccharides are natural polymers produced by microorganisms that are secreted extracellularly. They are a large group of biopolymers used in various applications, namely, food, pharmaceutical, and medical field (Giavasis 2013). They pose prodigious range of variety in both structural and functional properties and are commercially available. Specifically, curdlan, cyanobacteria, dextran, pullulan, schizophyllan, xanthan, and xylinan polysaccharides are commercially available microbial polysaccharides (Morris and Harding 2009). The key producers of microbial polysaccharides are fungi of the *Basidiomycetes* family and several gram-negative bacteria such as *Xanthomonas, Pseudomonas*, *Alcaligenes*, etc., and gram-positive bacteria such as lactic acid bacteria. Yeast belonging to *Saccharomyces* genus also synthesizes polysaccharides (Giavasis 2013). Presently, marine microbial extracellular polysaccharides have novel properties in structure and chemical composition that have specific applications in adhesives, textiles, pharmaceuticals, and medicine fields as anticancer drugs, food additives, oil recovery, and metal removal (Manivasagan and Kim 2014).

2.2 Fungal Polysaccharide

One of the common and well-studied fungal polysaccharide is pullulan. Pullulan is a white, tasteless, water-soluble homo-polymer of glucose. Genus of mushroom *Ganoderma* is rich in bioactive compounds whose polysaccharides are greatly consumed in Asia. It reveals most distinctive biological properties, as well as anti-inflammatory, antitumor and immunomodulatory activities (Ren et al. 2021). It is used as traditional medicine in China for health and longevity. Presently, compounds such as polysaccharides, alkaloids, amino acids, triterpenes, steroids, ergosterols, furan derivatives, nucleosides, fatty acids, and lipids from *Ganoderma* have been isolated and identified. In that mainly polysaccharides and triterpenoids are accountable for the pharmacological activities of *Ganoderma*. They are responsible for mitogen-activated protein kinase (MAPK) pathways in immune cells and also for cancer immunotherapy through nuclear factor-κB (NF-κB) (Ren et al. 2021).

Even though most of the microbial polysaccharides are derived from fungi and bacteria, yeast such as *Saccharomyces cerevisiae* produces polysaccharide glycan, which is extracted from yeast cells walls (Giavasis 2013). In the fruiting body of *Flammulina velutipes*, a novel water-soluble polysaccharide (*Flammulina velutipes* Polysaccharide1 – FVP1) is seen, which aids in Inflammatory Bowel Diseases. This polysaccharide is the active component showing bioactivity like immune regulation, antimicrobial activity, antineoplastic. The novel water-soluble polysaccharide has the potential to act as a functional food ingredient or a preventive drug for Inflammatory Bowel Diseases (IBD) (Zhang et al. 2020).

2.3 Cellulose

Cellulose, a bacterial-derived natural polymer produces 3D nano-fibril networks, which are biocompatible. It forms very narrow pore size and this obstructs cell adhesion and gas/fluid transportation. The meticulous designing of biomaterial with an appropriate pore size and pore interconnectivity can lead to cell adhesion, cell infiltration, migration, vascularization, and host integration therefore mimicking the natural extracellular matrix (ECM) (Dubey et al. 2021). The drug should provide a specific targeted therapy than developing a generalized effect. Nanotechnology in biomedical field involves enhanced drug targeting, imaging, and diagnosis. Thus, the use of nanoparticles has become the latest trend since it provides result (Unnikrishnan et al. 2020).

Bacterial cellulose was made into scaffolds using desired shape molds and achieved a porous network of scaffold. This scaffold showed very slow degradation behavior due to lack of cellulase enzymes in human and it is found that even after 60 days there was no change in its structure/decomposed. Thus, it can be employed for medical conditions that require long-term healing like large bone fractures. Growth factors are crucial for cellular process and tissue regeneration. Consequently preconditioning the cells before depositing in scaffolds plays a role in cellular repair and regeneration (Dubey et al. 2021).

2.4 Xanthan Gum

Xanthan gum is an external polysaccharide derived from fermentation of glucose and sucrose by the bacteria *Xanthomonas campestris* (Fig. 1). It is mainly utilized as food additive as stabilizer and thickening agent. Recently, xanthan gum was used along with other polysaccharides as drug delivery molecules. Ngwabebhoh et al. (2021) reported a crosslinking between chitosan, xanthan gum, and hydroxypropyl methylcellulose using Schiff's base reaction for controlled antibiotic drug delivery of minocycline, rifampicin, and ampicillin to inhibit microbial growth. They showed cumulative sustained release up to 80%.

3 Cell Delivery

Currently, nanoemulsions have been widely functional in the various areas of targeted drug delivery, cosmetic industry and food industry. They are non -equilibrated, kinetically stable systems containing oil, water and an emulsifier. One of the main uses of nanoemulsion is the delivery of drugs and bioactive materials to the specific target site. Nanoemulsions involve two synthesis steps: macro-emulsion and conversion to a nanoemulsion. The techniques involve high energy methods like high pressure homogenization, ultrasonication, and low energy method like phase inversion temperature, emulsion inversion point. Richa and Choudhury (2019) proposed a nanoemulsion with fucoid and κ-carrageenan polysaccharide, which

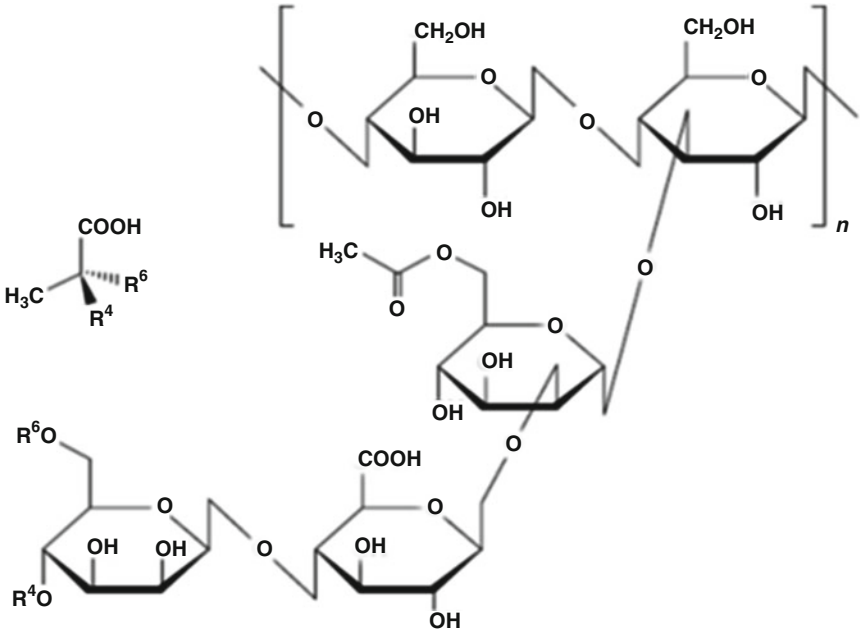


Fig. 1 Structure of xanthan gum (Giavasis 2013)

showed potential biological safety, stability, and biodegradability compared to synthetic emulsifiers. Microbe-based drug delivery systems are responsible for safe, nontoxic, site-specific targeted action with fewer side effects taken by conventional drug delivery system. Microbes, especially whole bacteria, bacterial toxin, viruses, or fungi express some validated results in drug delivery system for cancer and inflammatory bowel disease. Due to accumulation in tumor and tumor imaging, both bacteria and viruses are equally shown to be an interesting candidate for drug delivery. It will be helpful in futuristic advanced system for dynamic improvement in patient's health (Shende and Basarkar 2019).

Probiotics are beneficial microbes which promote gut health and are recommended as treatment for Crohn's disease, colorectal cancer therapy, and antibiotic-associated diarrhea. *Lactobacilli* and *Bifidobacterium sp*. are the most used probiotics and keeping them viable during formulation and storage is the crucial part in its preparation. So a novel microbial polysaccharide and sunflower oil-based emulsion gel formulation was developed to simultaneously deliver these anaerobes as well as a lipophilic antimicrobial drug metronidazole (Pandey et al. 2016). The use of microbial polysaccharide has many advantages. It promotes targeted delivery since they are indigestible in stomach or small intestine and digested only in colon. The sugars in polysaccharides provide a dual role (i) an anaerobic condition and (ii) as a nutrient to maintain its viability. The polysaccharides used are xanthan gum and guar beans-derived guar gum. The study showed

stability in oil-in-water type emulsion gels and their viability retained after 30 days of refrigeration. It also helped in controlled release of metronidazole drug in colon (Pandey et al. 2016).

3.1 Dextran

The dextran is a complex microbial polysaccharide made of branched α −1, 6 linked d-glucopyranoside residues, which has lots of medicinal use (Fig. 2). The most prevalent lactic acid bacteria like *Leuconostoc mesenteroides* and *Streptococcus mutans* produce dextran. This polysaccharide cannot be digested by human since we lack dextranase enzyme produced by colon residing bacteria. The degradation product of dextran is a biocompatible molecule, thus nontoxic in nature. This polymer protects the drug delivery particle from cell and protein adhesion thus avoiding any immune response from the system. It is an approved plasma substitution and plasma volume expansion molecule (Yao et al. 2014). One way of administering is by covalently binding the drug with dextran macromolecule, forming a prodrug. Alternatively, nanoparticles can be synthesized.

Colorectal cancer is the third most common cancer type (Tiryaki et al. 2020) affecting people and the therapy does not provide permanent relief from the disease. This was addressed by Tiryaki et al. by designing an enzyme triggered controlled drug delivery system. The drug encapsulated silica gel has dextran in the outer coating and 5-fluorouracil is used as the anticancer drug. 5-Fluorouracil inhibits the nucleotide pathway by controlling thymidylate synthase activity (Tiryaki et al. 2020). But it can also affect the normal cells and thus it is essential to deliver drug only at the intended site. This drug carrying silica gel was prepared by sol-gel method and dextran outer coating provides the enzyme triggered control release in colon.

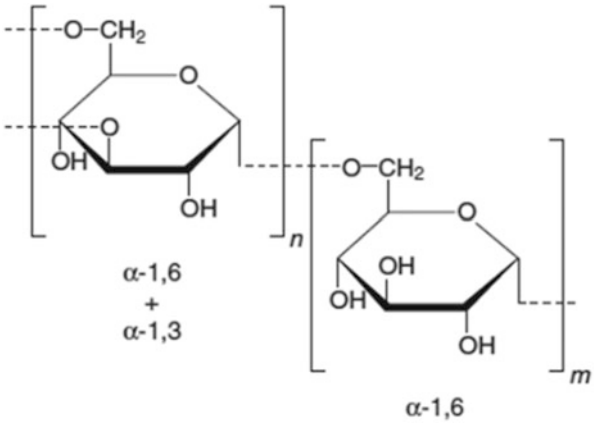


Fig. 2 Structure of dextran (Giavasis 2013)

The drug release pattern in gastric-, intestinal- and colon-simulated regions showed that silica aerosols released 85–89% of drug in all the regions within 24 h. At the same time silica aerosol coated with dextran and dextran-CHO released around 1.7% and 3.4%, in gastric and intestinal fluid, respectively, and 4.2% and 0.97% in dextranase free colon fluid medium. In presence of dextranase enzyme the release was increased to 24% and 13.4% for dextran and dextran-CHO-coated silica aerosols. This confirms the controlled drug release triggered by enzyme for the cancer therapy. MTT assay showed the nontoxic nature of the produced biomolecule in vitro (Tiryaki et al. 2020). Dang and Guan concluded that image-guided drug delivery is the best example in which it incorporates magnetic resonance imaging (MRI) with drug delivery nanoparticles to monitor the bio-distribution, circulation, and targeting the behavior of nanoparticles because they are not only required to deliver drugs but also to offer diagnosis and drug monitoring during cancer treatment (Dang and Guan 2020).

3.2 Dextran-Based Cryogels

One more dextran-based biomaterial involves the fabrication of cryogels. Cryogels are hydrogels differing in the production temperature that is below the freezing point of the solvent giving rise to high elastic and macroporous biomaterial (Pacelli et al. 2021). Interconnectivity between the pores can help in the movement of molecules and cell adhesion. They are prepared using natural or synthetic polymers or in combination to achieve the expected result. The solvent used is mostly water and thus the cryogels are formed at −15 °C associated with immediate freeze drying to get spongy like porosity. Since many parameters are involved in the making process, variation in any of the parameters may produce the desired result. The dextran methacrylate with the cross-linking agent as synthetic polymer polyethylene glycol dimethacrylate and water as solvent were used to produce cryogels. The polymer concentration and rate of reaction contributes to the main parameter for proper formation of cryogels. Unlike a hydrogel, the cryogel can withstand heavy mechanical stress and can resist deformation. It did not compromise on the biomolecule release profile due to its high porosity and it exhibited no cytotoxicity (Pacelli et al. 2021).

3.3 Dextran-Based Colon Cancer Therapy

The on-off kind of controlled drug release can be formed using a hydrogel with addition of electro-active aniline trimer with dextran polysaccharide and hexamethylene diisocyanate. They are external stimuli-based functional hydrogels, which can respond to electric stimuli. The external stimuli generally used are pH, temperature, light, enzymes, magnetic field, and electrostatic potential (Qu et al. 2019). In these, the electrostatic stimulation can be a great advantage since it can regulate the drug release based on its oxidation/reduction characteristics. The use of

aniline is biocompatible since it can be excreted through renal filtration and thus can act as smart biomaterial.

The electric potential driven release of the drug showed higher cumulative effect in a 120 min duration at 3 V than the 35% released drug in case of no external electric stimuli. Instead of continuous electric potential, intermittent voltage potential of 3 V per 30 min was given, which released drug in the same effect. The in vitro and in vivo cell culture showed biocompatibility. The release effect is due to the movement of charged molecules in the electric field and the changes in net charge of hydrogel due to oxidation/reduction reactions. This kind of controlled stimuli-based release helps in localized sustained drug release therapy (Qu et al. 2019).

Dextran polysaccharide can assist in prolonging the circulation time of drug in the blood stream that is usually not stable in vivo. An antiviral drug zidovudine (AZT) is the first approved drug for AIDS-HIV disease. But its effect is diminished due to its short half-life in blood circulation as it can easily dissolve in most of the pH. To increase the life span and to control its release a suitable vehicle needs to be designed. An encapsulation method of core- and shell-based nanoparticles was prepared with AZT present in the core. Dextran and stearic acid-coated polyethylene glycol (PEG) forms the outer shell and AZT is presented in the core. This nanoparticle preparation promotes circulation time, protects from phagocytosis, and controlled release. The delivery of anticancer drug and its cellular uptake also proved to be efficient in normal and cancer cells. Thus, a novel dextran using hybrid nanoparticle can be used in the positional delivery of the drug (Joshy et al. 2018). Drug delivery system formulated using liposomes and natural polysaccharide conjugates have great potential to improve drug-like properties and curative efficacy improving oral absorption, controlling the release, enhancing the in vivo retention ability, targeting the delivery, and exerting synergistic effects (Li et al. 2017).

Targeting cancer cells can be done by its characteristic lower pH, higher temperature, and high concentration of glutathione in the cells. The various therapies that make use of this property can be designed to produce external stimuli-based controlled drug delivery system. A system that uses pH as external stimuli was performed by hydrophobic camptothecin drug encapsulated by dextran with POGMEA through atom transfer radical polymerization (Bai et al. 2018), histidine cross-linked cholesterol–dextran micelles (Yao et al. 2014), and vicinal diol modified with hydrophobic acid-labile phenylboronic ester groups-based dextran B-Dex polymer, which is shown in Fig. 3 (Li et al. 2013).

These micelles can be designed to target specific cellular or subcellular locations for drug delivery and making it tumor-specific. They can enter the targeted tumor cell plasma membrane through enhanced permeability and retention effect (EPR) and it unloads its cargo when it encounters endosomes or lysosomes thereby lysing the cell (Bai et al. 2018). The disulfide cross-linking between dextran and the drug increased the tumor cell apoptosis. These micelles were found to possess high drug loading capacity, stable micelle structure in circulation fluid, rapid cellular internalization, and targeted drug delivery (Bai et al. 2018). The disulfide cross-linking is desired for stability and targeted drug delivery. It readily degrades in reducing environment making it subcellular-specific lysosomes (Lian et al. 2017). pH and

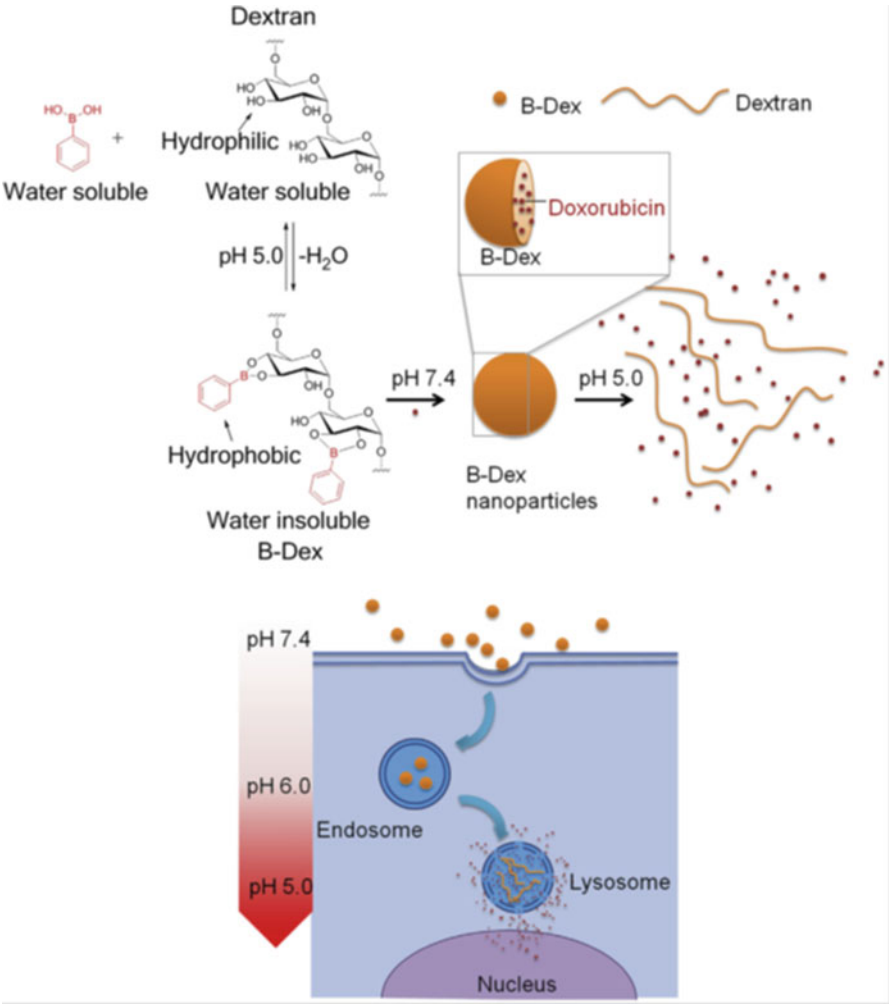


Fig. 3 Schematic of B-Dex polymer production and its pH stimuli-based doxorubicin drug delivery in lysosomes (Li et al. 2013)

reductive environment-based external stimuli provides dual targeting. Histidine cross-linked with cholesterol dextran conjugate micelles Doxorubicin (DOX) was the drug used in this study and it was greatly internalized and released its drug load inside the cell thus killing it (Lian et al. 2017).

Polyethylene glycol (PEG) is a FDA-approved molecule for biomedical use. In spite of its biocompatibility, opsonization, and longer half-life, it was found that use of PEG gives rise to hypersensitivity reaction, Anti-PEG antibody production, and activation of complement system. So it is desired to use an alternative for PEG, and research on dextran is becoming popular for the production of nanoparticles. So a drug delivery for the specific colorectal cancer cells was designed to use dextran with

poly-o-nitrobenzyl alcohol (PNBA), a light-sensitive polymer. The ester bond in this polymer absorbs 2 photons from UV light and converts into carboxylic acid functional group, which modifies its polymeric structure. The structure modification causes the nanoparticle to spill its contents at the specific site. The photo-radiation study revealed the disappearance of nanoparticles in the solution and the disappearance depends on the irradiation time and nanoparticle chemical composition (El Founi et al. 2018).

Multifunctionality nanoparticle combinations like diagnostic and therapy together in a single micelle can contribute to advanced cancer therapy. One such research provides anticancer drug doxicillin DOX and diagnostic super-paramagnetic iron oxide (SPIO) for magnetic resonance imaging (MRI) in an encapsulated nanoparticle for cancer therapy. The application of manganese ferrite $MnOFe_2O_3$ (Mn-SPIO) aids in the precise MRI scans, since it possesses superior MR transverse relaxation (T2) shortening effects. The nanoparticle is micellar in structure, which is made by dextran coated with stearic acid. This kind of micelle can carry hydrophobic drug in its core and can help in imaging and therapy of cancer cells simultaneously. Cellular internalization when compared between free and nanoparticle DOX, free DOX displayed the concentration with 24 h incubation while nanoparticle-based DOX achieved the same within 2 h of incubation in breast cancer MCF-7/Adr cells. The MRI signal provided a darkening effect than the control sample without Mn-SPIO when studied under clinical 3.0-T MRI scanner (Lin et al. 2015).

3.4 Hydrogels

Hydrogels are water insoluble matrix made up of cross-linked hydrophilic polymers, which are termed as smart biomaterials (Chandra et al. 2020; Qi et al. 2020). Hydrogels imbibe a lot of liquid and as a result it expands in volume and size. The hydrogels due to its swelling nature lack mechanical strength and collapse easily. To improve its stability cross-linking with either natural or synthetic polymers are used. Many polysaccharides derived from microbes, plants, and animals are used as a green technology to achieve biocompatible, biodegradable materials for tissue engineering application (Qi et al. 2020).

3.5 Gellan Gum

Gellan gum is a negatively charged linear polysaccharide produced by the bacteria *Pseudomonas/Sphingomonas elodea* (Lee et al. 2012). It consists of repetitive units of sugars like D-glucose, D-glucuronic acid, D-glucose, and L-rhamnose (Fig. 4). It is chiefly used in the food industry as a stabilizer and gelling agent. Currently, it is exploited in biomedicine field owing to its biocompatibility and biodegradation profile and it has been approved as an excipient in pharmaceutical preparations.

Cross-linking gellan gum with cations or chemical molecules generate hydrogels with malleable property. One such nontoxic cross-linker used is an endogen

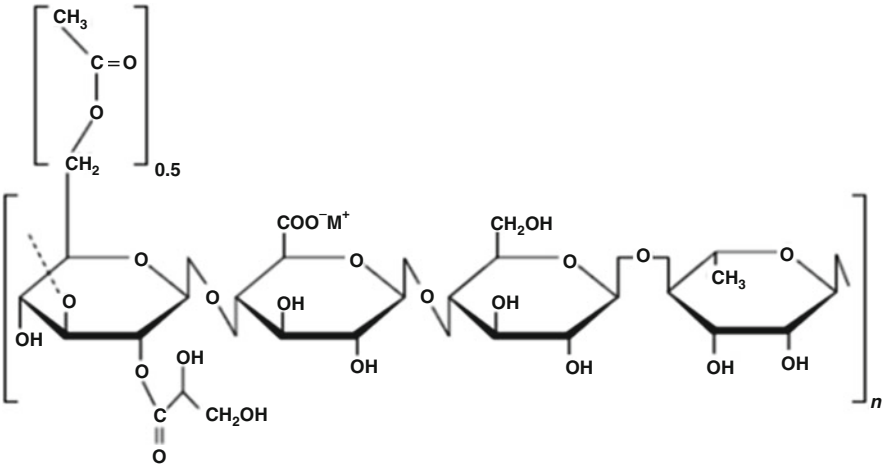


Fig. 4 Structure of gellan gum (Giavasis 2013)

spermidine, and hydrogels are constructed by ionotropic gelation technique, which promotes tissue regeneration and biocompatibility (Psimadas et al. 2012). This hydrogel can be used as a vehicle for supplying growth factors for cellular repair and regeneration. When a growth factor BMP-2 was supplied through the gellan gum-based hydrogel in a slow release manner to a healthy muscle cells, it developed ectopic bone formation (López-Cebral et al. 2017). Another research employed a photo-dimerization method to create hydrogel using cinnamate as a cross-linker with gellan gum. Cinnamate occurs naturally in plants like *Erythroxylum coca* and is a natural tropane alkaloid, which is nontoxic and exhibits anti-inflammatory properties. This research aimed at producing an anti-adhesion film to reduce postoperative inflammation, adhesion fibrosis, and to escalate general well-being. The anti-adhesion film should possess softness, flexibility, ease of application, transparency, be nonreactive and fixed in position, and have resolvable properties. The prepared film possessed high tensile strength, owing to the extent of cross-linking, which is an important property of anti-adhesion film (Lee et al. 2012). The visual examination of postoperative anti-adhesion film showed nil to mild adhesion in a group of operated rats, and experiment on number of inflammatory neutrophil cells showed less than the control group of cells. Thus, cinnamate also provides its anti-inflammatory property to the healing tissues (Lee et al. 2012).

A separate research used Chlorine 6 drug in the form of Chlorine 6 (Ce6)-fucoidan/alginates@gellam gum (Ce6-Fu/AL@GG)-based hydrogel as the anticancer drug in treating early colorectal cancer by way of photodynamic therapy. This hydrogel constitutes few naturally occurring polysaccharides like fucoidan, alginate, and gellen gum and used as a vehicle to carry the photosensitizer chlorine 6 to the tumor cells. Irradiation of laser light to this photosensitizer develops reactive oxygen species (ROS) and free radicals. This ROS can destroy the tumor cells through apoptosis and is believed to effectively heal wounds through vascularization and

cellular regeneration. The MTT assay with the HT-29 cells reveled exceptional cell death through augmented drug delivering hydrogel and laser irradiation. The hydrogels without photosensitizer drug showed no toxicity. Thus, it was concluded that the produced hydrogel can be effectively used as a carrier for chlorine 6 while it showed no cytotoxicity on its own (Karuppusamy et al. 2019).

By using gellan gum as a chief polymer and polyvinyl alcohol (PVA) as supporting polymer by a process of facile electro-spinning, gellan gum-based nano-fibrous transdermal substitute carrying antimicrobial drug amoxicillin was fabricated to combat microbial infection at wound site. The gellan/PVA nano-fibers have more potential as growth scaffold compared to PVA nano-fibers (Vashisth et al. 2016).

3.6 Salecan

Salecan, a linear polysaccharide belonging to the family of β-glucan, is a novel molecule isolated from *Agrobacterium sp*. ZX09. It is made up of repetitive glucopyranosyl units and has the tendency to absorb a great deal of water. This polysaccharide was found to possess antioxidant activity, nontoxicity, and is mostly used in food and biomedical field. Poly (*N*-(3-dimethylaminopropyl) acrylamide-co-acrylamide) is cross-linked with salecan polysaccharide by semi-interpenetrating polymer network to derive a biocompatible and a durable hydrogel. Incorporation of salecan resulted in less pores with bigger pore sizes. The drug delivery of model drug amoxycilin showed increased drug release due to large pore size (Qi et al. 2017). A novel method of salecan and gellan gum mixture produced a biocompatible hydrogel scaffold by physical method of cross-linking through heating-cooling. Different ratios of the polymer gave rise to varying mechanical strength and swelling properties, which help in desirable design flexibility and biocompatibility with dense cell population (Qi et al. 2020).

3.7 Heparosan Polysaccharide

Heparosan polysaccharide, a precursor to heparin and heparin sulfate, is derived from *Escherichia coli.* It is metabolized in lysosomes into simpler monosaccharides, thus constituting its low toxicity and good biocompatibility. It can be used in the cancer treatment by synthesizing heparosan-based micelles carrying the anticancer drug. The anticancer drug doxorubicin was employed in a study and the highest cellular uptake of the drug was observed in B16 cells, greatest cytotoxicity took place in MGC80-3 cells (Qiu et al. 2019). This diversifying effect is related to its drug delivery pathway into cells like phagocytosis, pinocytosis, clatherin receptor-mediated endocytosis, and caveolae-mediated endocytosis. The antitumor drug after internalization into cell enters into either mitochondria or nucleus to induce apoptosis (Qiu et al. 2019).

3.8 Pullulan

Pullulan is a fungal extracellular polysaccharide *Aureobasidium pullulans* consisting of repetitive maltotriose units connected through α-1, 6 and α-1, 4 glycosidic bonds. The Pullulan polymer does not cause immunogenicity in humans and thus it can act as a plasma expander. It has an innate affinity for uptake by liver cell (Constantin et al. 2020). This FDA-approved polymer exhibits enhanced water solubility, biocompatibility, hemocompatibility, oil resistance, flexibility, ability to form fibers, adhesion property, edible nature, and oxygen impermeable film formation (Su et al. 2020). Since pullulan is a macromolecule containing many surface hydrophilic groups, in association with a hydrophobic molecule can be fabricated to create a nanoparticle micelles for drug delivery. The shell and core type nanoparticle can accommodate the drug in core while the shell can have pullalan and derivatives (Constantin et al. 2020).

A study on the use of pullulan for drug delivery involved the synthesis of dibutylaminopropyl carbamate pullulan octanoate, an amphiphilic molecule. Nanoparticle was synthesized in two steps and loaded with model hydrophobic drug diclofenac by core-shell assembly. The diclofenac drug release profile of synthesized nanoparticle was extremely slow and it followed biphasic pattern. It took more than 100 h when kept under pH 7.4 and 5.4 at physiological temperature of 37 °C, on the contrary, free diclofenac was entirely released within 4 h of incubation in phosphate buffer (Constantin et al. 2020). This may be contributed to the hydrophobic nature of the drug and its dissipation in the reduced environment. They showed sustained and systemic release of drug in the specific pH.

Cytotoxicity studies revealed that administering low concentration of drug-loaded and drug-free nanoparticle does not produce cell death; rather it increased the cell viability. Around 80% cytotoxicity was observed when the drug containing nanoparticle was above 62.5 μl/ml concentration. Free polymer also showed similar results and it developed cytotoxicity above 62.5 μl/ml concentration (Constantin et al. 2020). Pullulan in combination with chitosan is used in electro-spinning of nano-fibers for preparing fast dissolving oral film (FDOF). FDOF is a rapid dissolving thin film containing a drug molecule for absorption of drug in buccal cavity. The drugs that cause bowel irritation; GI tract susceptible drugs and saliva activated drugs are formulated for absorption in the abundant buccal vasculature. Electro-spinning utilizes the surface charges in polymer to conduct high electricity, and jet expulsion of the polymers by spinneret at room temperature produces nano-fibers once solvent is evaporated. Chitosan and pullulan combined nano-fibrils are formed by electro-spinning and without use of any nonedible substances. The diameter of the nano-fiber altered based on the chitosan concentration. At first the diameter increased, then reduced with increase in chitosan content. A model drug aspirin was encapsulated in it and its dissolving capability showed it has FDOF nature (Qin et al. 2019).

3.9 Schizophyllan

Schizophyllan is a microbial β-glucan containing exo-polysaccharide produced by *Schizophyllum commune* which possesses medicinal property and bioactivity. It shows many properties including hepatoprotective immune stimulation, antimicrobial, anti-inflammatory, antitumoral, cholesterol- lowering, anti-fibrotic, anti-diabetic, and hypoglycemic activities (Negahban et al. 2021). It is believed that the collaborative effect of schizophyllan carrier and the drug promotes the efficacy of the treatment. Pirzadeh-Naeen et al. (2020) studied about ellagic acid, a natural antioxidant and anticancer agent used in the treatment of breast cancer. This drug was encapsulated separately in chitin (EA/Ch-NP) and schizophyllan (EA/SPG-NP). The nanoparticle was synthesized, and its size was around an average of 39.82 nm and 217.8 nm, respectively. Before investigating the treatment of breast cancer MCF-7 cells, the ellagic acid release was considered in 95% ethanol and in various pH mediums (range from 1.5 to 7.4) portraying digestive environment. The study revealed that schizophyllan-based nanoparticle showcased enhanced encapsulation efficiency and drug-loading capacity than chitin-based nanoparticle. The release study showed high drug release in 95% ethanol than in other pH mediums. The antioxidant activity determined by DPPH assay showed higher scavenging activity for free ellagic acid than both schizophyllan and chitin drug-loaded nanoparticle. But interestingly the chitin nanoparticle at a pH of 7.4 produced higher antioxidant activity than the free drug. The MTT assay was carried to comprehend the cell viability via mitochondrial dehydrogenase assay. The IC50 value obtained for schizophyllan and chitin were 60 and 115 μg/mL, respectively. Thus, targeting the breast cancer through ellagic acid-schizophyllan-based nanoparticle can be used in a potential cancer therapy (Pirzadeh-Naeeni et al. 2020).

A novel schizophyllan-based micelle system carrying the encapsulated drug paclitaxel (PTX) was developed to deliver highly hydrophobic drug to a target site. Negahban et al. studied the method of producing nano-micelles using schizophyllan and stearic acid for encapsulating hydrophobic drugs in tumor therapy. The produced nano-micelles showed self-assembly into a micelle. An esterification reaction of steric acid with schizophyllan converted it into an amphipathic molecule and they were self-assembled by simple ultrasound method. The synthesized nano-micelle displayed proper size in nm range and provided high PTX loading capacity. This drug carrying nano-micelles revealed high cytotoxicity against cancer cells. The schizophyllan by itself has anti-inflammatory and immune boosting property, along with the anticancer drug can produce synergistic effect in tumor therapy. Thus, the produced nano-micelle has potential as a new drug carrier for hydrophobic drugs (Negahban et al. 2021).

3.10 Curdlan

Curdlan is a linear polysaccharide produced by *Alcaligenes faecalis* and Rhizobium, which consist of β-1, 3 glucans. Curdlan epitope is recognized and internalized by macrophages and dendritic cells. This can activate the secretion of cytokines from

activated macrophages and dendritic cells, which successively help in differentiation of CD^{4+} cells to TH9 cells. This TH9 cells release IL9 and many granzymes when activated and all these will attack tumor cells. Injection of curdlan intratumorally enhances tumor cell necrosis and inhibits its progression by reprogramming the dendritic cells to provoke differentiation of CD^{8+} cells expressing CD103, a ligand for cancer cells. Herein, we report, for the first time, the peptide aptamer functionalization of curdlan and the tumor cell-targeted siRNA delivery by the curdlan derivative (Ganbold et al. 2019).

Natural carbohydrate polymer-based nanoparticles have great biocompatibility that is required for the safe delivery of various drugs including nucleic acid therapeutics. Herein, we designed curdlan-based nanoparticles for cancer cell-targeted delivery of short interfering RNA (siRNA). Ganbold et al. suggested that iRGD functionalized curdlan may provide a biocompatible carrier for siRNA delivery. Curdlan was chemically modified in order to complex with siRNA and specifically enter cancer cells. The first, chemical modification of 6-amination, the second, modification in peptide bearing cationic molecules. The cellular uptake of 6AC-iRGD/siRNA complex was inhibited by chloroquine suggesting that the complex enters the cells through clathrin-dependent endocytosis mechanism. The iRGD-functionalized curdlan was not only taken up by integrin-presenting cells but also carried and released siRNA in the cytoplasm, inducing significant silencing of a disease-related gene Plk1. Collectively, data demonstrated that the curdlan-based nanoparticle may provide a siRNA carrier with the ability of cancer cell-specific delivery (Ganbold et al. 2019).

Mycobacterium tuberculosis is the causative agent for tuberculosis disease, which has the tendency to replicate inside macrophages. The drugs used for its therapy cannot be internalized by the macrophage and due to the sub-therapeutical level of drug inside the immune cell can cause microbial resistance. Macrophage receptor dectin-1 recognizes curdlan and internalizes it. During the drug delivery of rifampicin, polysaccharide is conjugated to poly (D, L-lactide-co-glycolide) nanoparticles to bestow immunostimulatory activity (Gopinath et al. 2018).

A nanoparticle carrying antituberculosis drugs such as rifampicin and levofloxacin was prepared through conjugated cyclodextrin for targeting macrophage. It is a straightforward method for the production of curdlan-based nanoparticles. The drugs encapsulated in cyclodextrin remained inactive after conjugation with curdlan nanoparticles and got transformed into a sustained release of the drugs over a prolonged time. The minimal inhibitory concentration showed as good as free drug release. These nanoparticles affect the macrophages, but not the fibroblast cells because they are noncytotoxic to RAW 264.7 and L929 cells (Fig. 5) (Yunus Basha et al. 2019). The cellular internalization of antituberculosis drugs was 1.8 times higher in macrophages via dectin-1 receptor than the fibroblast cells. They could kill more than 95% of a tuberculosis model organism *Mycobacterium smegmatis* residing inside macrophages within 4 h. Basha et al. concluded that the drug-loaded nanoparticles targeting *Mycobacterium smegmatis*-infected macrophages provide effective bactericidal activity as it delivered the drugs more effectively than free drugs. Thus, curdlan-cyclodextrin conjugated nanoparticles are a potential nano-carrier for targeted drug delivery to macrophages (Yunus Basha et al. 2019).

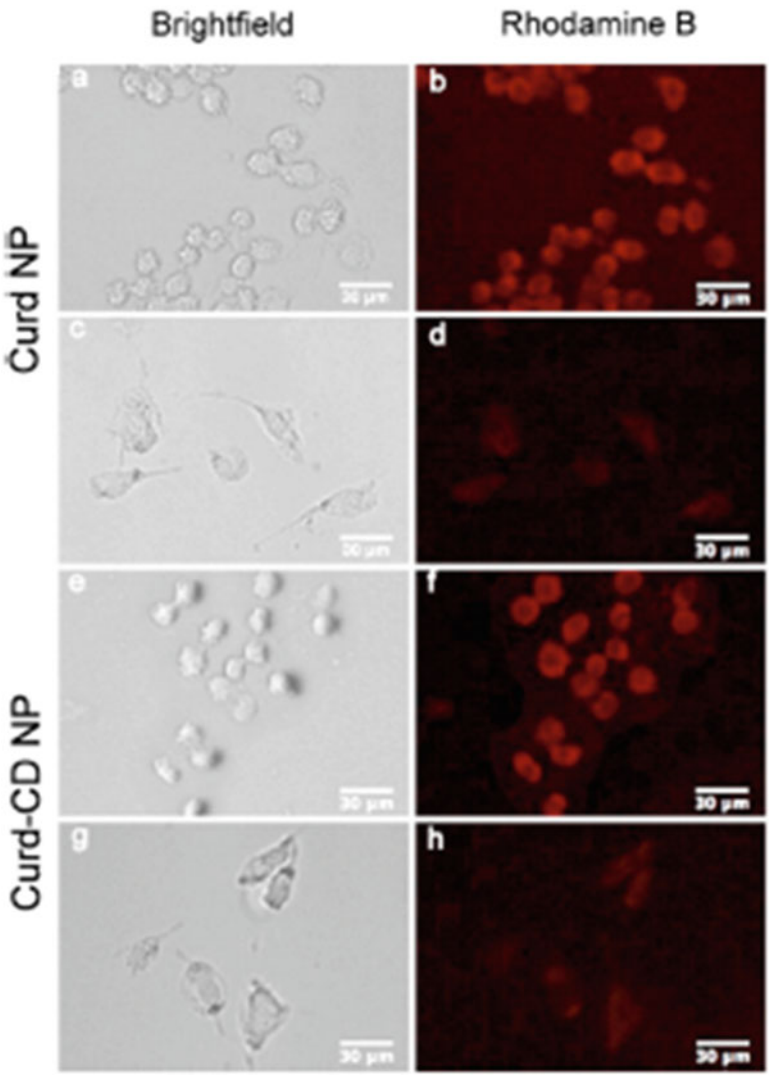


Fig. 5 Microscopic image of RAW264.7 macrophages (a, b, e, and f) and L929 fibroblast (c, d, g, and h) (Yunus Basha et al. 2019)

4 Conclusion

Microbial polysaccharides are a large group of extracellular polymers produced from microbial origin. They have various uses in various fields and currently it is developing into a promising field of research in biomedicine. They contain numerous hydrophilic functional groups in its surface, which can be utilized for preparation

of hydrogels or nanoparticles. The conjugation of this polymer with a hydrophobic fatty acid molecule can create a micelle offering microenvironment for stable transportation of hydrophobic drug. The hydrogels and nanoparticles derived from the wide selection of polysaccharides can help in controlled/sustained release of drug during cancer therapy. This fabrication can not only simply discharge the drug at target site but can also be devised to release the drug under external stimuli. Many parameters such as temperature, pH, electrostatic potential, concentration of a certain molecule, magnetic particles, etc., can act as the external stimuli for releasing the drug molecules from its carriers. Recently, dual stimuli-based nanoparticle/hydrogels were prepared, which could unload the drug when both conditions were met, for example, pH and reducing environment, pH and temperature. Even an electrostatic-based stimulus can be an advantage for the treatment. Recently, in tumor therapy, a combination of diagnostic imaging and drug delivery was performed for simultaneous characterization. The drugs and cells can be made to deliver to the respective sites with the help of microbial polysaccharides.

References

Bai S, et al. Reduction stimuli-responsive unimolecular polymeric prodrug based on amphiphilic dextran-framework for antitumor drug delivery. Carbohydr Polym. 2018;182:235–44.

Chandra PK, Soker S, Atala A. Tissue engineering: current status and future perspectives. In: Principles of tissue engineering; 2020. https://doi.org/10.1016/B978-0-12-818422-6.00004-6.

Constantin M, Bucatariu S, Sacarescu L, Daraba OM, Anghelache M, Fundueanu G. Pullulan derivative with cationic and hydrophobic moieties as an appropriate macromolecule in the synthesis of nanoparticles for drug delivery. Int J Biol Macromol. 2020;164:4487–98.

Dang Y, Guan J. Nanoparticle-based drug delivery systems for cancer therapy. Smart Mater Med. 2020;1(February):10–9.

Dubey S, Mishra R, Roy P, Singh RP. 3-D macro/microporous-nanofibrous bacterial cellulose scaffolds seeded with BMP-2 preconditioned mesenchymal stem cells exhibit remarkable potential for bone tissue engineering. Int J Biol Macromol. 2021;167:934–46.

El Founi M, et al. Light-sensitive dextran-covered PNBA nanoparticles as triggered drug delivery systems: formulation, characteristics and cytotoxicity. J Colloid Interface Sci. 2018;514:289–98.

Ganbold T, Han S, Hasi A, Baigude H. Receptor-mediated delivery of therapeutic RNA by peptide functionalized curdlan nanoparticles. Int J Biol Macromol. 2019;126:633–40.

Giavasis I. Production of microbial polysaccharides for use in food. Woodhead Publishing; 2013. https://doi.org/10.1533/9780857093547.2.413.

Gopinath V, Saravanan S, Al-Maleki AR, Ramesh M, Vadivelu J. A review of natural polysaccharides for drug delivery applications: special focus on cellulose, starch and glycogen. Biomed Pharmacother. 2018;107:96–108.

Joshy KS, et al. Novel core-shell dextran hybrid nanosystem for anti-viral drug delivery. Mater Sci Eng C. 2018;93:864–72.

Karuppusamy S, Hyejin K, Kang HW. Nanoengineered chlorin e6 conjugated with hydrogel for photodynamic therapy on cancer. Colloids Surf B: Biointerfaces. 2019;181(May):778–88.

Lee MW, Tsai HF, Wen SM, Huang CH. Photocrosslinkable gellan gum film as an anti-adhesion barrier. Carbohydr Polym. 2012;90(2):1132–8.

Li L, Bai Z, Levkin PA. Boronate-dextran: an acid-responsive biodegradable polymer for drug delivery. Biomaterials. 2013;34(33):8504–10.

Li Z, Wang LN, Lin X, Shen L, Feng Y. Drug delivery for bioactive polysaccharides to improve their drug-like properties and curative efficacy. Drug Deliv. 2017;24(2):70–80.
Lian H, et al. Core cross-linked poly(ethylene glycol)-graft-dextran nanoparticles for reduction and pH dual responsive intracellular drug delivery. J Colloid Interface Sci. 2017;496:201–10.
Lin B, et al. Multifunctional dextran micelles as drug delivery carriers and magnetic resonance imaging probes. Sci Bull. 2015;60(14):1272–80.
López-Cebral R, et al. Gellan gum based physical hydrogels incorporating highly valuable endogen molecules and associating BMP-2 as bone formation platforms. Carbohydr Polym. 2017;167: 345–55.
Manivasagan P, Kim SK. Extracellular polysaccharides produced by marine bacteria. Adv Food Nutr Res. 2014;72:79–94.
Morris G, Harding S. Polysaccharides, microbial. Encycl Microbiol. 2009:482–94.
Moscovici M. Present and future medical applications of microbial exopolysaccharides. Front Microbiol. 2015;6(SEP):1–11.
Negahban Z, Shojaosadati SA, Hamedi S. A novel self-assembled micelles based on stearic acid modified schizophyllan for efficient delivery of paclitaxel. Colloids Surf B: Biointerfaces. 2021;199:111524.
Ngwabebhoh FA, Zandraa O, Patwa R, Saha N, Capáková Z, Saha P. Self-crosslinked chitosan/dialdehyde xanthan gum blended hypromellose hydrogel for the controlled delivery of ampicillin, minocycline and rifampicin. Int J Biol Macromol. 2021;167(December):1468–78.
Pacelli S, et al. Dextran-polyethylene glycol cryogels as spongy scaffolds for drug delivery. Int J Biol Macromol. 2021;166:1292–300.
Pandey S, et al. Natural gum modified emulsion gel as single carrier for the oral delivery of probiotic-drug combination. Int J Biol Macromol. 2016;92:504–14.
Pirzadeh-Naeeni S, Mozdianfard MR, Shojaosadati SA, Khorasani AC, Saleh T. A comparative study on schizophyllan and chitin nanoparticles for ellagic acid delivery in treating breast cancer. Int J Biol Macromol. 2020;144:380–8.
Psimadas D, Georgoulias P, Valotassiou V, Loudos G. Molecular nanomedicine towards cancer. J Pharm Sci. 2012;101(7):2271–80.
Qi X, et al. Design of Salecan-containing semi-IPN hydrogel for amoxicillin delivery. Mater Sci Eng C. 2017;75:487–94.
Qi X, et al. Sustainable, flexible and biocompatible hydrogels derived from microbial polysaccharides with tailorable structures for tissue engineering. Carbohydr Polym. 2020;237 (March):116160.
Qin ZY, Jia XW, Liu Q, Kong BH, Wang H. Fast dissolving oral films for drug delivery prepared from chitosan/pullulan electrospinning nanofibers. Int J Biol Macromol. 2019;137:224–31.
Qiu L, et al. Elucidation of cellular uptake and intracellular trafficking of heparosan polysaccharide-based micelles in various cancer cells. Int J Biol Macromol. 2019;130:755–64.
Qu J, Liang Y, Shi M, Guo B, Gao Y, Yin Z. Biocompatible conductive hydrogels based on dextran and aniline trimer as electro-responsive drug delivery system for localized drug release. Int J Biol Macromol. 2019;140:255–64.
Ren L, Zhang J, Zhang T. Immunomodulatory activities of polysaccharides from Ganoderma on immune effector cells. Food Chem. 2021;340:127933.
Richa R, Choudhury AR. Exploration of polysaccharide based nanoemulsions for stabilization and entrapment of curcumin. Int J Biol Macromol. 2019;156:1287–96.
Shende P, Basarkar V. Recent trends and advances in microbe-based drug delivery systems. DARU J Pharm Sci. 2019;27(2):799–809.
Su T, Zhao W, Wu L, Dong W, Qi X. Facile fabrication of functional hydrogels consisting of pullulan and polydopamine fibers for drug delivery. Int J Biol Macromol. 2020;163:366–74.
Tiryaki E, Başaran Elalmış Y, Karakuzu İkizler B, Yücel S. Novel organic/inorganic hybrid nanoparticles as enzyme-triggered drug delivery systems: dextran and dextran aldehyde coated silica aerogels. J Drug Deliv Sci Technol. 2020;56:101517.

Unnikrishnan BS, et al. Self-assembled drug loaded glycosyl-protein metal nanoconstruct: detailed synthetic procedure and therapeutic effect in solid tumor treatment. Colloids Surf B: Biointerfaces. 2020;193(April):111082.

Vashisth P, et al. Drug functionalized microbial polysaccharide based nanofibers as transdermal substitute. Nanomed Nanotechnol Biol Med. 2016;12(5):1375–85.

Yao X, Chen L, Chen X, He C, Zheng H, Chen X. Intercellular pH-responsive histidine modified dextran-g-cholesterol micelle for anticancer drug delivery. Colloids Surf B: Biointerfaces. 2014;121:36–43.

Yunus Basha R, Sampath SK, Doble M. Dual delivery of tuberculosis drugs via cyclodextrin conjugated curdlan nanoparticles to infected macrophages. Carbohydr Polym. 2019;218 (January):53–62.

Zhang R, et al. Polysaccharide from flammuliana velutipes improves colitis via regulation of colonic microbial dysbiosis and inflammatory responses. Int J Biol Macromol. 2020;149: 1252–61.

Injectable Polymeric System Based on Polysaccharides for Therapy

44

Guy Decante, Joaquim Miguel Oliveira, Rui L. Reis, and Joana Silva-Correia

Contents

Abstract

Microbial polysaccharides are inexpensive natural polymers synthesized by various microorganisms such as bacteria, fungi, and microalgae. They have been generally used in the food industry as emulsifier, gelling agents, and thickeners. Nowadays, their favorable biological properties, similarity to native ECM, and wide range of molecular weights, and functional groups have sparked the interest of researchers for their use as injectable scaffolds used in drug delivery, tissue engineering, and regenerative medicine. Notable applications of microbial

G. Decante · J. M. Oliveira · R. L. Reis
3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Zona Industrial da Gandra, Barco, GMR, Portugal

ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães, Portugal
e-mail: guy.decante@i3bs.uminho.pt; miguel.oliveira@i3bs.uminho.pt; rgreis@i3bs.uminho.pt

J. Silva-Correia (✉)
3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Zona Industrial da Gandra, Barco, GMR, Portugal
e-mail: joana.correia@i3bs.uminho.pt

J. M. Oliveira et al. (eds.), *Polysaccharides of Microbial Origin*,
https://doi.org/10.1007/978-3-030-42215-8_55

polysaccharides are vaccine preparations and intra-articular injections of hydrogels in osteoarthritis therapy. These systems provide minimally invasive routes of implantation in vivo to locally deliver drugs, cells, and other therapeutics in a controlled manner. They may also provide a stable environment to support cell differentiation and migration to enhance tissue regeneration. The various uses of microbial polysaccharides and their derivatives as injectable scaffolds used in therapy are reviewed herein.

Keywords

Hydrogels · Polysaccharides · Bacteria · Drug delivery · Tissue engineering

1 Introduction

Polysaccharides extracted from microorganisms have generated a vivid interest in the last three decades due to their wide range of structural and functional properties, and generally low costs. As natural-based carbohydrate macromolecules formed by repeated monosaccharide units linked by glycosidic bonds, they are intrinsically biocompatible and biodegradable, and possess functional groups that allow for their easy chemical modification. Additionally, polysaccharides have similar structures to those of the glycans which constitute the native extracellular matrix (ECM). Monosaccharide units can also mediate cellular processes at molecular level thanks to their biological role in cell signaling. These substances have been used in numerous fields, including the food industry, and in pharmaceutical research. As an example, microbial polysaccharides have been extensively used to form the integral component of many vaccines (Lee et al. 2001b; Jennings and Pon 2010; Patel and Patel 2011). Microbial polysaccharides are classified according to their morphological localization: Cell wall, intercellular and exocellular polysaccharides. Their morphological localization will influence their properties and subsequent applications. Cell wall polysaccharides, such as chitin, mainly contribute to the structural stability of cells. Intracellular polysaccharides are concentrated to the cytoplasm of cells and act as carbon and energy reserves. Finally, exocellular polysaccharides are secreted by cells and can be subdivided into capsular polysaccharides and exopolysaccharides. Capsular polysaccharides are defined as polymers covalently bound to the cells surface, producing capsules around the cells that act as physical barriers against bacteriophages. Exopolysaccharides produce a slime loosely bond to the surface of cells, allowing cells to adhere to other surfaces, while acting as carbon or water reserves (Patel and Patel 2011; Freitas et al. 2021). Fig. 1 shows the basic anatomy of bacteria and where each of these polysaccharides are located within.

Microbial polysaccharides gained prominence in biomedical applications thanks to their properties such as their capability to form intermolecular

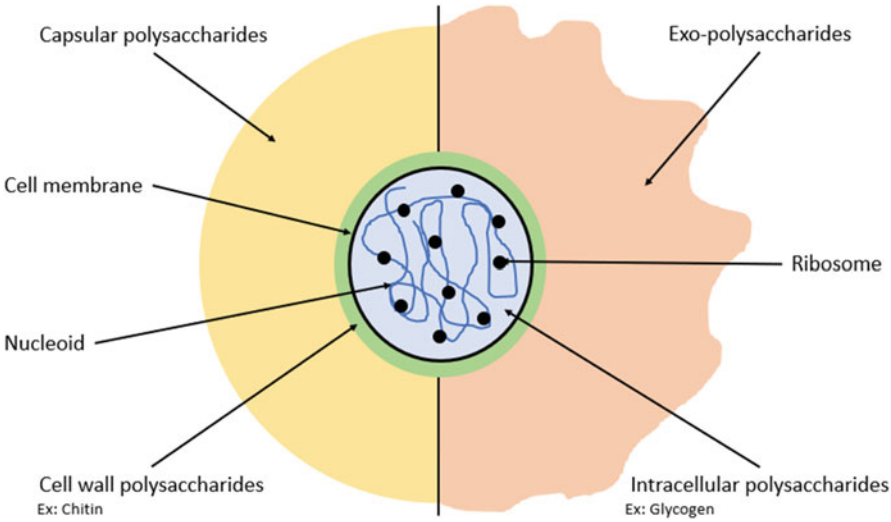


Fig. 1 Basic anatomy of bacteria. Bacteria may secrete capsular polysaccharides or exo-polysaccharides as external layers

interactions to create polymeric matrices, which can easily be loaded with inorganic materials to allow for drug encapsulation, or to create composite hydrogels with enhanced properties (Freitas et al. 2021). Herein, the important advances dealing with injectable polymeric materials based on polysaccharides, and their applications in pharmaceutic and medical fields are overviewed.

2 Injectable Polymeric Systems

Injectable polymeric systems cover a broad range of materials which can be inserted into tissues using percutaneous procedures for various medical applications. These systems, which including injectable hydrogels, porous hydrogels, and micelles, are implanted in the body through minimally invasive procedures to create structures that will fill complex tissue defects, and/or deliver drugs, cells and/or other therapeutics in a sustained manner (Fig. 2). These injectable polymeric systems are extensively used in tissue engineering, regenerative medicine, and in drug and cell delivery. Although these systems have similar polymeric structures, their properties and characteristics differ, allowing for their use in different applications.

An injectable hydrogel is a polymeric structure which can be extruded as a solution directly into the desired location in vivo, where it will polymerize to

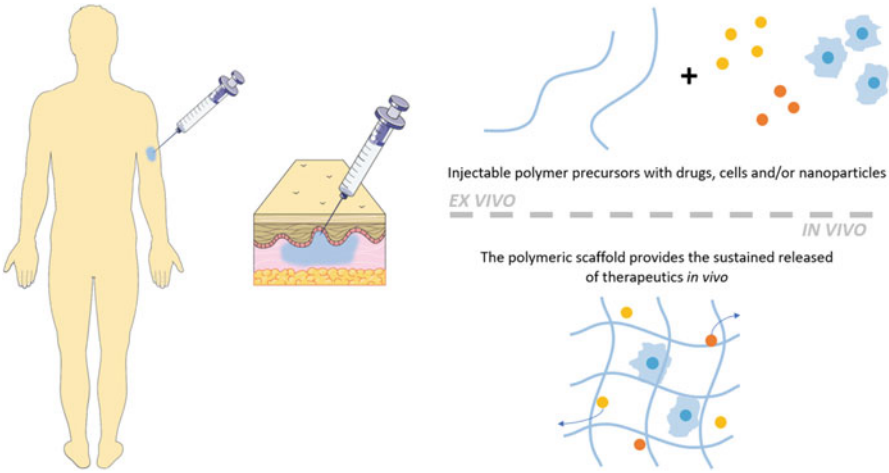


Fig. 2 Principle of injectable polymeric systems. Polymer precursors loaded with cells, drugs, nanoparticles and/or other therapeutics are injected into the patient's body. The polymer precursor will polymerize in situ through different chemical and/or physical processes to create polymeric structures that will fill tissue defects and/or deliver therapeutics. This figure was created using images from Servier Medical Art Commons Attribution 3.0 Unported License (https://smart.servier.com/). Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License (https://creativecommons.org/licenses/by/3.0/)

transition from a solution to a gel. As it is well known, hydrogels have high water contents which grant them with good biocompatibility and low cytotoxicity, making them ideal to reproduce the ECM in applications such as tissue engineering, regenerative medicine, or drug and cell delivery. Such hydrogels meet great success as carrier matrices for biologics, thanks to their ability to provide a controlled release at targeted areas. Compared to polymeric scaffolds produced ex vivo and subsequently implanted, injectable hydrogels can alleviate the need for surgical interventions, which reduces the patient discomfort, risk of infection, recovery time while offering better aesthetic outcomes. They also have better handling properties and a better adaptability to the irregular shapes of the defects. Hydrogels possess a degree of high customizability which allows them to be tailored to better suit their intended applications. Controlling the viscosity of such systems for example, is critical to ensure that they can be injected into the desired area without clogging the injection device, damaging their potential loaded biologics, or leaking into surrounding tissues. These systems possess self-assembling or stimuli-responsive gelation mechanisms for their networks to polymerize in situ, resulting in stronger and more stable hydrogels (Yang et al. 2014). These chemistries must be carefully selected to not display any toxicity during polymerization. Photo-crosslinking for example, is a widely used crosslinking methodology for in situ polymerization. These chemistries rely on photo-

crosslinkers which may present toxicity issues if they are left unreacted (Yang et al. 2014). In tissue engineering and regenerative medicine for example, it is essential that the scaffolds produced in situ offer a stable environment for cells to grow, differentiate and proliferate. For biomedical applications, these crosslinking mechanisms must occur under mild conditions, and not generate damages to surrounding tissues or the possible biologics embedded within them.

Porous hydrogels cover a broad variety of materials used in biomedical applications, especially in drug and cell delivery. Also referred to as micro/nanogels, or granular hydrogels, these materials are defined as hydrogels produced from micro- to nano-sized particles of polymeric networks. Regular hydrogels consist of polymeric materials crosslinked in swollen meshes, which mesh size range from the tens to hundreds of nanometers. Because the size of most cells ranges in micrometers, regular hydrogels must be degraded before cellular infiltration can occur. Due to their particulate structures, porous hydrogels possess intrinsic porosities, relative to their particle size, which improve the diffusion of loaded cells and drugs. These particles can be dispersed in solvent or larger polymeric networks, and possess tunable sizes, geometries, properties, and characteristics. The literature on these systems is extremely diverse, and there are already many excellent articles that thoroughly cover this subject such as the review from Chiriac et al. (Chiriac et al. 2019). Summarily, due to their particulate structures, these gels also possess very large surface-to-volume ratios that allow for high loading of therapeutics and multivalent bioconjugation, high-exchange rate, and a fast response to environmental cues. Their characteristics such as their sizes and degradation mechanisms can be tuned, allowing for the controlled delivery of loaded therapeutics at targeted sites. Additionally, micro- and nano-gels possess attractive properties for localized surgical injections, such as large viscosities, shear-thinning behavior, and yield stresses (Riederer et al. 2016). Moreover, these systems meet great success for drug and cell delivery applications thanks to their ability to protect their loaded biologics from host immune response, degradation, and shear stress, while also being able to contain them at the implantation sites. However, nanogels are generally expensive to produce, and limited at experimental production, as they are currently produced using organic solvents and additives which may remain in the medium and cause toxicity issues. Non-harsh reagents should be developed to offer safer processing routes (Wang et al. 2017).

Micelles are self-assembled colloidal nanostructures composed of a hydrophilic shell surrounding a hydrophobic core (Torchilin 2007). They are widely used as carriers for delivering drugs with poor water solubilities. Thanks to their small size and hydrophilic shells, micelles remain undetected by macrophages and the reticuloendothelial system, which enhances the circulation time of their encapsulated drug. Moreover, encapsulating these drugs in hydrophilic micelles can allow increasing their bioavailability, which lower the doses required and potential side effects (Amin et al. 2017). Micelles possess tunable sizes which allows to direct the loaded biologics to tissues where permeability is enhanced, particularly tumor and

inflammatory tissue. Furthermore, micelles can be functionalized to recognize specific molecular signals associated with diseased sites, which allows for the targeted delivery of their encapsulated drugs (Xiong et al. 2011). Micelles are extensively used in drug delivery as they enable to entrap and deliver hydrophobic drugs and proteins thanks to enhanced solubility and stability. For drug delivery applications, micelles should have high stability, high biocompatibility, biodegradations properties and low immunogenicity (Zhang et al. 2013). Microbial polysaccharides then appear as good materials to develop micelles since they possess such properties. Microbial polysaccharides can also possess natural positive, negative, or neutral charges, or they may be modified to.

Despite the great diversity of polymers and their properties, modifications are often required for hydrogels to have better suiting characteristics for their applications. Microbial polysaccharides are no exception to that matter. Many applications of microbial polysaccharides imply their modification by direct immobilization of molecular functional groups, or their grafting with other polymers (Bacelar et al. 2016; Hu et al. 2017; Kirschning et al. 2018). In addition, hydrogels based on microbial polysaccharides can easily be loaded with nanoparticles, microspheres, etc. to enhance their mechanical and/or biological properties (Ng et al. 2020).

3 Applications of Injectable Materials Based on Microbial Polysaccharides

Microbial polysaccharides can create polymeric structures with a wide range of properties depending on their types, the species they have been extracted from, their possible modifications, the materials they are combined with, etc. All microbial polysaccharides possess natural biocompatibilities, low toxicities and the capacity to gel under mild, physiologically relevant, conditions (Velasco et al. 2012) which make them attractive to create injectable in situ gelling hydrogels to reproduce the ECM. They may also possess immunoregulatory, anticancer, antiviral, anticoagulant, antithrombotic, and many other properties (Filomena et al. n.d.; Matsuda et al. 1999; Patel and Patel 2011; Moscovici 2015; Yu et al. 2018; Carvalhal et al. 2019; Khan et al. 2019; Ng et al. 2020; Zhou et al. 2020; Freitas et al. 2021), making them very interesting for various applications in tissue engineering, regenerative medicine, and controlled local delivery of drugs, genes, cells, or other therapeutics.

3.1 Drug and Cell Delivery

Microbial polysaccharides have met great success in delivering therapeutics in vivo through injectable polymeric systems (Zhang et al. 2013; Huh et al. 2017; Suner et al. 2018; Cadinoiu et al. 2019; Kumari and Badwaik 2019). These systems represent a substantial share of the global pharmaceutical market, only preceded by oral medication. They offer the possibility to rapidly target specific areas by minimally invasive routes such as intravenous, subcutaneous, intradermal,

intramuscular, intracutaneous, intraperitoneal, intrathecal, intraarterial, intraspinal, and intracardiac. Generally, these delivery systems are preferred if drugs have short half-lives and/or poor absorption by conventional routes of administration (Cadinoiu et al. 2019). Additionally, using injectable hydrogels for delivery allows to bypass first-pass metabolism, which mainly occurs with the oral administration of drugs which then enter the portal circulation before entering the systemic circulation, resulting in rapid decreases in their concentrations (Mathew et al. 2018). Microbial polysaccharides are attractive materials for delivery systems thanks to their multiple functional groups which allow for their bioconjugation with various therapeutic agents, and the wide range of their molecular weights which allows to tune the biodegradation kinetics and the therapeutic release of subsequent hydrogels (Thambi et al. 2016). Thanks to their carbohydrate structures, hydrogels produced from microbial polysaccharides can undergo total degradation within the body, avoiding additional surgeries to remove residual material (Mathew et al. 2018). Moreover, microbial polysaccharides possess attractive immunoregulatory properties for delivery applications. For instance, microbial polysaccharides have been extensively used as antigens for vaccine preparations (Lee et al. 2001a). However, they can only induce the production of low-affinity antibodies by themselves, therefore immunization with bacterial polysaccharides only leads to T cell-independent immune responses (Hütter and Lepenies 2015). Bacterial polysaccharides are often conjugated with proteins to promote long-term antibodies and memory response (Robbins et al. 1983; Finn 2004). Many currently commercialized vaccines are based on conjugated capsular polysaccharides and have proved to be very efficient in preventing a large range of human diseases (Mettu et al. 2020).

In situ gelling hydrogels are attractive systems for delivery applications. Therapeutic agents can be mixed with the precursor polymer solutions before injection, resulting in their entrapment within the hydrogel networks which can provide their sustained release at the target sites. Local and controlled delivery of therapeutics is associated with lowered dosing frequencies and side effects (Singh and Hari Kumar 2012). However, since polymer networks may act as diffusion barriers for therapeutics, particulate structures with intrinsic porosities are preferable to allow for their diffusion through the interior of polymeric matrices. Nanogels, microgels, and micelles based on microbial polysaccharides have been extensively studied as delivery vehicles (Zhang et al. 2013; Suner et al. 2018; Chiriac et al. 2019; Kumari and Badwaik 2019). Numerous currently available vaccines systems are based on chitosan micro- and nano-particles, for example, and can increase the efficacy and response to immunization with model protein antigens, viral antigens, bacterial-derived antigens and toxins, and DNA plasmids (Chua et al. 2012). Such structures possess attractive advantages for drug delivery. Their small scale allows for the penetration of tissues by paracellular or transcellular pathways, and their fast environmental responses allow for a highly controlled release of their loaded therapeutics (Suner et al. 2018). As aforementioned, microbial polysaccharides are also particularly attractive to develop micelles used in drug delivery thanks to their natural properties and ease of modification. Micelles can be tailored to have adequate size, charge, and shape for delivery, but they can also be further modified with peptides,

molecules or other polysaccharides to improve site accumulation and uptake (Zhang et al. 2013). They possess a number of benefits over other drug delivery carriers, in particular: (1) they allow to encapsulate and deliver hydrophobic drugs, which allows to reduce the required doses and therefore minimizes the side effects; (2) their small size and hydrophilic shells render them invisible to macrophages and the reticuloendothelial system, which increases the circulation times of their embedded therapeutics; (3) micelles can take advantage of the enhanced permeability and retention effect to deliver drugs through passive targeting; and (4) micelles are also easy and rapid to produce, allowing them to be readily prepared in large quantities owing.

However, micelles are also generally unstable due to their dilution by body fluids, and require modification to alleviate this issue (Amin et al. 2017). Moreover, due to their size, micro-, nanogels and micelles have short diffusional paths which results in a rapid release of their encapsulated therapeutic compared to larger carriers (Chiriac et al. 2019). Furthermore, nanoscale materials are often subjected to fast clearance by body defense mechanisms due to their size.

Injectable hydrogels have also met great success in gene delivery thanks to their ability to prevent genes from aggregation and extracellular degradation, which allows for their long-term release in a controlled and sustained manner (Mathew et al. 2018). Chitosan for example, has been used as a non-viral vector for gene delivery thanks to its natural cationic charges able to make complexes with negatively charged DNA, siRNA and oligonucleotides (Riederer et al. 2016; Huh et al. 2017). Despite their numerous functional groups, polysaccharides generally require modification to increase their affinity with cells, genes, proteins, etc. Resulting nanoparticles have demonstrated improved delivery capacities by effectively targeting the implantation sites, while protecting the different genes from degradation, and with efficient gene transfection (Huh et al. 2017).

Microbial polysaccharides can be used alone or combined with other materials to better tailor the subsequent hydrogels to the intended applications (Alves et al. 2016; Ng et al. 2020). Especially, thanks to their multiple functional groups, microbial polysaccharides can be modified or supplemented with other polymers to create "smart" or "stimuli-responsive" hydrogels (Thambi et al. 2016). These hydrogels are designed to react various stimuli such as temperature, pH, ionic concentration, enzymes, and magnetic or electric field, which can be externally applied or specific to the targeted site. They will then respond to these stimuli with structural changes such as shape morphing, or the modification of their properties like their solubilities and sol-gel transitions. Such types of changes can be reversible or irreversible. Stimuli-responsive hydrogels are very attractive to create delivery vehicles capable of providing a controlled release of therapeutics in vivo which may lead to considerable advantages over traditional medications such as greater drug efficiency, rapid drug absorption, reduced side effects (Bhardwaj et al. 2015). Although they may be based on similar materials, in situ gelling hydrogels, micro- and nanogels, and micelles have their strengths and weaknesses that researchers must consider when developing a novel injectable system to deliver drugs, cells or other therapeutics. Table 1 summarizes these pros & cons of injectable polymeric structures for applications in drug and cell delivery.

Table 1 Strengths and weaknesses of injectable polymeric systems for drug and cell delivery applications (Torchilin, 2007; Xiong et al. 2011; Chua et al. 2012; Zhang, Wardwell and Bader 2013; Yang et al. 2014; Riederer et al. 2016; Amin et al. 2017; Wang, Qian and Ding 2017; Chen et al. 2017b; Mathew et al. 2018; Suner et al. 2018; Chiriac et al. 2019; Kumari and Badwaik 2019; Ng et al. 2020; Wang et al. 2020)

Injectable polymeric system	Description	Advantages	Disadvantages	References
In situ gelling hydrogels	Polymer precursors are dispersed in a solution which is injected into the body and undergoes a sol-gel transition in situ	Ease of modification: Can be modified with other materials, and/or loaded with particles. Control over crosslinking also provides tunable mechanical and drug release properties	The nanosized mesh of the polymeric network acts as a diffusion barrier to larger cells and therapeutics, unreacted crosslinkers are often toxic	Yang et al. (2014), Chen et al. (2017b), Mathew et al. (2018), Ng et al. 2020 and Wang et al. (2020))
Microgels	Microscale particles of polymeric networks	High loading capacities, they can protect encapsulated biologics from external factors, possess attractive properties for localized surgical injections, such as large viscosities, shear-thinning behavior, and yield stresses, they also have fast environment response, easy functionalization, active and passive targeting abilities, intrinsic microscale porosity to allow for the diffusion of cells	Micro-sized carriers have short diffusional paths which result in rapid release of the loaded cargo	Chua et al. (2012), Riederer et al. (2016), Wang et al. (2017), Chen et al. (2017b), Suner et al. (2018), Chiriac et al. (2019) and Wang et al. (2020)).
Nanogels	Nanoscale particles of polymeric networks	High loading capacities, they can protect encapsulated biologics from external factors, possess attractive	Their porosity is too fine for cells to diffuse, nanosized materials often present a fast clearance due to	Chua et al. (2012), Riederer et al. (2016), Wang et al. (2017), Suner et al. (2018), Chiriac et al.

(continued)

Table 1 (continued)

Injectable polymeric system	Description	Advantages	Disadvantages	References
		properties for localized surgical injections, such as large viscosities, shear-thinning behavior, and yield stresses, they also have fast environment response, easy functionalization, active and passive targeting abilities, intrinsic porosity which allows for the diffusion of oxygen and nutrients. Their nanoscale allows them to penetrate tissues by paracellular or transcellular pathways	body defense mechanisms, nanosized carriers also have short diffusional paths which results in a rapid release of the loaded cargo. Nanogels are expensive to produce, and their production methods generally require organic solvents and additives which may remain in the medium	(2019) and Kumari and Badwaik (2019)
Micelles	Hydrophilic polymer aggregates with a hydrophobic core	Can encapsulate and deliver hydrophobic drugs, easy functionalization, active and passive targeting abilities. Their nanoscale allows them to penetrate tissues by paracellular or transcellular pathways	Nanosized materials often present a fast clearance due to body defense mechanisms, nanosized carriers also have short diffusional paths which results in a rapid release of the loaded cargo, subject to dilution by body fluids	Torchilin (2007), Xiong et al. (2011), Zhang et al. (2013), Amin et al. (2017), Chiriac et al. (2019) and Kumari and Badwaik (2019)

3.2 Tissue Engineering and Regenerative Medicine

Hydrogels produced from microbial polysaccharides possess numerous attractive properties to be used for injectable systems in tissue engineering. Hydrogels are biocompatible and can crosslink in situ through mild processes to create soft structures that resemble that of natural ECM. These structures are permeable to allow for the diffusion of nutrients and the clearance of waste products, their

mechanical properties can be tailored to match those of targeted tissues, and they are able encapsulate bioactive molecules and/or cells during injection while providing the latter with suitable environments for their proliferation and differentiation. However, microbial polysaccharides generally display limited performance in these aspects, therefore they are often modified and/or supplemented with other materials to better comply to the requirements of tissue engineering (Ng et al. 2020). Injectable nanocomposites made from microbial polysaccharides and mineral nanoparticles are an effective way to create biomimetic polymeric scaffolds with suitable mechanical properties and bioactivity. Bones for example, are nanocomposite materials consisting of minerals (60%), organic materials (30%) and cellular components (10%) (Tran et al. 2011). The inorganic components of bones provide them with the required strength to withstand their dynamic environment while still being able to maintain cellular function. It also provides them with cellular binding sites for integrin and/or growth factors, which promotes osteoconductivity, osteoinductivity, and osseointegration that polysaccharides generally lack (Tran et al. 2011; Ng et al. 2020).

So far, injectable hydrogels based on polysaccharides have been used for numerous applications of tissue engineering including wound healing (Li et al. 2015), soft tissue augmentation (Silva et al. 2015), bone defects repair (Jung et al. 2018), and cartilage regeneration through intra-articular injections to treat osteoarthritis (Chen et al. 2017a). Osteoarthritis is a degenerative joint disease characterized by the wear and tear of joint cartilage, leading to their erosion and the inflammation of underlying bone. This disease can affect any joint and results in pain and stiffness. Intra-articular injections of hydrogels (or viscosupplementation) are considered effective treatments against osteoarthritis. A hydrogel is injected between the cartilages to relieve pain, improve joint lubrication, and delay further cartilage damage (Fig. 3). Microbial polysaccharides met great success in this latter application thanks to their compositions and structures which resemble to that of chondrocyte ECM, providing a biomimetic environment to maintain chondrocytes function, and which degradation can provide elements required for new tissue synthesis (Chen et al. 2017a). Moreover, some polysaccharides present high rheological and water-retention characteristics, improving joint lubrication and protecting the cartilage from mechanical degradation (Elmorsy et al. 2014). Hyaluronic acid (HA) is a natural component of synovial fluid and has been considered as an effective treatment for osteoarthritis. However, HA is unstable and prone to degradation by hydrolytic or enzymatic reactions in vivo (Zhong et al. 1994), leading to its short-term efficacy (<6 months) and the need of repeated injections (Altman et al. 2018). Other polysaccharides have recently been studied as longer-lasting alternatives. Local injections of xanthan gum for example, have been demonstrated to relieved pain induced by osteoarthritis, while being able to protect articular cartilage and reduce its degradation and death in a rabbit osteoarthritis model (Li et al. 2019).

The performance of scaffolds for tissue engineering is directly linked to their ability to provide a stable environment for cells to attach, grow, differentiate and proliferate. In situ gelling hydrogels have limited performance in tissue engineering and regenerative medicine due to their nanoscale networks which may act as

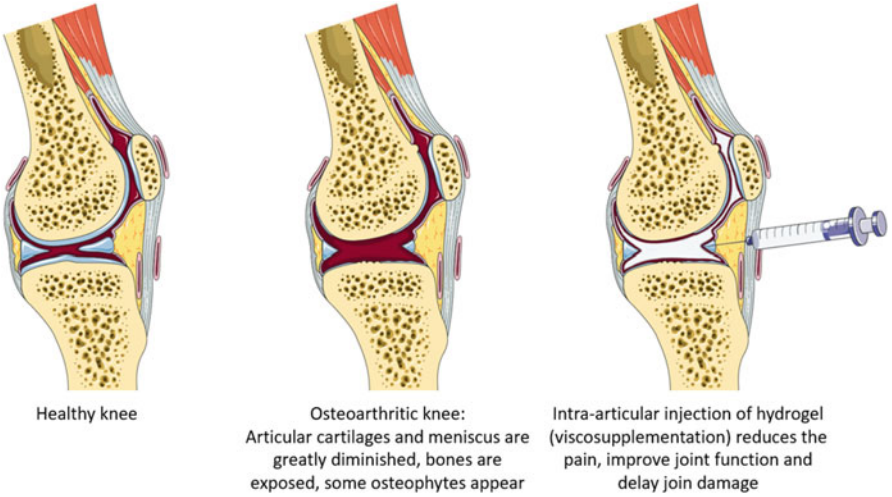


Fig. 3 Intra-articular injection of hydrogel in osteoarthritic knee (also known as viscosupplementation). Hyaluronic acid is usually used in these procedures as it is a natural component of synovial fluid, but other natural polymers are currently being studied, such as xanthan gum. This figure was created using images from Servier Medical Art Commons Attribution 3.0 Unported License (https://smart.servier.com/). Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License (https://creativecommons.org/licenses/by/3.0/)

diffusion barriers to micro-sized cells, leading to reduced ECM formation and secretion throughout the engineered tissues. Ideal scaffold architectures used in tissue engineering and regenerative medicine should have porosities higher than 90% with pore sizes greater than 300 μm while being able to maintain structural viability (Tran et al. 2011). Injectable microgels and their intrinsic microscale porosities recently drawn a lot of interest because of their better cell seeding capacities and ability to provide adequate environments for cells to develop and migrate, resulting in better outcomes than regular nonporous injectable hydrogels (Chen et al. 2017b). Additionally, microgels can allow to encapsulate and protect cells to deliver them in vivo. Similarly, nanogels are able to entrap macromolecules, and prevent proteins and enzymes from denaturing or aggregating. The use of injectable nanogels for tissue engineering applications is still very recent and limited, but injectable microgels based on modified polysaccharides have been used for many applications in soft and hard tissue engineering so far. However, polysaccharide microparticles generally require inadequate processing methods to be used for injectable applications (Chen et al. 2017b). Recently, Wang et al. developed a novel approach to create injectable microporous hydrogels by creating an in situ bubble forming hydrogel based on HA (Wang et al. 2020). Their hydrogels achieved a combination of injectability and pore formation. This porous structure was achieved without lyophilization and pore size could be modified according the molecular weight and concentration of HA (Fig. 4). These hydrogels showed adequate biocompatibility to direct cell behaviors and mild host response, and they are believed

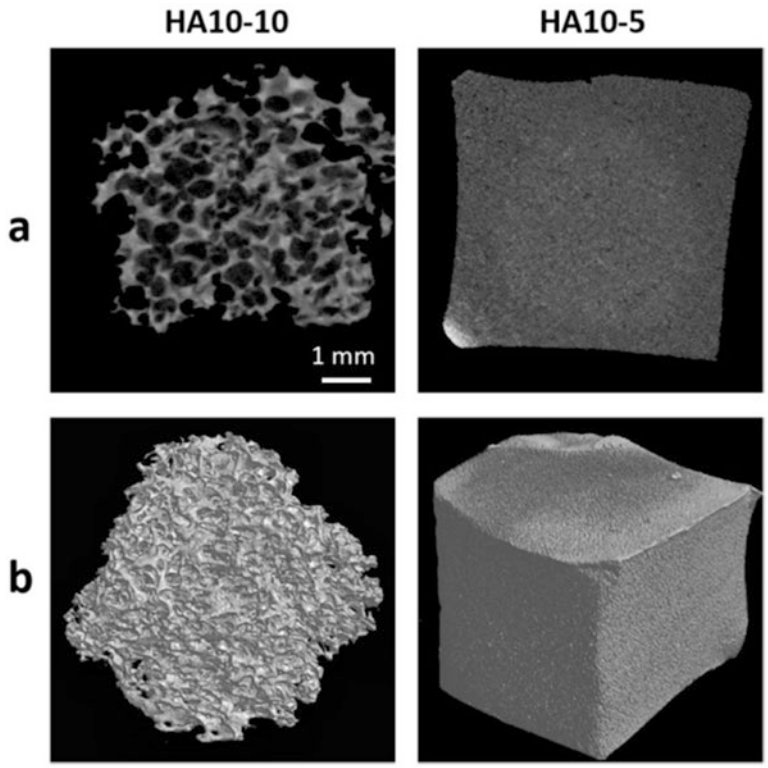


Fig. 4 (**a**) 2D micro-CT images of the HA hydrogels; (**b**) 3D micro-CT images of the HA hydrogels. (scale bar: 1 mm). The HA10–10 hydrogel was produced from HA with a molecular weight of 100,000 Da at a concentration of 10 wt.%, and the HA10–5 hydrogel was produced from HA with a molecular weight of 100,000 Da at a concentration of 5 wt.%. (Reproduced from Wang et al. (2020) with permission of MDPI, Copyright © 2020, licensed under CC BY 4.0 (http://creativecommons.org/licenses/by/4.0/))

to provide a novel versatile approach for injectable systems used in tissue engineering and regenerative medicine. As for drug and cell delivery applications, these polymeric structures have their own strengths and weaknesses that must be considered when developing an injectable system for tissue engineering and regenerative medicine (Table 2).

4 Conclusion and Outlook

Hydrogels based on natural hydrogels and their derivatives have been widely used for biomedical applications thanks to their general biocompatibility and safety of implantation into the body. Microbial polysaccharides, which have been extensively used in the food industry, sparked vivid interest for their use in biomedical

Table 2 Strengths and weaknesses of injectable polymeric systems for tissue engineering and regenerative medicine applications (Tran et al. 2011; Chua et al. 2012; Velasco, Tumarkin and Kumacheva 2012; Yang et al. 2014; Li et al. 2015; Riederer et al. 2016; Wang, Qian and Ding 2017; Chen et al. 2017b; Jung et al. 2018; Mathew et al. 2018; Suner et al. 2018; Chiriac et al. 2019; Kumari and Badwaik 2019; Wang et al. 2020; Ng et al. 2020)

Injectable polymeric system	Description	Advantages	Disadvantages	References
In situ gelling hydrogels	Polymer precursors are dispersed in a solution which is injected into the body and undergoes a sol-gel transition in situ	Ease of modification: Can be modified with other materials, and/or loaded with particles. Control over crosslinking also provides control over mechanical properties	The nanosized mesh of the polymeric network acts as a diffusion barrier to larger cells, composites are often required to provide god mechanical and/or biological properties. Unreacted crosslinkers are often toxic	Tran et al. (2011), Yang et al. (2014), Li et al. (2015), Jung et al. (2018), Mathew et al. (2018), Ng et al. (2020) and Wang et al. (2020)
Microgels	Microscale particles of polymeric networks	High cell loading capacities, they can protect encapsulated biologics from external factors, their intrinsic microscale porosity allows for the diffusion of cells and nutrients	Their production methods are often inadequate for injectable applications	Chua et al. (2012), Velasco et al. (2012), Riederer et al. (2016), Chen et al. (2017b), Suner et al. 2018, Chiriac et al. (2019) and Wang et al. (2020).
Nanogels	Nanoscale particles of polymeric networks	High loading capacities, they can protect encapsulated biologics from external factors, their intrinsic porosity allows for the diffusion of oxygen and nutrients. Their nanoscale allows for penetration of the tissues by paracellular or transcellular pathways	The porosity is too fine for cells to diffuse, nanosized materials often present a fast clearance due to body defense mechanisms. Nanogels are expensive to produce, and their production methods generally require organic solvents and additives which may remain in the medium	Chua et al. (2012), Riederer et al. (2016), Wang et al. (2017), Suner et al. (2018), Chiriac et al. (2019) and Kumari and Badwaik (2019)

applications. Hydrogels based on microbial polysaccharides generally possess a wide range of attractive factors such as favorable biological properties, structural similarity to natural ECM, a wide range of molecular weights, and degradability. Additionally, some microbial polysaccharides may possess specific immunoregulatory, anticancer, antiviral, anticoagulant, or antithrombotic properties that make them particularly interesting in numerous applications such as vaccine preparation, and cancer therapy. Researchers have taken benefit from all these properties to create scaffolds used in drug delivery, tissue engineering and regenerative medicine. In the last few years, injectable polymeric systems based on microbial polysaccharides have also met extensive development to create minimally invasive routes of implantation, alleviating the need for surgical operations, reducing recovery times, risks of infection, while having better aesthetic outcomes. These polymeric systems encompass in situ gelling hydrogels, micro- and nano-gels, and micelles. These different structures all have their own pros & cons which should be considered relatively to the intended application. Generally, polymeric systems based on microbial polysaccharides lack mechanical strength and/or bioactivity for optimal performances. Current research on microbial polysaccharides utilizes their ease of modification to supplement them with other biomaterials and alleviate these issues. These modifications also allow to implement additional properties such as stimuli sensitivity into these polymeric systems. Altogether, microbial polysaccharides readily compete with other inexpensive natural polymers thanks to their novel or improved properties which allows them to be used in numerous biomedical applications. Moreover, novel microbial sources which synthesize polysaccharides with distinctive properties continue to be isolated. These new microbial polysaccharides may translate into the development innovative and advanced biomaterials differentiation to be used as injectable systems or in other fields such as 3D bioprinting. Modified microbial polysaccharides are extensively studied in 3D bioprinting applications to take advantage of their unique benefits while compensating for their weaknesses. Novel microbial polysaccharides with improved properties would prove effective to provide inexpensive methods for personalized scaffolds used in biomedical applications.

Interestingly, extremophilic bacteria drew a lot of attention thanks to the stability of the polysaccharides they yield, which can be used for the encapsulation of drugs and their effective delivery under different pH, and thus opening up new frontiers for chemotherapy and biopharmaceutics. Similarly, we can expect new microbial sources to be isolated in the future, which may yield polysaccharides with closer better mechanical and biological properties, allowing their use with fewer modifications.

Acknowledgments The authors acknowledge the financial support provided through the projects B-FABULUS (PTDC/BBB-ECT/2690/2014) and Fun4TE (PTDC/EMD-EMD/31367/2017), financed by the Portuguese Foundation for Science and Technology (FCT) and co-financed by European Regional Development Fund (FEDER) and Operational Programme for Competitiveness and Internationalisation (POCI). This work has been co-funded through the project 3BioMeD (FCT/4773/4/5/2017/S). G. Decante acknowledges for his research fellowship (BI_ PTDC/BBB-

ECT/2690/2014_2020_01) attributed under B-FABULUS project. The FCT distinction attributed to J. Silva-Correia Oliveira (IF/00115/2015) under the Investigator FCT program is also greatly acknowledged.

References

Altman R, et al. Efficacy and safety of repeated courses of hyaluronic acid injections for knee osteoarthritis: a systematic review. Semin Arthritis Rheum. 2018:168–75. https://doi.org/10.1016/j.semarthrit.2018.01.009.

Alves VD, Torres CAV, Freitas F. Bacterial polymers as materials for the development of micro/nanoparticles. Int J Polym Mater Polym Biomater. 2016;65(5):211–24. https://doi.org/10.1080/00914037.2015.1103239.

Amin MCIM, et al. Polymeric micelles for drug targeting and delivery. In: Nanotechnology-based approaches for targeting and delivery of drugs and genes; 2017. p. 167–202. https://doi.org/10.1016/B978-0-12-809717-5.00006-3.

Bacelar AH, et al. Recent progress in gellan gum hydrogels provided by functionalization strategies. J Mater Chem B. 2016:6164–74. https://doi.org/10.1039/c6tb01488g.

Bhardwaj A, et al. Stimuli-sensitive systems-an emerging delivery system for drugs. Artif Cells Nanomed Biotechnol. 2015;43(5):299–310. https://doi.org/10.3109/21691401.2013.856016.

Cadinoiu AN, Rata DM, Atanase LI. Biocompatible injectable polysaccharide materials for drug delivery. Polysaccharide Carriers Drug Delivery. 2019:127–54. https://doi.org/10.1016/B978-0-08-102553-6.00006-4.

Carvalhal F, et al. Antithrombotics from the sea: polysaccharides and beyond. Mar Drugs. 2019;17(3):170. https://doi.org/10.3390/md17030170.

Chen Q, et al. Recent advances in polysaccharides for osteoarthritis therapy. Eur J Med Chem. 2017a:926–35. https://doi.org/10.1016/j.ejmech.2017.08.048.

Chen Y, et al. Facile formation of a microporous chitosan hydrogel based on self-crosslinking. J Mater Chem B. 2017b;5(47):9291–9. https://doi.org/10.1039/c7tb02736b.

Chiriac AP, et al. Advancement in the biomedical applications of the (nano)gel structures based on particular polysaccharides. Macromol Biosci. 2019; https://doi.org/10.1002/mabi.201900187.

Chua BY, et al. Chitosan microparticles and nanoparticles as biocompatible delivery vehicles for peptide and protein-based immunocontraceptive vaccines. Mol Pharm. 2012;9(1):81–90. https://doi.org/10.1021/mp200264m.

Elmorsy S, et al. Chondroprotective effects of high-molecular-weight cross-linked hyaluronic acid in a rabbit knee osteoarthritis model. Osteoarthr Cartil. 2014;22(1):121–7. https://doi.org/10.1016/j.joca.2013.10.005.

Filomena M, et al. Bioactivity and applications of polysaccharides from marine microalgae. n.d.; https://doi.org/10.1007/978-3-319-03751-6_47-1.

Finn A. Bacterial polysaccharide–protein conjugate vaccines. Br Med Bull. 2004;70(1):1–14. https://doi.org/10.1093/bmb/ldh021.

Freitas F, et al. Advanced microbial polysaccharides. In: Biopolymers for biomedical and biotechnological applications; 2021. p. 19–62. https://doi.org/10.1002/9783527818310.ch2.

Hu Y, Li Y, Xu FJ. Versatile functionalization of polysaccharides via polymer grafts: from design to biomedical applications. Acc Chem Res. 2017;50(2):281–92. https://doi.org/10.1021/acs.accounts.6b00477.

Huh MS, et al. Polysaccharide-based nanoparticles for gene delivery. Top Curr Chem. 2017:31. https://doi.org/10.1007/s41061-017-0114-y.

Hütter J, Lepenies B. Carbohydrate-based vaccines: an overview. Methods Mol Biol. 2015:1–10. https://doi.org/10.1007/978-1-4939-2874-3_1.

Jennings HJ, Pon RA. Bacterial polysaccharide vaccines: glycoconjugates and peptide-mimetics. Microbial Glycobiol. 2010:933–56. https://doi.org/10.1016/B978-0-12-374546-0.00048-1.

Jung SW, et al. Multivalent ion-based in situ gelling polysaccharide hydrogel as an injectable bone graft. Carbohydr Polym. 2018;180:216–25. https://doi.org/10.1016/j.carbpol.2017.10.029.
Khan T, et al. Polysaccharides as potential anticancer agents—a review of their progress. Carbohydr Polym. 2019:412–28. https://doi.org/10.1016/j.carbpol.2019.01.064.
Kirschning A, Dibbert N, Dräger G. Chemical functionalization of polysaccharides—towards biocompatible hydrogels for biomedical applications. Chem Eur J. 2018:1231–40. https://doi.org/10.1002/chem.201701906.
Kumari L, Badwaik HR. Polysaccharide-based nanogels for drug and gene delivery. Polysaccharide Carriers Drug Delivery. 2019:497–557. https://doi.org/10.1016/B978-0-08-102553-6.00018-0.
Lee CR, Grodzinsky AJ, Spector M. The effects of cross-linking of collagen-glycosaminoglycan scaffolds on compressive stiffness, chondrocyte-mediated contraction, proliferation and biosynthesis. Biomaterials. 2001a;22(23):3145–54. https://doi.org/10.1016/S0142-9612(01)00067-9.
Lee CJ, et al. Bacterial polysaccharides as vaccines – immunity and chemical characterization. Adv Exp Med Biol. 2001b:453–71. https://doi.org/10.1007/978-1-4615-1267-7_30.
Li Z, et al. Injectable polysaccharide hybrid hydrogels as scaffolds for burn wound healing. RSC Adv. 2015;5(114):94248–56. https://doi.org/10.1039/c5ra16912g.
Li J, et al. Xanthan gum ameliorates osteoarthritis and mitigates cartilage degradation via regulation of the Wnt3a/β-catenin signaling pathway. Med Sci Monit. 2019;25:7488–98. https://doi.org/10.12659/MSM.916092.
Mathew AP, et al. Injectable hydrogels for delivering biotherapeutic molecules. Int J Biol Macromol. 2018:17–29. https://doi.org/10.1016/j.ijbiomac.2017.11.113.
Matsuda M, Shigeta S, Okutani K. Antiviral activities of marine pseudomonas polysaccharides and their oversulfated derivatives. Mar Biotechnol. 1999;1(1):68–73. https://doi.org/10.1007/PL00011753.
Mettu R, Chen CY, Wu CY. Synthetic carbohydrate-based vaccines: challenges and opportunities. J Biomed Sci. 2020:1–22. https://doi.org/10.1186/s12929-019-0591-0.
Moscovici M. Present and future medical applications of microbial exopolysaccharides. Front Microbiol. 2015:1012. https://doi.org/10.3389/fmicb.2015.01012.
Ng JY, et al. Biomimicry of microbial polysaccharide hydrogels for tissue engineering and regenerative medicine – a review. Carbohydr Polym. 2020:116345. https://doi.org/10.1016/j.carbpol.2020.116345.
Patel RM, Patel VP. Microbial polysaccharides: current innovations and future trends in medical science. CPR. 2011;1(2):204–9.
Riederer MS, et al. Injectable and microporous scaffold of densely-packed, growth factor-encapsulating chitosan microgels. Carbohydr Polym. 2016;152:792–801. https://doi.org/10.1016/j.carbpol.2016.07.052.
Robbins JB, et al. Considerations for formulating the second-generation pneumococcal capsular polysaccharide vaccine with emphasis on the cross-reactive types within groups. J Infect Dis. 1983;148(6):1136–59. https://doi.org/10.1093/infdis/148.6.1136.
Silva AKA, et al. Polysaccharide-based strategies for heart tissue engineering. Carbohydr Polym. 2015;116:267–77. https://doi.org/10.1016/j.carbpol.2014.06.010.
Singh K, Hari Kumar SL. Injectable in-situ gelling controlled release drug delivery system. Int J Drug Dev Res. 2012;4(2):56–69.
Suner SS, et al. Responsive biopolymer-based microgels/nanogels for drug delivery applications. In: Stimuli responsive polymeric nanocarriers for drug delivery applications: volume 1: types and triggers; 2018. p. 453–500. https://doi.org/10.1016/B978-0-08-101997-9.00021-7.
Thambi T, Phan VHG, Lee DS. Stimuli-sensitive injectable hydrogels based on polysaccharides and their biomedical applications. Macromol Rapid Commun. 2016:1881–96. https://doi.org/10.1002/marc.201600371.
Torchilin VP. Micellar nanocarriers: pharmaceutical perspectives. Pharm Res. 2007:1–16. https://doi.org/10.1007/s11095-006-9132-0.
Tran RT, et al. Biodegradable injectable systems for bone tissue engineering. RSC Green Chem. 2011:419–51. https://doi.org/10.1039/9781849733458-00419.

Velasco D, Tumarkin E, Kumacheva E. Microfluidic encapsulation of cells in polymer microgels. Small. 2012:1633–42. https://doi.org/10.1002/smll.201102464.

Wang H, Qian J, Ding F. Recent advances in engineered chitosan-based nanogels for biomedical applications. J Mater Chem B. 2017:6986–7007. https://doi.org/10.1039/c7tb01624g.

Wang L, et al. Fabrication of injectable, porous hyaluronic acid hydrogel based on an in-situ bubble-forming hydrogel entrapment process. Polymers. 2020;12(5) https://doi.org/10.3390/POLYM12051138.

Xiong XB, et al. Engineering of amphiphilic block copolymers for polymeric micellar drug and gene delivery. J Control Release. 2011:248–61. https://doi.org/10.1016/j.jconrel.2011.04.028.

Yang JA, et al. In situ-forming injectable hydrogels for regenerative medicine. Prog Polym Sci. 2014:1973–86. https://doi.org/10.1016/j.progpolymsci.2014.07.006.

Yu Y, et al. Biological activities and pharmaceutical applications of polysaccharide from natural resources: a review. Carbohydr Polym. 2018:91–101. https://doi.org/10.1016/j.carbpol.2017.12.009.

Zhang N, Wardwell PR, Bader RA. Polysaccharide-based micelles for drug delivery. Pharmaceutics. 2013:329–52. https://doi.org/10.3390/pharmaceutics5020329.

Zhong SP, et al. Biodegradation of hyaluronic acid derivatives by hyaluronidase. Biomaterials. 1994;15(5):359–65. https://doi.org/10.1016/0142-9612(94)90248-8.

Zhou W, et al. The antidiabetic effect and potential mechanisms of natural polysaccharides based on the regulation of gut microbiota. J Funct Foods. 2020:104222. https://doi.org/10.1016/j.jff.2020.104222.

The Role of Hyaluronic Acid in Tissue Engineering

45

Maurice N. Collins, Fernanda Zamboni, Aleksandra Serafin, Guang Ren, A. V. Thanusha, and Mario Culebras

Contents

M. N. Collins (✉) · F. Zamboni
School of Engineering, University of Limerick, Limerick, Ireland

Stokes Laboratories, Bernal Institute, University of Limerick, Limerick, Ireland

Health Research Institute, University of Limeric, Limerick, Ireland
e-mail: Maurice.collins@ul.ie

A. Serafin · G. Ren · A. V. Thanusha · M. Culebras
School of Engineering, University of Limerick, Limerick, Ireland

Stokes Laboratories, Bernal Institute, University of Limerick, Limerick, Ireland

J. M. Oliveira et al. (eds.), *Polysaccharides of Microbial Origin*,
https://doi.org/10.1007/978-3-030-42215-8_56

Abstract

Hyaluronic acid (HA) is a glycosaminoglycan that is found in extracellular tissue in many parts of the body. It is a material of increasing importance to biomaterial science and is finding applications in diverse areas ranging from tissue culture scaffolds to cosmetic materials. This chapter considers the recent research on the role of HA in tissue engineering and the importance of HA as an immunomodulatory material. The chemical modifications and processing methods employed to produce HA-modified tissue scaffolds are discussed, thus giving a better understanding of the structure-function-property relationships that influence scaffold performance, tissue growth, and regeneration. The chapter concludes with a vision for the future of HA in tissue-engineered constructs.

Keywords

Hyaluronic acid · Chemical modification · Scaffolds · Tissue engineering · Vascularization · Immunomodulatory · Antimicrobial

1 Introduction

Hyaluronic acid (HA) is a nonsulfated glycosaminoglycan that is found in the extracellular matrix of many tissues of the human body. HA owes its name to its transparent and bright appearance, named after hyaloid (meaning glassy in Greek) and uronic acid. HA was first mentioned in the literature in 1918, when a new polysaccharide consisting of glucosamine and glucuronic acid was published (Levene and López-Suárez 1918). A few years later, another independent publication reported the isolation of an extremely high-molecular weight polysaccharide from the vitreous eye of cows (Meyer and Palmer 1934). HA was initially produced by extraction from various animal parts. However, the growing demand for HA shifted the production to microbiological sources. In 1937, Kendall isolated HA from streptococci A and C (Kendall et al. 1937), which remains to date the most economical and the main reliable source for the industrial production of HA. HA is a material of ever-increasing importance to biomaterials science and engineering while

it is finding applications in diverse areas ranging from tissue culture scaffolds to cosmetics. Its properties, both physical and biochemical, in hydrogel form are extremely attractive for technologies associated with body repair. Here, we consider important chemistries underpinning its role in tissue engineering and how its immunomodulatory properties can tailor performance. Latest scaffold processing technologies are discussed with specific interest on HA's role in 3D bioprinting. Finally, the role of HA is discussed in the regeneration of skin, peripheral, and central nervous system.

1.1 Tissue Scaffolds

Briefly, tissue scaffolds are temporary supporting structures for growing cells and tissues. They can sometimes be referred to as synthetic extracellular matrices (ECM). Cells undergo proliferation, migration, and differentiation in three dimensions within scaffolds, which leads to the formation of specific tissue with functions as would be found in the human body. Scaffolds are required to meet several design criteria as follows and are discussed in more detail in (O'brien 2011):

- The surface should permit cell adhesion, promote cell growth, and allow the retention of differentiated cell functions.
- Scaffolds should be biocompatible; neither the polymer nor its degradation by-products should provoke inflammation or toxicity in-vivo.
- The scaffold should be biodegradable and eventually eliminated from the body.
- Implanted scaffolds need mechanical integrity to function from the time of implantation to the completion of the remodeling process with a balance between mechanical properties and sufficient porosity to allow cell infiltration and vascularization.
- Porosity should provide sufficient space for cell adhesion, extracellular matrix (ECM) regeneration with minimal diffusional constraints during culture, and the pore structure should be interconnected to allow even spatial homogeneous tissue formation.
- Cells primarily interact with scaffolds via chemical groups (ligands) on the material surface. Scaffolds synthesized from natural extracellular materials (e.g., collagen) naturally possess these ligands in the form of Arg-Gly-Asp (RGD)-binding sequences, whereas scaffolds made from synthetic materials may require deliberate incorporation of these ligands through, for example, protein adsorption. For any scaffold, a critical range of pore sizes exists which may vary depending on the cell type used and tissue being engineered (Collins and Birkinshaw 2013a).
- The material should be reproducible and processable into three-dimensional structures with properties or design variables tailored for the intended scaffold application and environment into which the scaffold will be placed.

1.2 The Extracellular Matrix (ECM)

The ECM is the noncellular component of all tissues, responsible for the provision of physical support to cells and for the regulation of diverse cell functions through biochemical and biomechanical cues. There are two major types of ECM, each with distinct architecture and composition: (i) interstitial matrices that surround cells, providing mechanical support; and (ii) pericellular matrices, which are in closer contact with cells (e.g., basement membranes), protecting them from rupture (Theocharis et al. 2016). Nevertheless, ECM composition varies from tissue to tissue, as a result of multiple signals, which dictate the ECM structure and its biomechanical properties.

HA plays an important role in the ECM of many soft connective tissues, where it acts as a space filler, lubricant, and osmotic buffer (Clegg et al. 2013; Laurent et al. 1996). When in physiological solutions, mutual repulsions between the carboxyl groups of HA occur, causing it to swell forming a hydrated network. The associated plasticity influences tissue reorganization and embryonic development.

2 Degradation and Chemical Modifications of HA

HA – the only nonsulfated GAG – is anionic and contains alternate units of the disaccharide β-1,4-D-glucuronic acid-β-1, and 3-*N*-acetyl-D-glucosamine. Among all GAGs, only HA is biosynthesized at the cell membrane and not at the Golgi apparatus. Moreover, HA is also the only GAG not covalently attached to proteoglycans (Theocharis et al. 2016). It binds either to its own synthases or to cell surface receptors (CD44 and RHAMM) (Acharya et al. 2008) and is involved in several cell function responses (Nasreen et al. 2002).

The size of HA depends on the relative activity of HA-synthesizing and -degrading enzymes. In mammals, there are three hyaluronan synthase (HAS) isoforms (HAS1, 2, and 3) that mainly differ in their enzymatic ability to produce HA of different sizes. HA clearance in vivo starts when it binds to HA receptor for endocytosis (HARE) on the cell membrane (Pandey and Weigel 2014). Then, HA rapidly degrades in vivo by physiological enzymes called hyaluronidases (HYAL). The majority of HA degradation is obtained by HYAL-1 and HYAL-2 to low-molecular weight fragments that possess size-dependent functions (Stern 2004). HYAL-1 is found in the lysosome and utilizes HA of any size as substrate to generate tetrasaccharides whereas HYAL-2 which is found in the plasma membrane (anchored by glycosilphosphatidyl-inositol – GPI) degrades HA to fragments of about 20 kDa (Stern 2004).

Due to its biological and gel-forming properties (Collins and Birkinshaw 2008; Collins and Birkinshaw 2013a), HA has found a variety of biomedical applications including tissue engineering (Song et al. 2013), wound healing, drug delivery systems (Choh et al. 2011), cell encapsulation (Peroglio et al. 2012), and microfluidics applications (Burdick and Prestwich 2011). As previously described, HA is readily degraded into bioactive fragments. For that reason, a variety of chemical

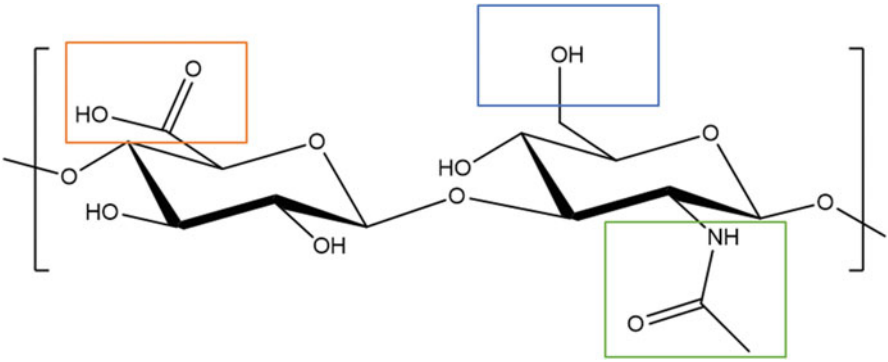


Fig. 1 Molecular structure of HA

modifications can be performed in order to increase HA-structural stability (Collins and Birkinshaw 2007). HA modification targets the following functional groups: the carboxylic acid group and the hydroxyl group (Fig. 1). An amino group can also be recovered by de-acetylation of the *N*-acetyl group.

HA can be chemically modified in two different ways: crosslinking or conjugation (Table 1). HA conjugation and HA cross-linking are based on similar chemical reactions and only differ in that, in the first case, a compound is grafted onto one HA chain by a single bond only, whereas in the second case, different HA chains are linked together by two bonds or more.

2.1 Conjugation of HA: Chemical Modifications Through the Carboxylic Acid Group

2.1.1 Amidation

Amidation with carbodiimides is one of the most common synthetic routes to chemically modify HA through the COOH group (Bulpitt and Aeschlimann 1999; Oh et al. 2010) Prestwich (Prestwich et al. 1998; Vercruysse and Prestwich 1998). The predominant cabodiimide in the vast majority of studies is 1-ethyl-3-[3-(dimethylamino)-propyl]-carbodiimide (EDC) since it is water soluble. The mechanism of this reaction was studied by Nakajima and Ikada in 1995 (Nakajima and Ikada 1995). The first step of the amidation reaction is the activation of the carboxylic acid by EDC, and as a result, an O-acyl isourea intermediate is formed. The second step of the reaction is the nucleophilic attack by the amine on the activated HA, which leads to the formation of the amide bond as shown in Fig. 2.

However, the intermediate is highly reactive and reacts with water, in which case it quickly rearranges into a stable *N*-acyl urea by-product. In addition, the reaction is highly pH dependent (Nakajima and Ikada 1995; Danishefsky and Siskovic 1971; Follain et al. 2008).

Table 1 Summary of all the chemical modification techniques described in this section

Group	Reaction type	Activator	Reagents
–COOH	Amidation	Carbodiimides	EDC, NHS
	Amidation	CMPI	CMPI, triethylamine
	Amidation	CDMT	CDMT, NMM
	Amidation	1,1 -Carbonyl-diimidazole	1,1 -Carbonyl-diimidazole
	Ugi condensation		Formaldehyde, diamine,
	Ugi condensation		and cyclohexyl isocyanide
	Esterification	Diazomethane	Trimethylsilyl diazomethane, acetic acid
	Esterification	Alkyl halides	Alkyl iodides or bromides
	Esterification	Tetraethylene glycol tosylate	Tetraethylene glycol tosylate
	Esterification	Bisepoxides	Butanediol-diglycidyl ether
	Oxidation	Sodium periodate	Sodium periodate
–OH	Ether formation	Bisepoxides	1,2,3,4-Diepoxybutane
	Ether formation	Bisepoxides	Butanediol-diglycidyl ether
	Ether formation	Bisepoxides	Ethyleneglycol diglycidyl ether and polyglycerol polyglycidylether
	Ether formation	Bisepoxides	Epichlorohydrin diepoxyoctane
	Ether formation	Bisepoxides	diepoxyoctane
	Ether formation	Divinyl sulfone	Divinyl sulfone
	Ether formation	Ethylenesulfide	Ethylenesulfide, dithiolthreitol (DTT)
	Hemiacetal formation	Glutaraldehyde	Glutaraldehyde
	Esterification	Alkyl succinic anhydrides	Octenyl succinic anhydride
	Esterification	Acyl-chloride-activated carboxylate	
	Esterification	Methacrylic anhydride	
	Carbamate formation	Cyanogen bromide (CNBr)	
$NHCOCH_3$	Deacetylation/ amidation	Hydrazine sulfate	

Alternative routes for amidation involve using 2-chloro-1-methylpyridinium iodide (CMPI) as the activating agent of the carboxyl groups of HA (Magnani et al. 2000). This reaction is typically carried out in dimethylformamide (DMF) to minimize CMPI hydrolysis. The HA sodium salt must first be converted into a tetrabutylammonium (TBA) salt to allow its solubilization in the organic solvent. 1,3-Diaminopropane can be used to form crosslinks between the HA chains. First, CMPI reacts with a carboxyl group of HA; this forms a pyridinium intermediate and releases a chloride ion, which is neutralized by tetrabutylammonium. The nucleophilic diamine then attacks the activated HA carboxyl and forms the amide bond

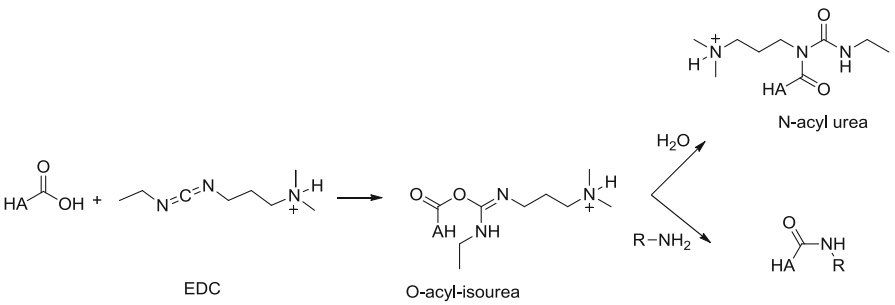


Fig. 2 Amidation with EDC

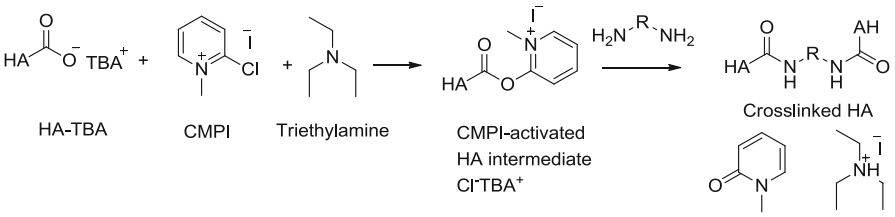


Fig. 3 Amidation with CMPI

(Fig. 3). Triethylamine neutralizes the iodide ion released. One of the problems of this method is the organic solvent, which requires additional purification steps and also the conversion of HA into TBA salt.

Amidation with 2-chloro-dimethoxy-1,3,5-triazine (CDMT) reported by Bergman et al. (Bergman et al. 2007) is another method for HA amidation. This reaction is carried out using a solvent mixture water: acetonitrile (3:2). CDMT reacts with the carboxylic acid to form a CDMT-activated HA intermediate. *N*-methylmorpholinium (NMM) is added to the mixture to neutralize the chloride ions which are formed. The CDMT-activated HA intermediate then reacts with the amine to form the amide bond (Fig. 4). Substitution degrees of up to 25% were obtained using a ratio HA:CDMT of 2:1 suggesting that higher degrees could be obtained by increasing the amount of CDMT. This represents a potential method to obtain high grafting yields.

Finally, amidation with carbonyldiimidazole as the activating agent of the HA carboxyl groups is another strategy worth mentioning (Schanté et al. 2011). This reaction is performed in DMSO from HA–TBA salt. Carbonyldiimidazole reacts with HA to form a highly reactive intermediate which quickly rearranges into a more stable HA–imidazole intermediate. This last intermediate reacts with an amine to form the amide bond (Fig. 5).

2.1.2 Ugi Condensation

Ugi condensation has been reported by many authors to crosslink HA with a diamine (Crescenzi et al. 2003a, b; de Nooy et al. 2000; Maleki et al. 2007). Typically, the reaction is carried out in water at pH 3 with formaldehyde, cyclohexyl isocyanide,

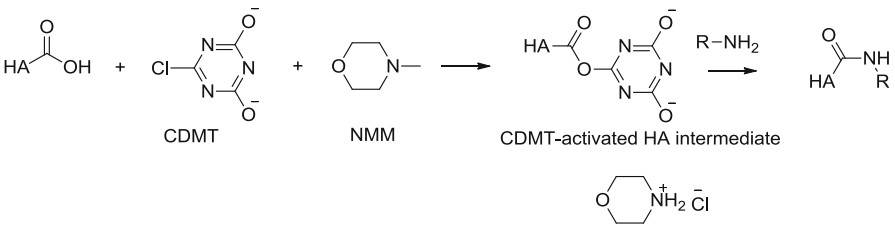


Fig. 4 Amidation with CDMT

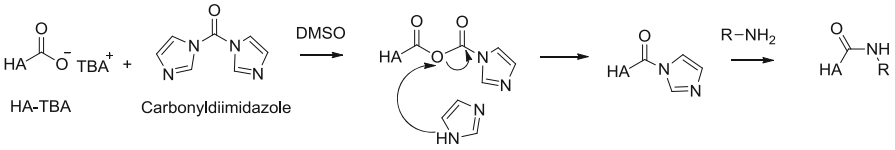


Fig. 5 Amidation with carbonyldiimidazole

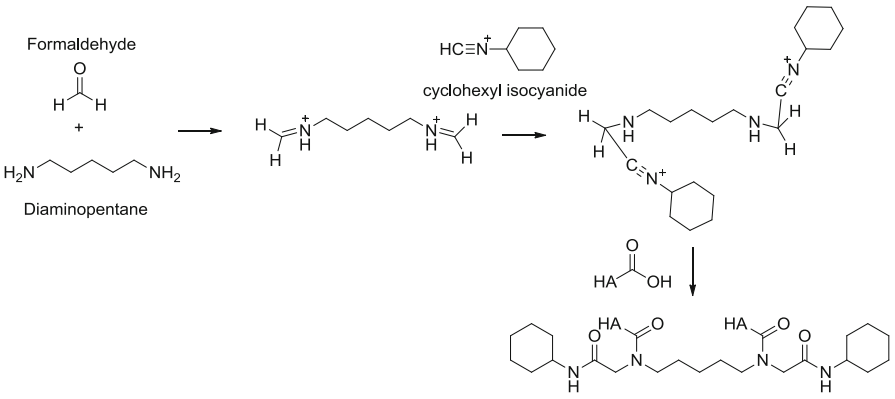


Fig. 6 Ugi condensation

and the diamine. First of all, the diamine condenses with formaldehyde to form a protonated diimine which then reacts with the cyclohexyl isocyanide. The carboxyl group of HA then eliminates the activated cyanide intermediate to form an (acylamino) amide bond (Fig. 6). However, this method leads to the formation of a secondary amide, adding a second pending group, in this case, a cyclohexyl.

2.1.3 Ester Formation

Several chemical strategies can be used for the esterification of the carboxylic acid group of HA such as using alkyl halides (Della Valle and Romeo 1997), tosylate

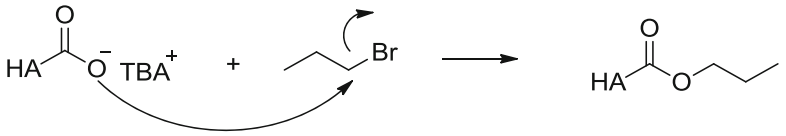


Fig. 7 Chemical modification of HA by Alkylbromide

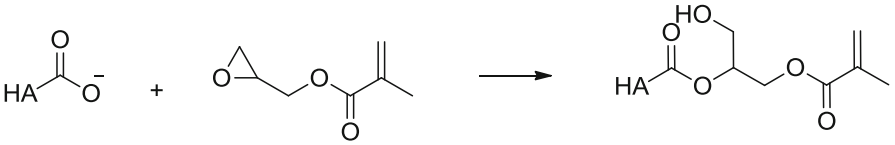


Fig. 8 Chemical modification of HA by glycidyl methacrylate

(Huin-Amargier et al. 2006), and diazomethane (Hirano et al. 2005; Bencherif et al. 2008; Baier Leach et al. 2003; Prata et al. 2010; Weng et al. 2008).

Using alkyl bromide ester formation occurs over 24 h in DMSO as shown in Fig. 7.

Using epoxides as a reaction between HA and glycidyl methacrylate can be used to prepare methacrylated HA. The reaction is performed in water in the presence of excess triethylamine as a catalyst (Bencherif et al. 2008). The reaction occurs mainly on the carboxylic groups of HA and a secondary transesterification on the hydroxyl groups is reversible (Fig. 8).

2.2 Chemical Modifications Through the Hydroxyl Group

2.2.1 Ether Formation

The use of diglycidyl ethers is a common way to crosslink HA through the hydroxyl group (Schanté et al. 2011). As an example, 1,2,3,4-diepoxybutane can be used as a crosslinking agent doing the reaction in strong alkaline conditions at pH 13–14 (0.2 M NaOH and 0.1% sodium borohydride) and at 50 °C for 2 h. Another example is the use of butanediol-diglycidyl ether (BDDE) in a 0.25 M NaOH solution. The reaction consists of the epoxide ring opening to form ether bonds with the HA hydroxyl groups (Fig. 9).

One of the factors to be considered using these types of compounds is the pH since the carboxylic acid and the hydroxyl groups could be reaction points. When HA is at high pH values (pH $>$ 13) above the pKa value of the hydroxyl groups (approximately 10), almost all those functional groups are deprotonated, and therefore their nucleophilic character increases to higher than the deprotonated carboxyl groups. The epoxides therefore react preferentially with the hydroxyl groups to form ether bonds. However, when the pH is lower than the pKa value of the hydroxyl group, only a small quantity of hydroxyl groups is deprotonated. Therefore, the reaction through the carboxyl group is predominant.

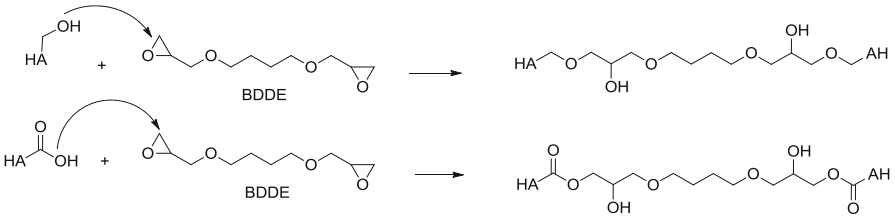


Fig. 9 Chemical modification of HA by diglycidyl ether compounds

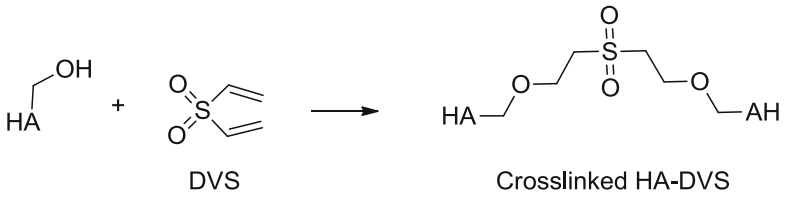


Fig. 10 Chemical modification of HA by DVS

The use of divinyl sulfone (DVS) has been established as an efficient method to crosslink HA for many authors (Collins and Birkinshaw 2007; Ramamurthi and Vesely 2002). Typically, the reaction is driven at high pH values (higher than 13) and creates sulfonyl bis-ethyl linkages between the hydroxyl groups of HA (Fig. 10). The advantage of this method is that it occurs at room temperature, which decreases the degradation of HA at high pH compared to higher temperatures, and it is a fast reaction.

Another type of strategies that can be used for ether group formation in HA through reaction of the hydroxyl group is via reaction with ethylene sulfide (Serban, Yang, and Prestwich (2008)] or using glutaraldehyde for HA crosslinking (Crescenzi et al. 2003a; Collins and Birkinshaw 2007; Tomihata and Ikada 1997).

2.2.2 Ester Formation

Ester formation using alkyl succinic anhydrides has been described by several authors (Eenschooten et al. 2010; Tommeraas and Eenschooten 2009). As an example, the reaction between HA and octenyl succinic anhydride (OSA) is illustrated in Fig. 11. Under alkaline conditions, the hydroxyl groups of HA react with the anhydride to form ester bonds.

Acyl-chloride activated carboxylate compounds can be also used to form ester bonds through reaction with the hydroxyl group of HA. This method can be carried out by reaction of the carboxyl groups of the compound to be grafted (first activated by chloroacylation with thionyl chloride) and HA at room temperature in an organic solvent (Pravata et al. 2008) (Fig. 12).

Esterification with methacrylic anhydride is also possible in order to obtain a photocrosslinked version of HA. Several authors have reported this reaction

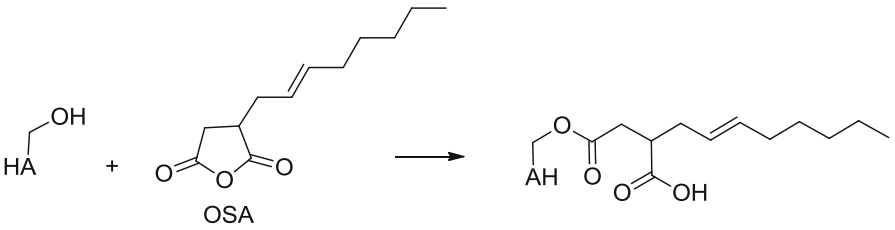


Fig. 11 Chemical modification of HA by OSA

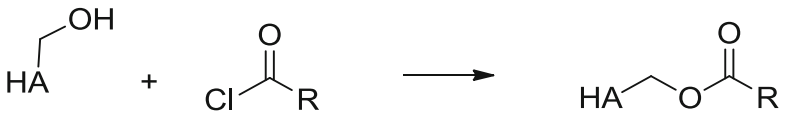


Fig. 12 Chemical modification of HA by acyl-chloride-activated carboxylate compounds

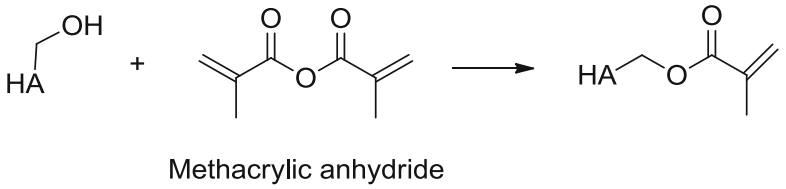


Fig. 13 Chemical modification of HA by acyl-chloride-activated carboxylate compounds

(Burdick et al. 2005; Seidlits et al. 2010; Smeds and Grinstaff 2001). Typically, the reaction is performed in ice cold water for 12 h at pH 8–10 (Fig. 13).

2.3 Modification of the –$NHCOCH_3$

N-acetyl group of HA can be deacetylated in order to recover the amino group which can then react with an acid following the amidation strategies described previously. Deacetylation is usually performed using hydrazine over a period of 5 days at 55 °C, which induces severe chain fragmentation (Schanté et al. 2011). Then, the deacetylated HA can react with one carboimide-activated acid in order to form a new amide compound based on HA (Fig. 14).

2.4 Other HA-Crosslinking Strategies for Biomedical Applications

For photo-initiated systems, hydrogels result mainly from the polymerization of water-soluble monomers in the presence of a multifunctional crosslinker by UV radiation (photo-crosslinking) or by the reaction between complementary groups as shown in Fig. 15 (Bae et al. 2014; Choi et al. 2015). Although the rationale behind

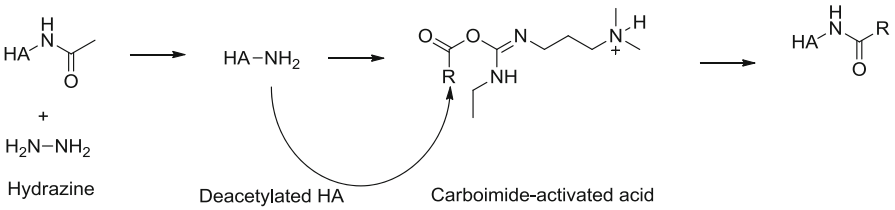


Fig. 14 Deacetylation of HA by hydrazine

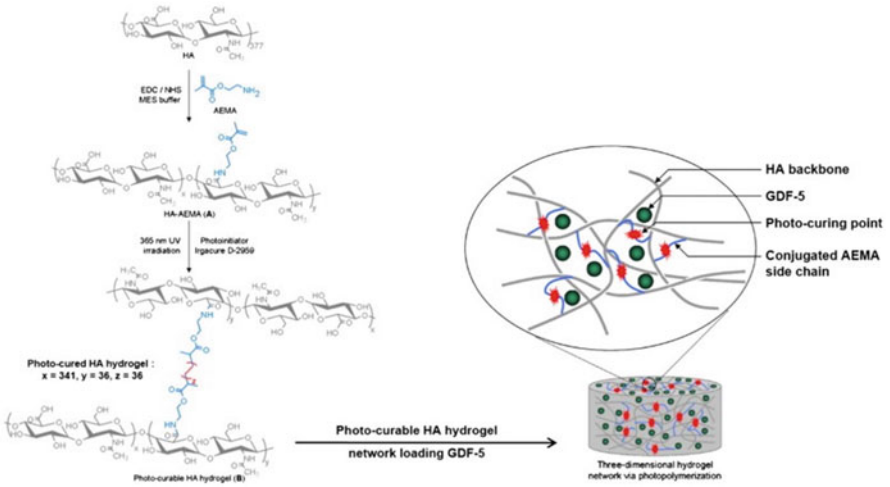


Fig. 15 A photo-curable HA system. (Reprinted with permission from Bae et al. 2014)

the development of these hydrogels is very simple, the resulting hydrogels commonly have an increased structural stability, longer degradation, and high swelling profiles (Collins and Birkinshaw 2008).

However, photo-polymerized hydrogels bring a few concerns relating cell viability for cell encapsulation. Cell exposure to UV radiation increases the cellular production of ROS [e.g., superoxide (O_2^-) and hydrogen peroxide (H_2O_2)], which may cause lipoperoxidation and damage to DNA (Markovitsi et al. 2010) and increase the oxidative degradation of HA (Valachova et al. 2016; Valachova et al. 2015). The use of photo initiators (such as Irgacure 2959, 2,2-dimethoxy-2-phenylacetophenone – DMPA, lithium phenyl-2,4,6-trimethylbenzoylphosphinate – LAP, and eosin Y) in HA gelation has been well described (Poldervaart et al. 2017; Jha et al. 2010; Gwon et al. 2017; Rosales et al. 2017). However, it is known that these photo-initiators are cytotoxic in a time- and concentration-dependent manner, although LAP is shown to be the most benign (Kessler et al. 2017). In relation to the use of photo-initiators in HA gelation processes for cell encapsulation strategies, the cell type being encapsulated can influence the construct outcome. For instance, different cell types respond differently to photo-initiator's toxicity which is

thought to be due to the variable intracellular antioxidative machinery that different cell types use to quench ROS (Williams et al. 2005). For example, pancreatic β-cells are particularly sensitive to oxidative stress because of their low-antioxidant capacity (Drews et al. 2010). Therefore, when using photo-polymerization in HA gelation processes for cell encapsulation applications, additional protective measures from oxidative stress, such as antioxidants and scavenger enzymes, should be put in place to ensure the maintenance of β-cell viability (Weaver and Stabler 2015; Asami et al. 2013).

Smart hydrogel systems which were reported to behave in a stimuli-responsive manner, where polymerization is triggered by environmental changes, such as temperature and pH, have been reviewed (Lim et al. 2014). They are mainly crosslinked via weak, noncovalent interactions, such as hydrophobic and electrostatic interactions between oppositely charged biomolecules (Wu and Gong 2011). For example, HA conjugated to Pluronic® F127 exhibited thermo-sensitive sol-gel transitions and is being envisaged as a suitable candidate for cell delivery systems (Fig. 16) (Lee and Park 2009).

Interpenetrating polymer networks (IPN) consisting of two or more networks of different components self-assemble into entangled arrangements, displaying synergistic effects from the simultaneous operation of the two networks (Kheirabadi et al. 2015). HA-based semi-IPN hydrogels have been reported for bioprinting

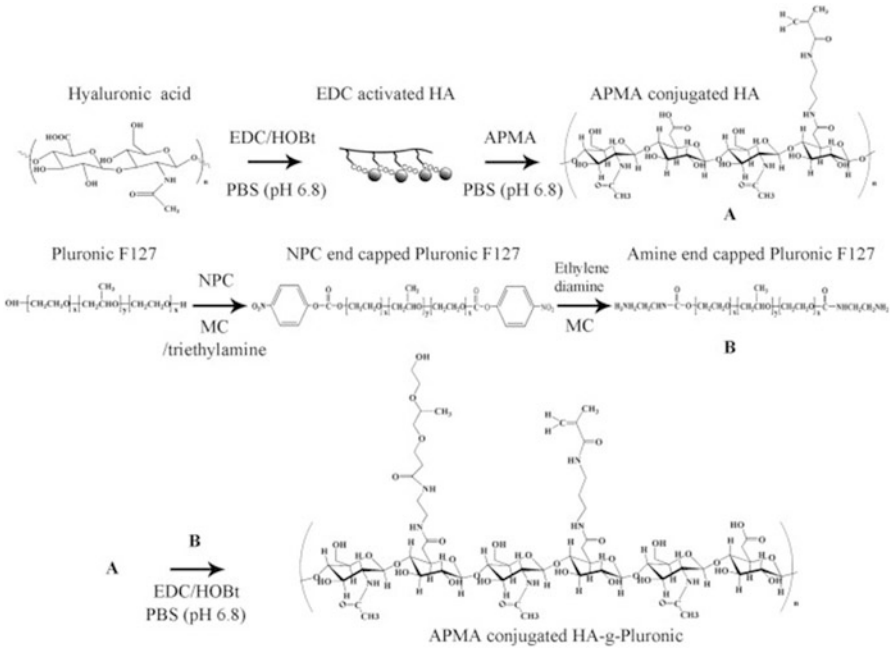


Fig. 16 A thermosensitive HA grafted with Pluronic® F127. (Adapted with permission from Lee and Park 2009)

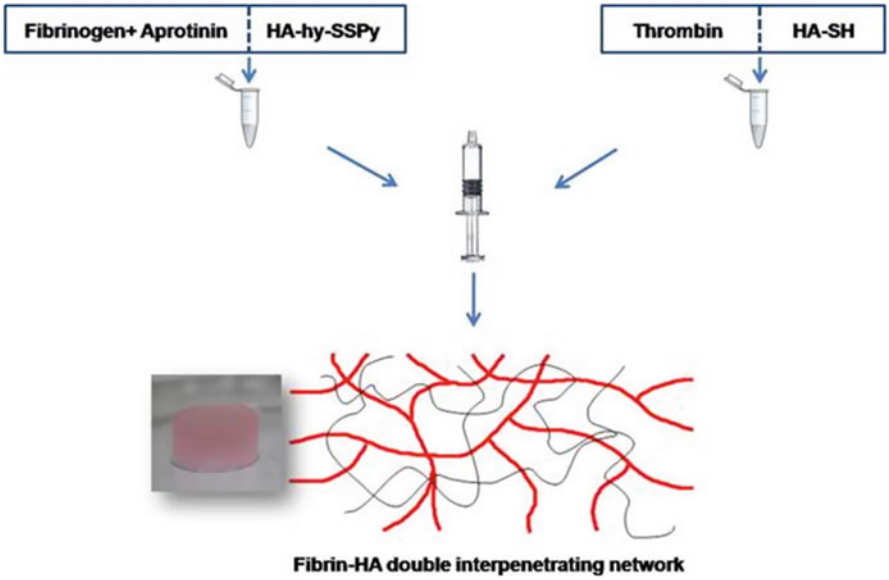


Fig. 17 In situ formation of double IPN of HA with fibrin (Reprinted with permission from Zhang et al. 2016)

(Pescosolido et al. 2011) and cell encapsulation (Zhang et al. 2016; Chen et al. 2016a) as shown in Fig. 17.

Recently, increasing attention is being directed toward guest-host assemblies (Fig. 18a). HA has been conjugated to cyclodextrin (host) and adamantane or azobenzene (guest), curcubit(6)uril (host) and polyamine (guest), such as spermine and 1,6-diaminohexane, to self-assemble into an IPN by reversible guest-host interactions (Rodell et al. 2013; Park et al. 2012a; Rosales et al. 2018). The noncovalent character of guest-host networks confers to hydrogels full reversibility under shear, allowing association or self-healing when shear is removed. These materials offer exciting possibilities for in situ minimally invasive injectable procedures. In addition, their use in bioprinting strategies is also appealing as these hydrogels are easily printed, i.e., show an increase of flowability when passing through the nozzle and fast recovering after that without requiring UV curing, which can be more beneficial to cells.

Double network (DN) hydrogels, a subset of IPN, with a combination of physical, covalent, or ionic bonds are also gaining importance (Fig. 18b) (Highley et al. 2016). DN hydrogels exhibit resistance to mechanical failure because they combine a shear-thinning self-assembly hydrogel network and a crosslinked hydrogel network that provides self-adhesive properties (Lu et al. 2013). HA DN hydrogels were developed for cell encapsulation and drug delivery systems (Mealy et al. 2015; Rodell et al. 2015a). It is possible to tune their degradation through modification of amino acid tethers (Rodell et al. 2015a), and porosity is controlled by HA concentration/modification (Rodell et al. 2016).

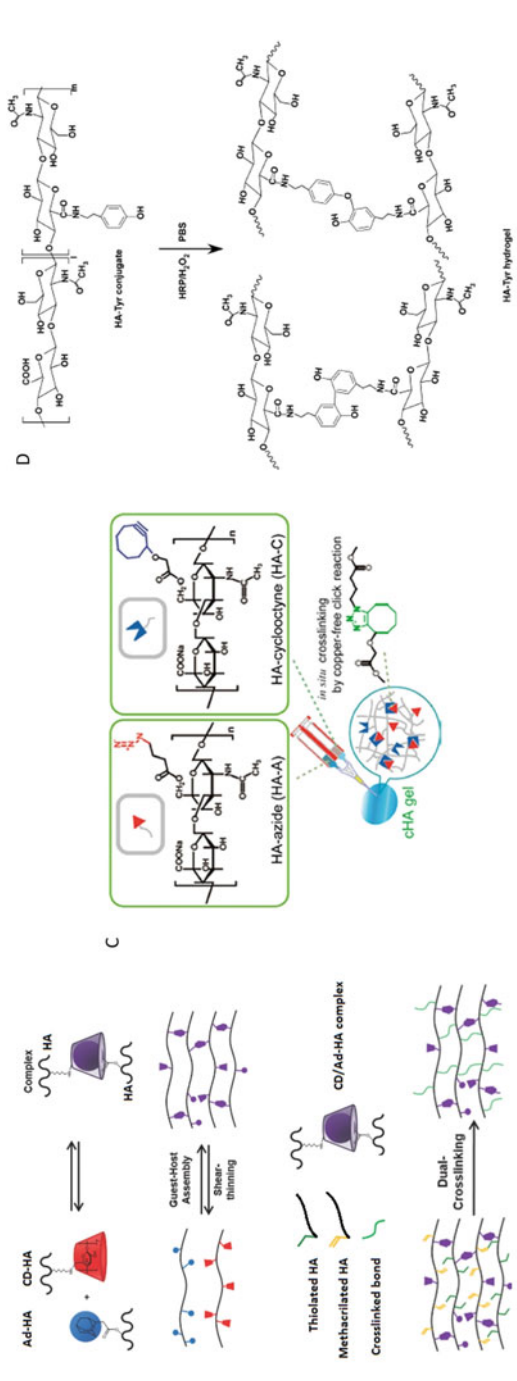


Fig. 18 Hydrogels produced by: (**a**) guest-host crosslinking. (Adapted from Rodell et al. 2015); (**b**) DN crosslinking. (Adapted from Rodell et al. 2015); (**c**) click chemistry crosslinking. (Reprinted with permission from Takahashi et al. 2013); and (**d**) enzymatic crosslinking. (Adapted from Schante et al. 2011)

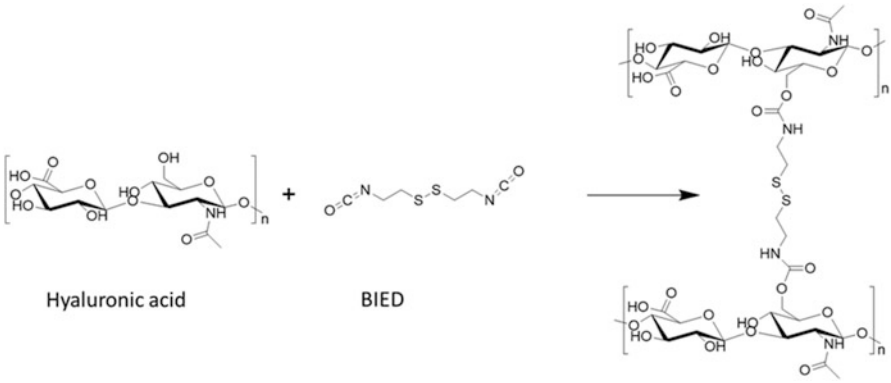


Fig. 19 Schematic reaction between HA and BIED. (Reproduced with permission from Zamboni et al. 2020)

Other smart hydrogels also under the spotlight are polymers with complementary functional groups which can avail of click chemistries (Fig. 18c) or enzymatic crosslinking under physiological conditions (Fig. 18d) while displaying minimal toxicity. In situ click chemistry crosslinking was reported for azide-modified HA and cyclooctyne-modified HA and for tyramine-conjugated HA hydrogels formed by the oxidative coupling of tyramines catalyzed by hydrogen peroxide (H_2O_2) and horseradish peroxidase (HRP) (Takahashi et al. 2013; Ni et al. 2015; Abu-Hakmeh et al. 2016).

Labile crosslinking strategies of hyaluronic acid via urethane formation using bis(β-isocyanatoethyl) (BIED) disulfide have been recently developed (Zamboni et al. 2020). The alkyl-based isocyanate reacts readily with hydroxyl groups on the HA to form stable urethane crosslinks (Fig. 19). A centrally located disulfide bond allows versatility with respect to reversible crosslinking by disulfide bond hydrolysis, and this allows potentially controlled layer by layer deposition of the HA for tailored pharmaceutical applications. The study further shows how these materials can modulate the immune system. With cellular grafts of LMW HA-BIED showing decreased fibroblast production of GM-CSF, indicating these grafts could be transplanted into tissues abundant in fibroblasts. On the other hand, cellular grafts of HMW HA-BIED show no monocyte activation and TNF-alpha production, while protecting LPS induction, thus showing the potential of being transplanted in tissues with direct blood contact.

3 Potential HA-Based Scaffold-Processing Methods

Several approaches to the fabrication of porous degradable polymer scaffolds have been developed. Some commonly used techniques are discussed here.

3.1 Phase Separation

Phase separation is a swift, controllable, and scalable approach to fabricate two- and three-dimensional scaffolds with interconnected porous structures. Phase separation has been classified as nonsolvent-induced phase separation (NIPS) (Wang et al. 2019a), chemically induced phase separation (CIPS), and thermally induced phase separation (TIPS) (Jeon et al. 2018). The TIPS method is composed of two components, polymer and solvent. Phase separation is induced by removing the thermal energy from the dope solution. Upon the removal of solvent by means of extraction, evaporation, or sublimation, the residual polymer solidifies into the skeleton, while the space previously occupied by the solvent becomes pores (Jung et al. 2016). The TIPS method is typically employed to fabricate highly porous membranes, which are inherently more reproducible and less prone to defects (Kim et al. 2016). The pore morphology varies depending on the polymer, solvent, concentration of the polymer solution and the phase separation temperature. Recently, polymerization-induced phase separation was used to formulate poly(ethylene glycol)/hyaluronic acid (PEGdA/HA) semi-IPNs that could provide dynamic microenvironments with improved mechanical properties to support cell survival, spreading and sustained migration, showing great potential in orthopedic tissue engineering (Lee et al. 2015).

3.2 Supercritical Fluid Technology

Supercritical fluid (SCF) technology provides a cost-effective and eco-friendly alternative to conventional solvent-based methodologies for polymer synthesis in pharmaceutical and biomedical applications, such as drug delivery and tissue engineering. As the use of organic solvents can be drastically reduced or eliminated, it is regarded as a nontoxic process. In this process, an SCF is used, which turns into a homogeneous phase where the vapor/liquid boundary disappears above its critical point in terms of temperature and pressure. This supercritical phase shows an intermediate behavior between that of a liquid and a gas and induces the formation of small gas bubbles homogeneously throughout the polymer in response to the thermodynamic driving force (Collins and Birkinshaw 2013a). Carbon dioxide (CO2) is the most widely used SCF due to its low toxicity, chemical inertness, and nonflammability, as well as its readily availability at high purities and low cost. The SC-CO_2 polymerization can be operated at rather mild conditions due to its low critical point, i.e., at temperature of 31.1 °C and pressure of 7.38 MPa, as shown in Fig. 20 (Kankala et al. 2017). The latest progress of SCF technology in biomedical engineering can be read in this review, while further information on engineering scaffold materials utilizing gas-foaming technologies can be read in the following articles: Diaz-Gomez et al. 2016; Costantini and Barbetta 2018; Poursamar et al. 2016; Maniglio et al. 2018; Manavitehrani et al. 2019; and Rao et al. 2019.

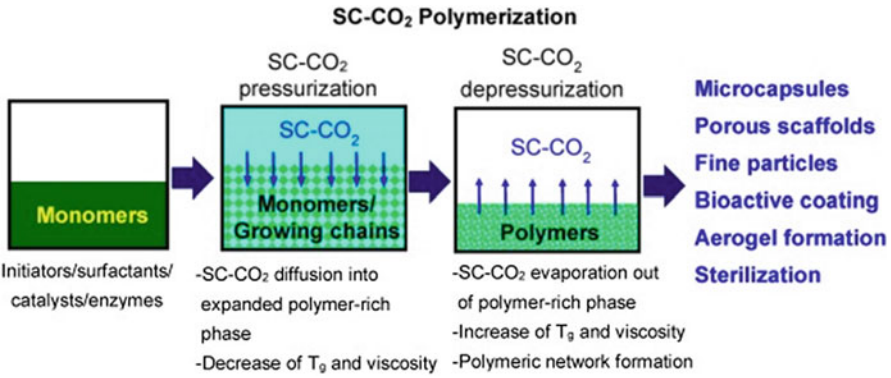


Fig. 20 Schematic showing the mechanism of SCF polymerization. (Reproduced with permission from Tsai and Wang 2019)

3.3 Porogen Leaching

Porogen leaching is known as an expedient and economical technique to process scaffolds with a homogeneous porous structure, as pore shape, size, and total porosity can be tailored (Yin et al. 2016). Recently, this method has been combined with other processing methods to engineer various porous scaffolds, such as polycaprolactone/graphene oxide (PCL/GO) nanocomposite scaffolds through a SC-CO_2 technology/porogen leaching route (Yildirim et al. 2018); poly(lactic acid) (PLA) with high mechanical performance assisted via a solid state extrusion/ porogen leaching approach (Yin et al. 2016); nanoporous polyacrylonitrile/calcium carbonate (PAN/$CaCO_3$) nanofiber through a solvent casting/porogen leaching technique (Mahmoodi and Mokhtari-Shourijeh 2016); and porous polyetheretherketone (PEEK) and bioactive hydroxyapatite (HA)-reinforced PEEK (HA-PEEK) by a compression molding/porogen leaching method (Conrad and Roeder 2020).

As shown in Fig. 21, Thomas *et al.* have developed HA-based scaffolds with complex 3D architecture using cheap sacrificial crystals (potassium dihydrogen phosphate or urea) that act as porogen (Thomas et al. 2017). This offers a route for the synthesis of biomimetic 3D tissue scaffolds for in vitro studies of pore architecture influences on cellular behavior, as well as to engineer implantable biomaterials for specific niches in the body, such as regeneration of the nervous system.

3.4 Electrospinning

Microfibers and nanofibers processed by electrospinning provide extremely high surface-to-volume ratios, complex porous structures, and diverse fibrous morphologies, which are coherent with the demands of advanced biomedical applications such as tissue engineering scaffolds and wound healing. The recent progress and potential developments of electrospinning in biomaterial engineering have been

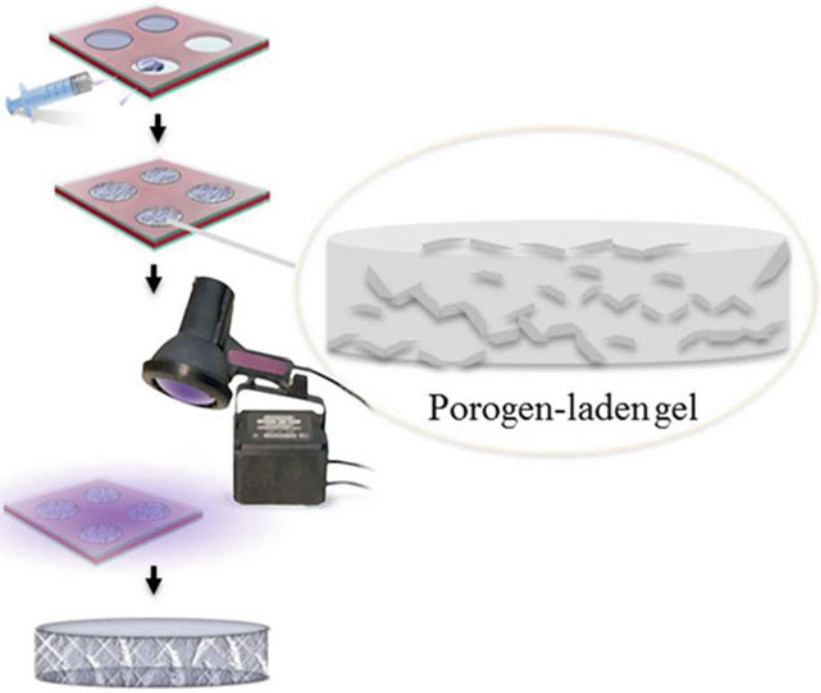


Fig. 21 Schematic showing the preparation process of porous HA-based scaffolds through porogen leaching. (Reproduced with permission from Thomas et al. 2017)

reviewed (Ding et al. 2019; Miguel et al. 2018). A range of nanofibrous (Sun et al. 2019; Chanda et al. 2018; Figueira et al. 2016; Seon-Lutz et al. 2019) HA-based scaffolds have been successfully produced by electrospinning, for potential wound healing and tissue regeneration applications. A distinct advantage of this method is the capability of producing core-shell nanofibers through coaxial electrospinning which exhibit desired properties from each of the ingredients. For example, core-shell-structured polyurethane/starch (HA) PU/St (HA) nanofibers have been fabricated by coaxial electrospinning (Movahedi et al. 2020). This unique core-shell structure represents excellent biological properties from starch, superb water absorption ability, and flexibility from HA which promotes cell adhesion, as well as mechanical strength from PU, which make it an ideal scaffold biomaterial for wound healing and skin tissue engineering. Another example worth noting is the development of a HA-based silk fibroin/zinc oxide (HA–SF/ZO) core-shell electrospun dressing, where the antibacterial agent ZO was loaded as the core to improve the drug release as well as to maintain its bioactivity, which is critical in burn wound management. In vivo studies indicate that the wound healing procedure was substantially improved while the inflammatory response at the wound site was significantly reduced by this ZO-loaded wound dressing.

3.5 Freeze Drying

One of the most common processes to prepare porous scaffolds in tissue engineering is freeze drying, also known as lyophilization. The material in hydrogel form is first frozen, while the water content forms nuclei of ice, which act as porogen, that are homogeneously distributed throughout the polymer. The frozen water is then removed through sublimation by reducing the pressure and adding heat, and a porous spongy structure is left behind. The pore size echoes with the nuclei size, which can be controlled by exposing the hydrogel to different temperatures as freezing conditions. Freeze drying is widely used in processing the spongy HA-based scaffolds that can provide the support required in tissue engineering and regenerative medicine (Kalam 2016; Lu et al. 2017; Yin et al. 2019; Zhang et al. 2017; Zapotocky et al. 2017; Kaczmarek et al. 2018).

3.6 Centrifugal Casting

The extensive existence of tubular tissues in the human body, such as capillaries, bones, kidney tubules, and genitourinary structures, suggests the potential of centrifugal casting in tissue engineering. Lee et al. have used a centrifugal casting technique to fabricate silk fibroin (SF) film for corneal tissue engineering. Compared with SF films prepared by dry casting methods, SF films fabricated with the aid of centrifugal force demonstrated superior surface roughness, better tensile strength, as well as better cell proliferation, which was speculated to be helpful in the regeneration of the corneal layer (Lee et al. 2016). The same research team later combined centrifugal casting with a digital light processing (DLP) 3D printer and enabled rapid, easy, and low-cost fabrication of sophisticated fixation systems, such as screws and plates, based on tailor-made geometric design (Kim et al. 2018). Nanofibrous porous tubular scaffolds can be fabricated by centrifugal casting combined with phase separation, as shown in Fig. 22, to provide an advantageous environment for cell adhesion and viability, which has significant potential for bone tissue regeneration (Chen et al. 2016a).

3.7 Scaffold-Templating Techniques

During the process of scaffold templating, HA-based solution is vacuum injected into templates made from sintered beads. Then the HA is crosslinked around beads, which are subsequently removed by leaching. Afterward, lyophilization is carried out, leaving adequate porosity for cell and tissue invasion. Shape and size of the beads can be controlled to obtain scaffolds with desired microporous morphology. Rodriguez-Perez et al. have adopted this methodology to fabricate a novel biomaterial with interpenetrating polymeric networks (IPNs) based on HA and poly(ethyl acrylate) (PEA). These scaffolds display a homogeneous interconnected morphology consisting of pores with size around 182 μm, and they show good cell response

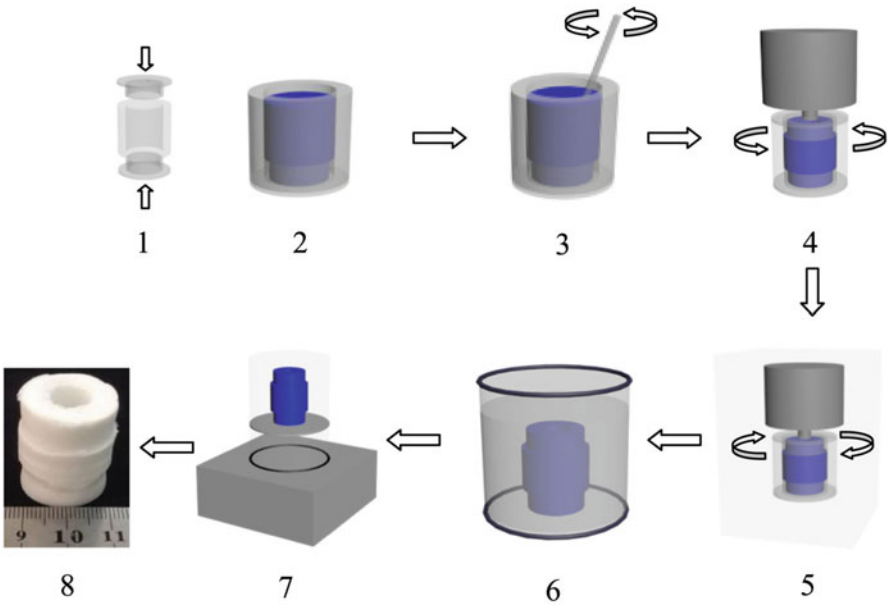


Fig. 22 Schematic illustrating the fabrication of nanofibrous tubular scaffolds: (1) mold, (2) polymer solution, (3) porogen addition, (4) motor assembly, (5) centrifuge and phase separation, (6) mold removal, solvent exchange, (7) freeze drying, and (8) acquisition. (Reproduced with permission from Chen et al. 2016a)

in in vitro studies (Rodriguez-Perez et al. 2016). As shown in Fig. 23, Singh et al. reported a magnetic templating technique, which used dissolvable magnetic alginate microparticles (MAMs) to form highly aligned, 3D, tubular microarchitecture under an applied magnetic field. Scaffolds based on glycidyl methacrylate hyaluronic acid (GMHA) and collagen I were tailored to mimic the microarchitecture of the native nerve, with the ability to guide cellular migration and potentially aid in peripheral nerve regeneration after injury (Singh et al. 2020).

3.8 Micropatterning Techniques

Micropatterning, initially a miniaturization technique used in electronics, has recently gained some momentum in biomaterial engineering and is applied in cellular biology (Ahmadian et al. 2020). HA-based micropatterns (HAP) can be fabricated using spatially controlled light exposure (Hui et al. 2019; Porras et al. 2016) and capillary force lithography (CFL) (Li et al. 2013; Han et al. 2019). These micropatterns are used to regulate the cells' behaviors, including their attachment, proliferation, distribution, morphology, and even cytokine secretion on the HA-based hydrogel scaffolds with submicrometer resolution. Credi et al. have

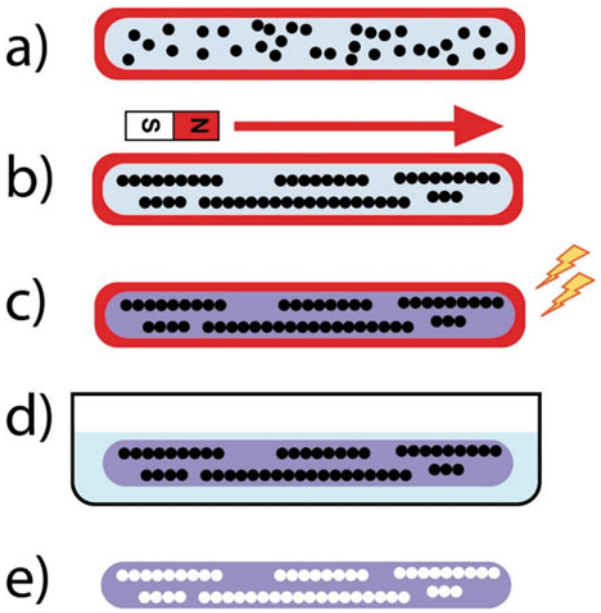


Fig. 23 Schematic illustrating magnetic templating: (**a**) mold is loaded with a mixture of MAMs and hydrogel precursor solution; (**b**) the hydrogel precursor is aligned under a static, uniform magnetic field; (**c**) the hydrogel is UV cured around the aligned MAMs; (**d**) MAMs are dissolved; and (**e**) hydrogel scaffolds patterned with aligned porous channels are formed. (Reproduced with permission from Singh et al. 2020)

optimized a single-step photolithographic process for selectively micropatterning glycidyl methacrylate-modified HA onto UV-curable perfluoropolyether (FPE)-based surfaces. HA micropatterns with complex geometrical features can be rapidly and cost-effectively designed, in order to affect cell adhesion efficiency, which can be utilized to selectively capture and immobilize cancer cells (Credi et al. 2016).

3.9 Rapid Prototyping: Solid Freeform Fabrication (SFF) and Bioprinting

Bioprinting is the most suitable technique to produce well-defined 3D structures with highly complex and patient specific geometries (Zhang et al. 2019; Gleadall et al. 2018). As shown in Fig. 24, this technique is based on a bottom-up approach, where hydrogel materials and cells are combined to form a bioink which is then deposited layer-by-layer in a controlled manner, making up a 3D complex structure (Pati et al. 2014). There are three common printing methods, i.e., inkjet, microextrusion, and laser-assisted bioprinting, while the selection of method should be based on the biomaterial to be used, cell viability to be achieved, and desired resolution (Sundaramurthi et al. 2016; Murphy and Atala 2014). In order to reproduce the complex and heterogeneous 3D architecture of functional organs, 3D printing and bioprinting techniques coupled to imaging modalities such as computer tomography (CT) and magnetic resonance imagining (MRI) provide unique spatially accurate

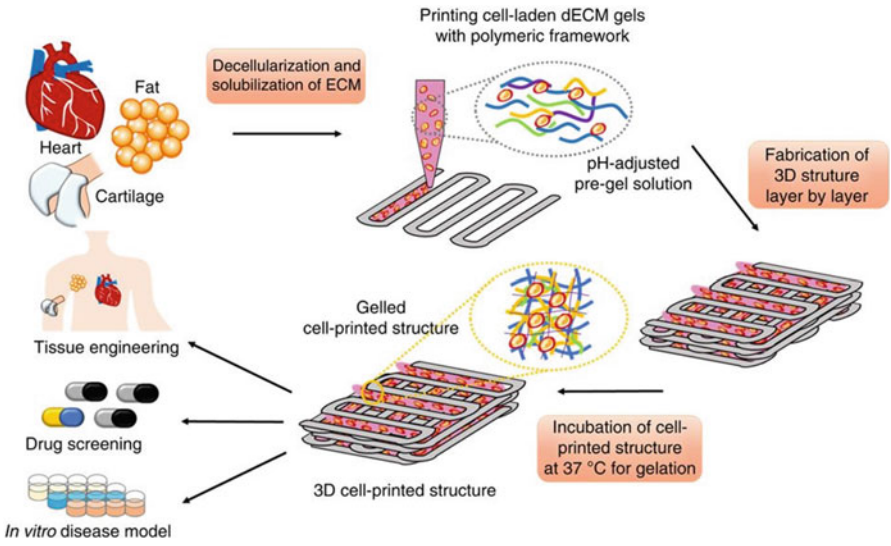


Fig. 24 Schematic elucidating bioprinting of a tissue-engineering construct using bioink. (Reproduced with permission from Pati et al. 2014)

models in regenerative medicine for the direct printing of tissue constructs (Cahill et al. 2019).

The application of pristine HA as bioink is limited by its high hydrophilicity, but this can be mitigated using chemical techniques described above or through a combination with other biomaterials. For example, semi-IPN of HA and modified dextran (Zoratto and Matricardi 2018), thiolated HA crosslinked with PEG (Godesky 2020), gelatin-methacryloyl (gelMA), gellan and methacrylated HA (HAMA) (Mouser et al. 2020), HA, hydroxyethyl acrylate (HEA) and gelatin-methacryloyl (Noh et al. 2019), HA and alginate (Antich et al. 2020), and methacrylated collagen I, thiolated HA, and gelatin nanoparticles (Clark et al. 2019) have been reported as successful bioinks.

Recently, increasing attention is drawn to the development of bioinks encapsulated with stem cells. For example, Sakai et al. have demonstrated the utility of the bioprinting technique to create cell-laden hydrogel constructs using HA-gelatin-based bioink crosslinked through irradiation by visible light. The hydrogelation rate and mechanical properties of the hydrogel can be controlled by adjusting the crosslinking condition. After printing, the human adipose stem cells (hADSCs) enclosed in the hydrogel elongated and proliferated while maintaining their differentiation potential, which demonstrated the great potential of the bioprinting technique in applications of tissue engineering and regenerative medicine (Sakai et al. 2018).

For further information on the fabrication techniques of tissue engineering scaffolds, the reader is referred to a review (Hokmabad et al. 2017) and a book chapter (Rey and St-Pierre 2019).

4 HA in Scaffold Vascularization Strategies

Natural tissues and organs exhibit a 3D architecture which allows cell-to-cell and cell-to-ECM interactions. The survival of large 3D architectures relies on blood perfusion within an intricate vascular network, delivering oxygen and nutrients while removing carbon dioxide and metabolites. The adequate vascularization of 3D tissue-engineered substitutes is a major engineering hurdle in creating artificial organs. Posttransplantation success of these tissue substitutes is highly dependent on the ability to promote rapid and stable neovascularization (formation of new blood vessels) to support growth, function, and viability. Researchers rely on increasing knowledge of angiogenic and vasculogenic processes to stimulate vascular network formation within 3D tissue constructs, such as the incorporation of proangiogenic materials or growth factors in the development of cellular scaffolds.

The influence of HA on the angiogenic process was reported in the early 1980s (West et al. 1985). While native HMW-HA was reported as an angiogenic inhibitor, LMW-HAs are highly bioactive and stimulate the angiogenesis process (Gao et al. 2019). The size of these HA oligomers also influences the proangiogenic nature of the fragments. Oligomers with 6–10 saccharide units were shown to be pro-angiogenic (Wang et al. 2019a) while fragments with only 4 saccharide units were unable to prompt a proangiogenic response (Cui et al. 2009).

In the endothelium, HA interacts with endothelial cells that line the interior surface of blood and lymphatic vessels. This angiogenic capacity comes from receptor-mediated interactions of HA oligomers with the CD44 and hyaluronan-mediated motility receptor (RHAMM) of endothelial cells, which triggers endothelial cell proliferation, migration, collagen synthesis, and cell sprouting (Pardue et al. 2008). Park et al. suggested a new mechanism of HA-promoted angiogenesis, where plasminogen activator-inhibitor-1 (PAI-1) is inducted through RHAMM and transforming growth factor β receptor I (TGFβR1) signaling (Park et al. 2012a).

As a result, HA oligomers have been used to increase the angiogenic capacity, and consequent vascularization, of other biomaterials (Silva et al. 2016; Perng et al. 2011), and also to enhance wound-healing strategies (Wang et al. 2016). Recently, a clinical trial demonstrated that HA hydrogels enabled the vascularization of free gingival grafts and functioned as a scaffold between the recipient's transplantation bed and the gingival graft, reducing graft shrinkage (Cankaya et al. 2020). In a lab-on-a-chip model for a functional blood-brain barrier system, a fibrin 3D-lumenized vasculature together with perivascular cells showed better astrocyte-endothelium interaction when the ECM of the lumen was modified to contain HA (Lee et al. 2020). In another microfluidic study, an in vitro assessment of biomaterial-based angiogenesis showed that a HA matrix was able to sequester growth factors and enabled endothelial cells derived from human-induced pluripotent stem cells to form stable, capillary-like networks (Natividad-Diaz et al. 2019). In addition, HA-based matrices were also reported to selectively bind to vascular endothelium growth factor (VEGF) (Lim et al. 2016) and release VEGF by mediated MMP degradation (Jha et al. 2016). Endothelial cells encapsulated in HA-dextran

hydrogels containing VEGF were able to form a functional vascular network integrated to the host-vascular system (Portalska et al. 2014).

HA hydrogels also upregulated the expression of angiogenic markers such as VEGF and FGF-2 in fibroblast and endothelial cells, resulting in a neovascular response in vitro (Ciccone et al. 2019). Scaffolds made of a microfibrous blend of poly(L-lactide-co-ε-caprolactone) and HA also increased CD34 expression of endothelial cells, resulting in the formation of small vessels (Kenar et al. 2019). Endothelial cells also increased their CD31 expression [also called platelet endothelial cell adhesion molecule (PECAM-1)], a marker for endothelial cell junctional integrity and vascular permeability barrier, when cultured on HA-modified collagen nanofibers for a vascular tissue-engineered scaffold (Kang et al. 2019). Furthermore, higher expression of CD31 by endothelial cells, when exposed to HA, shows immunomodulatory properties, as it inhibits the circulation of leukocytes (Lertkiatmongkol et al. 2016). HA-modified collagen nanofibers also promoted lymphatic endothelium formation by increasing the expression of lymph vessel endothelial hyaluronan receptor-1 (LYVE-1) (Gao et al. 2019).

Additionally, other strategies can be used to tackle hypoxia thus providing early oxygenation to the graft. For example, HA hydrogels functionalized with perfluorocarbon moieties (fluorinated oxadiazole) improve cell oxygenation and promote fibroblast growth under hypoxic conditions (Palumbo et al. 2014).

5 Immunomodulatory Properties of HA-Based Scaffolds

Combinatorial libraries of biomaterials and chemical modification offer a good strategy to determine optimum material selection for mitigation of the immune response while allowing the diffusion of nutrients, therapeutic agents, and cell wastes; see Fig. 25 (Vegas et al. 2016).

The immune system is a defense mechanism against environmental threats and pathogens; it can be divided in the innate and adaptive immune system. When the immune system experiences a biological imbalance, several chronic and acute inflammatory conditions can be triggered, as well as the development of several autoimmune conditions (Zamboni et al. 2018).

The immune system also plays an important role in the success of cell and organ transplantation. Normally, when an injured or failing organ needs to be repaired or replaced using allogenic (stem or somatic cells) and xenogeneic sources available, these foreign cellular sources are recognized by the host immune system leading to its rejection. However, the immune system is not the only player during an inflammation process; the remodeling of the ECM plays an important role during inflammation by promoting immune cell activation, tissue invasion, and destruction (Hallmann et al. 2015; Sorokin 2010).

The ECM is the noncellular component of all tissues, responsible for the provision of physical support to cells and for the regulation of diverse cell functions through biochemical and biomechanical cues. There are two major types of ECM, each with distinct architecture and composition: (i) interstitial matrices that surround

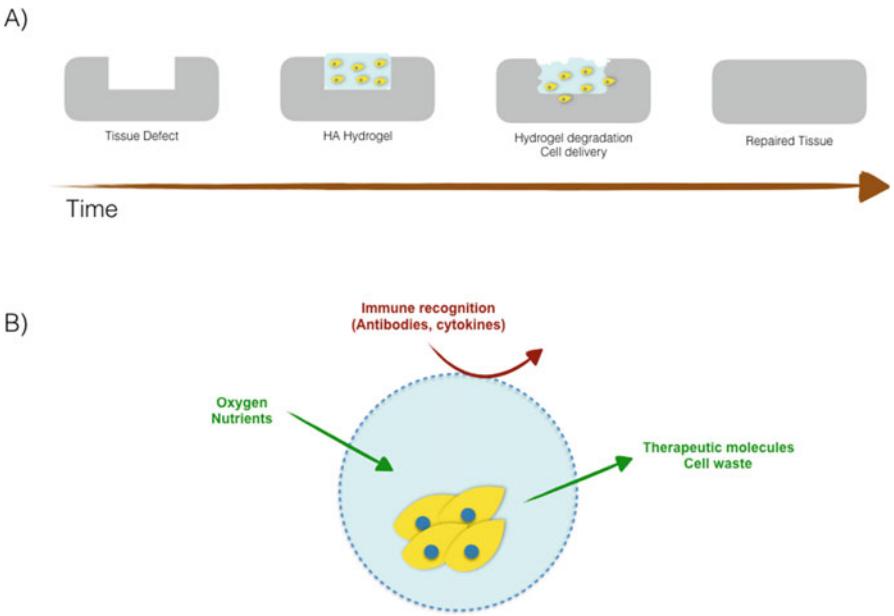


Fig. 25 Schematic representation of an encapsulation system for cells delivery, immunomodulation, and immunoprotection. (**a**) Cells are delivered by a carrier (e.g., hydrogel, microparticles, and microcapsules) and then are able to migrate to the diseased tissue/organ under controlled or stimuli-responsive release, and (**b**) cells are embedded within a semipermeable membrane that protects the biological material from immunological recognition while permitting the inflow of ant outflow of key elements for cell survival and function

cells, providing mechanical support; and (ii) pericellular matrices, which are in closer contact with cells (e.g., basement membranes), protecting them from rupture (Theocharis et al. 2016). Essentially, ECMs are composed of water and fibrous-forming proteins, such as collagens, elastin, and fibronectin; glycoproteins; proteoglycans (PGs); and glycosaminoglycans (GAGs) (Theocharis et al. 2016).

ECM remodeling plays an important regulatory role over the immune system. The degradation of ECM components by proteases fine tunes inflammatory responses by the generation of bioactive fragments called matrikines. Matrikines act as damage-associated molecular patterns (DAMPs) which are responsible for the activation of the immune system (Zamboni and Collins 2017). In order to avoid transplant rejection, the development of biomaterials, which are capable of intervening in immunological processes to achieve therapeutic results, is of critical importance in the overall aim to prevent or reverse the development of immune-mediated cell attack.

During HA remodeling, HMW-HA is degraded into LMW-HA; these HA fragments promote leukocyte recruitment by the activation of toll-like receptors (TLRs). However, when hyaladherin proteins crosslink HA into stable structures, they enhance CD44 binding on lymphocytes. Tumor necrosis factor stimulated gene

6 (TSG-6) is a hyaladherin that has an important function in preventing HA degradation by inhibiting HYALs and enhancing HA binding to the cell surface receptor CD44 on lymphocytic cell lines (Baranova et al. 2011). The effect of TSG-6 on diabetes development has shown to delay autoimmunity and enhance tolerogenicity of cells (Kota et al. 2013). When HMW-HA alone or in association to hyaladherins binds to CD44 receptors on Tregs, it promotes its immune suppressive capacity by increasing the transcription factor Foxp3 expression and increasing the production of anti-inflammatory cytokines, such as IL-10 (Ruppert et al. 2014).

Fibroblasts are predominantly present in connective tissues, and they elicit immunological responses by producing proinflammatory cytokines and chemokines that aid the immune system to induce and recruit inflammatory cells (Bautista-Hernandez et al. 2017). Scaffolds of porous freeze-dried poly(lactide-co-glycolic) acid (PLGA) and 3D printed poly lactide acid (PLA) showed enhanced fibroblast proliferation when coated using HMW-HA hydrogels (Zamboni et al. 2017; Souness et al. 2018). Fibroblasts when activated secrete cytokines that modulate the immune system. Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a cytokine secreted from fibroblasts that induces monocyte differentiation as part of the immune response chain. HAs of varying MW elicit contrasting production of GM-CSF by fibroblasts. LMW-HA shows basal fibroblast production of GM-CSF, while HMW-HA was able to elicit a rapid spike in production of GM-CSF within 24 hours of incubation often associated with an acute inflammatory response (Zamboni et al. 2020).

Hyaluronidase administration for in vivo acute inflammation and sepsis treatment showed that HA fragmentation suppressed neutrophil infiltration and cytokine production (IL-1β and IL-6) (Pereira et al. 2020). HA oligomers were able to regulate macrophage polarization into the anti-inflammatory M2 subtype (Wang et al. 2019a). However, HMW-HA hydrogels have been reported to increase lymphocyte and T-helper cell proliferation and activation into an anti-inflammatory environment. T-helper cells increased the secretion of IL-10, a known anti-inflammatory cytokine, while decreasing the secretion of IL-2 and interferon gamma (IFNγ), known proinflammatory cytokines. In addition, they also increased monocyte differentiation into M2 macrophage subtype (Gomez-Aristizabal et al. 2016).

Moreover, the complement system is part of the innate immune system and comprises a series of proteins that are activated in response to a pathogen to enhance the ability of antibodies (adaptive immune system) and phagocytic cells (innate immune system) to fight a threat. Its modulation can be linked to autoimmunity and transplant rejection. Thus, complement modulation can impact transplant rejection. GAGs have the ability to modulate complement activation, which is highly dependent on sulfate and negative charge. For example, it has been observed that highly sulfated GAGs can promote C3 activation while little or no effect was seen with negatively charged GAGs, such as HA (Meri and Pangburn 1994). Interestingly, HA is also reported to modulate platelet activation during inflammation. Platelets are the main cellular effectors for hemostasis, but they also possess a plethora for intracellular mediators and surface receptors (such as integrins, CD44, and TLR) known for

their involvement in inflammatory processes. Platelets actively degrade HA from the surface of endothelial cells, via HYAL-2; when they are exposed to ECM of a disrupted vessel wall, they initiate inflammatory and angiogenic signaling by stimulating mononuclear leukocytes in the immediate microenvironment to produce cytokines such as IL-6 and IL-8 (Zamboni et al. 2018).

The immunological effect that a material can have is also influenced by the intrinsic relationship between structure and function properties, such as polymer molecular weight, concentration, degree of substitution, backbone chemistry, and crosslinking density. Porosity and pore size fine tune the permeability of a 3D scaffold. Permeability will dictate the diffusion and permeation rates of molecules (such as antibodies and cytokines) that impact the immune response. The stability of the scaffold structure can also impact the immune response, as degradation can release DAMPs.

Molecular weight cut-off (MWCO) determines the minimum molecular weight of a solute that is completely excluded by this 3D matrix. However, these values can lead to misinterpretation as the size of solutes with the same molecular weight can vary dramatically between polysaccharides and proteins. Hence, other parameters such as diffusion coefficients are considered more accurate to characterize permeation.

Permeation is a vital factor to consider for the success of tissue-engineered constructs in order to avoid graft rejection. It will prevent the inflow of molecules such as antibodies as small as IgG (150 kDa), cytokines and chemokines such as IL-1 (17.5 kDa) and CCL-2 (8 kDa), respectively, while allowing the diffusion of oxygen and LMW biological compounds such as glucose (180 Da) and carbon dioxide (44 Da), essential for the cellular survival and function. For example, higher polymer and crosslink concentrations correlate to decreased pore sizes, which creates matrices with smaller MWCO and slower diffusion rates (Weber et al. 2009; Bal et al. 2014).

The MW of HA is very important regarding new vaccine delivery systems. For example, studies evaluated the delivery of different HA MW (between 7 and 741 kDa) to the lungs of mice. Regarding the pharmacokinetic parameters of HA, lower HA MW showed more rapid systemic distribution, while 67 and 215 kDa HA showed longer persistence in the lungs. Lung exposure appeared to be optimum in this size range due to the rapid absorption of <67 kDa HA, poor lung penetration, and mucociliary clearance of viscous solutions of HA >215 kDa (Kuehl et al. 2016).

Beside MW, the crosslinking degree (CD) can also modulate the physicomechanical properties of HA viscosupplements (Lee et al. 2014). HA crosslinked with divinyl sulfone (DVS) showed that higher CD increased the viscoelasticity and the extrusion force, where HA:DVS 1:1 (CD = 90%) showed extrusion force of 14.9 N and HA:DVS 5:1 (CD = 14.2%) showed an extrusion force of 6.8 N. Futhermore, increased CD showed reduced swelling ratios (SR) (90% CD = 23.6% SR and 14.2% CD = 73% SR). HA hydrogels also showed Newtonian pseudoplastic behavior and were classified as weak (Shimojo et al. 2015).

Regarding the relationship between the crosslinking densities and the HA hydrogel degradation, studies have shown that higher crosslinking densities reduce enzymatic degradation, suggesting that the slower degradation rate of the HA is

associated with limited access of the enzyme to the active sites of the biopolymer chains due to the presence of large amounts of covalently incorporated crosslinker (DVS) (Lai 2014). The increased stability of the matrix, due to the slower degradation profile, benefits immunoprotection and immunomodulation in tissue engineering by decreasing the release of LMW-HA fragments that act like DAMPs to the environment.

Studies have documented the effects that HA induces in the immune system by analyzing proinflammatory gene and cytokine expression of IL-1β and IL-6 and nitric oxide (NO) produced by lipopolysaccharide (LPS)-induced macrophages. The results indicate that lower DVS (up to 50%) and 1,4-butanediol diglycidyl ether (BDDE) at concentrations of 0.5 and 1.0 ppm do not induce the expression of genes nor cytokines (Choi et al. 2015). These results are promising for tissue engineering applications, where null immunological response is required.

6 HA in Tissue-Engineering Applications

HA has been used extensively to regenerate many different tissue types. Here, we focus on emerging nerve (peripheral and central) and skin regeneration strategies.

6.1 Peripheral Nerve Regeneration (PNS)

Recent developments on the use of HA for nerve regeneration applications comprise the delivery of neural stem cells embedded in HA matrices. Farrel et al. (2017) reported on injectable uncrosslinked HA as a suitable candidate for in situ delivery of neural stem cells (NSCs). The resulting gel, a blend composed by HA, Type-1 collagen, laminin, and chondroitin sulfate, maintained its integrity after 10 days but was rapidly degraded in the presence of HYAL (between 2 and 6 h). Interestingly, NSCs' differentiation toward neuronal or glial cells was driven by the relative concentration of the components present in the blend. Anti-FAS-conjugated HA microsphere gels were also reported to deliver NSCs (Shendi et al. 2017), protecting cell microspheres from deleterious cytokines. Moreover, the presence of anti-Fas motif decreased cell viability of inflammatory T cells, inducing their apoptosis. As T cells are the principal responsible for NSCs' death after transplantation, these HA-based microspheres work as cell vehicle and immunomodulators. In an experimental study for peripheral nerve scarring, the topical application of HA on adult Wistar rats at 4 and 12 weeks displayed significant reduction of neural scar thickness. Histomorphologic analysis and electrophysiological attributed this to the retardation of lymphocyte migration, degranulation, and chemotaxis. HA allowed the regeneration of nerve by inhibiting the epineural and extraneural scar formation at the repair site of the peripheral nerve (Ozgenel 2003). K. Ikeda et al. have conducted an experiment on rabbits to show HA can inhibit the adhesion of peripheral nerve on a rabbit model. At 6 weeks of histological and electrophysiological study, it was shown that HA can effectively reduce scar tissue after neurolysis (Ikeda et al. 2003).

Studies have shown that electrical stimulation aids the process of nerve repair. With this in mind, HA-based tubular scaffolds were seeded with Schwann cells with 450 μA and 900 μA current intensities applied to study the increase of cellular activity. Results show that 450 μA accelerate the cell number (Ramos et al. 2018). A scaffold composed of HA-collagen composite incorporated with neurotrophin-3 and BrdU-labeled neural stem cells conduit was inserted at the facial nerve ends of rabbits to study peripheral nerve injuries. At 12 weeks, it was found from the electrophysiology study that the composite incorporated with neural stem cells facilitates the reinnervations of the damaged facial nerve (Zhang et al. 2008).

6.2 Central Nervous System (CNS)

The CNS has very limited capacity for regeneration, particularly in the spinal cord, caused primarily by reactive activation of astrocytes and the presence of reactive oxygen species (ROS) microenvironment following injury (Zhang et al. 2020). Spinal cord injury (SCI) is a common CNS injury, often resulting in the paralysis of the patient at or below the injury site, caused by a primary injury to the spinal cord by means of traumatic contusion or laceration.

Upregulation of the surrounding cells and inhibitory factors, such as astrocytes and chondroitin sulfate proteoglycans, result in the formation of a glial scar around the periphery of the cyst. This scar acts as a barrier, both physically and chemically, to the reestablishment of axonal and neuronal tracts in the spinal cord (Liu et al. 2013). With limited and often ineffective treatment outputs, TE strategies for SCI repair have been explored by the research community. Among many biomaterials used for this treatment, HA shows promise. HA plays an important role not only as a CNS ECM component by influencing the viscosity of the tissue due to its affinity to bind with water, but also as a cell behavior modulator in the CNS by means of cell surface receptors such as CD44 or RHAMM (Gaudet and Popovich 2014; Burnside and Bradbury 2014; Toole 2004).

The molecular weight of HA appears to also play a vital role in the cellular response in the CNS. In SCI studies, the addition of HWM-HA prevents astrocyte proliferation, and LWM-HA promotes differentiation of cells, while degradation of the HA in the ECM by means of hyaluronidase promotes astrocyte proliferation (Struve et al. 2005; Khaing et al. 2011; Seidlits et al. 2019). These results have the potential to be utilized in various CNS repair strategies, especially in the inhibition of glial scar formation.

Traditional TE strategies focus on utilizing HA as a scaffold material, often utilizing the lyophilization technique to achieve scaffolds with controlled porosity and orientated channels for guided neuronal regrowth. The morphological aspect of a scaffold is crucial to the survival and proliferation of cells by allowing nutrient diffusion throughout the scaffold matrix. HA and PLLA fiber scaffolds studied by Martinez-Ramos et al. were created by first lyophilizing HA scaffolds with 400 μm poly-ε-caprolactone fibers in order to create channels within the scaffold. After lyophilization, the fibers were removed, and 20-30 μm PLLA fibers were placed in

the created channels. The scaffold was seeded with spinal cord-derived ependymal progenitor cells (epSPC). In vivo results indicated that the scaffold improved the regrowth of neuronal fibers at the epicenter of rat SCI lesions and reduced astrogliosis, though a longer study is recommended by the authors (Martinez-Ramos et al. 2019). Though an alternative technique for the fabrication of scaffolds for CNS repair could utilize 3D printing techniques, the use of HA as the printing material for such an application has not been thoroughly explored by the research community to date.

In other strategies, HA is often utilized as an injectable hydrogel which gels under physiological conditions by means of chemical modifications (Ho et al. 2019; Gupta et al. 2006; Fuhrmann et al. 2015). Führmann et al. combined HA with methylcellulose, RGD peptide, cell survival and differentiation factor PDGF-A, and human-induced pluripotent stem cell-derived oligodendrocyte progenitor cells (hiPSCs-derived OPCs). Injected into SCI lesions in rat in vivo models with the hydrogel gelling in situ, the transplanted cells in the hydrogel expressed OPCs migration to the lesion site, where the potential myelination of axons could occur. This is attributed to the promotion of cell attachment and hence cell survival by means of the RGD peptide. Also, at 9 weeks postinjury, the transplanted cells expressed markers associated with astrocytes (glial fibrillary acidic protein-GFAP) and OPCs (SOX10), though the presence of a teratoma in a number of the studied rats prevented extensive analysis (Führmann et al. 2016). The HA and methylcellulose hydrogel was further expanded in a study by He et al. A key aspect of any TE material is the retention of the desired shape and geometry in vivo to not induce any additional damage to the surrounding tissue with material swelling. In the study, the injected HA hydrogel swelled to only 15% in situ. In addition, when examining the tissue histology, it was reported that the presence of the hydrogel inhibited the expression of proinflammatory cytokines TNF-α, IL-1β, and IL-6 and upregulated the levels of anti-inflammatory cytokines, thus promoting the inhibition of glial scar formation (He et al. 2019).

The combination of the RGD peptide and HA has also been explored by Zaviskova et al. Hydroxyphenyl derivative of HA combined with RGD, fibrinogen, and Wharton's jelly-derived mesenchymal stem cells (hWJ-MSCs) was tested in vitro and in vivo in rat SCI models. Observed in vivo results have shown an infiltration of axons and blood vessels into the hydrogel-filled lesion site. GFAP staining showed the migration of astrocytes from the lesion periphery to the hydrogel lesion site, indicating that the material's presence is permissive toward glial cell infiltration. Though the authors did not observe any hWJ-MSCs cells 8 weeks posthydrogel implantation, it is speculated that neurotrophic and immunomodulatory effects can be observed for a long period of time postinjury due to the release of trophic factors by the initial presence of the transplanted cells at the site of injury (Zaviskova et al. 2018).

An addition of growth factors into the HA hydrogel as a combinatory TE strategy has also been explored. Xie et al. combined sodium hyaluronate with ciliary neurotrophic factor (CNTF) and tested the efficacy of this material in vivo in rat SCI lesions. Postinjury injections of BrdU aimed at labeling dividing cells showed

that Nestin+ and BrdU+ cells were observed in the lesion site of the HA-CNTF tested group, indicating the activation and migration of endogenous neural stem cells (NSCs) into the lesion site. New-born Tuj1+ & BrdU+ immature neurons and new-born NeuN+ & BrdU+ mature neurons were also observed in the lesion area 30 days post-SCI. Interestingly, Dil (a lipid membrane dye which labels ependymal cells) positive cells were observed in the parenchyma outside the central canal, with this result not being replicated in the control lesion SCI model. This indicates that the presence of the HA-CNTF hydrogel prompted the activation and migration of ependymal originating NSCs (Xie et al. 2018).

Novel HA-based hydrogels for CNS TE investigate the combination of HA as a biomaterial with novel molecules to further prompt and aid in the regenerative capacity of nerves. One recent study combined HA with 2,6,6-tetramethylpiperidinyloxy (TEMPO) with the aim of reducing the ROS environment in the SCI lesion site and promote regeneration. The results showed a positive ratio of NF (neurofilament marker) to GFAP, suggesting that the ROS environment was reduced, further evidenced with an improvement in the Basso, Beattie and Bresnahan (BBB) locomotor-rating scale scores (Zhang et al. 2020).

Recent biomedical engineering strategies for CNS repair have focused on the utilization of neural stem cells and biomolecules, though nowadays this strategy alone is not as popular due to the hostile nature of the environment around the lesion site, which prevents stem cell proliferation and differentiation (Mothe and Tator 2012). Therefore, more emphasis is being placed on tissue engineering strategies which support and promote cell growth within this specific stem cell environment.

The utilization of HA as a cell encapsulation material has shown promising results in SCI models. A study by Zarei-Kheirabadi et al. utilized a commercially available HA kit (HyStem-C Cell Culture Scaffold Kit) as the hydrogel material to encapsulate human embryonic stem cell derived neural stem cells (hESC-NS). After 1 week post-SCI in rat in vivo models, hESC-NS/HA hydrogel was injected into the lesion site. GFAP expression was lower than in the hESC-NS cell only group, indicating that the utilization of HA reduces the presence of the glial scar around the lesion site by inhibiting astrogenesis. The differentiation of the hESC-NS cells in the presence of the HA into oligodendrocytes was also elevated, suggesting that HA promotes oligogenesis, though maturation of oligodendrocytes appears to be inhibited by the presence of HA by means of TLR2 signaling (Zarei-Kheirabadi et al. 2020; Sloane et al. 2010).

Overall, the utilization of HA as a hydrogel material for CNS TE strategies appears to be promising, with HA playing a dual role as both an ECM environment component as well as providing complex cellular cues to the surrounding cells. The more that we study this material in CNS ailments, the more informed we can be as to how we can tailor the body's response to achieve the desired regenerative result.

6.3 Skin

HA plays a major role in hydration of the surface of the skin (Papakonstantinou et al. 2012). Full epidermal-dermal-based scaffolds have been developed using a porous HA

scaffold as the dermal component and poly-L-Lysine as the epidermal component utilizing a spray-assisted layer-by-layer assembly technique, with keratinocytes cultured on top of the epidermal layer which assists cell adhesion, and regeneration of skin layers (Monteiro et al. 2015). Hydrogels incorporated with hyaluronic acid have been utilized to treat second-degree burn wounds (Koul et al. 2016). Gelatin hydrogels in combination with hyaluronic acid, chondroitin sulfate, and asiatic acid with nanoparticles like ZnO and CuO were evaluated in Wistar rats with a second-degree burn wound and at 4 weeks, histopathology results revealed better healing of burn wounds in comparison with commercially available wound dressings (Thanusha et al. 2018), and these gels can also function as a skin substitute (Av et al. 2020). In another study with electrospun-based scaffolds composed of cationic gelatin, HA, chondroitin sulfate and sericin were used to coculture human-derived keratinocytes and mesenchymal stem cells (hMSCs). After 5 days of coculture in vitro, results showed that there was epithelial differentiation from hMSCs with expression of dermal protein markers (Bhowmick et al. 2016). An artificial skin-equivalent nanofibrous substitute was developed using HA and collagen with the programmed release of multiple angiogenic growth factors such as platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), endothelial growth factor (EGF), and basic fibroblast growth factor (bFGF) either encapsulated in the gelatin nanoparticles or directly embedded in the nanofibers by electrospinning technology. The tensile strength of these Col-HA-GN scaffolds was similar to that of human skin. In vivo studies were conducted on streptozotocin-induced diabetic rats; histopathology and immune-histochemical analysis revealed that faster epithelization rates and enhanced maturation of vessels were associated with scaffolds (Lai et al. 2014). In another study, an artificial nanofibrous skin substitute was developed using mPEG-PCL-grafted gelatin and incorporated with HA, chondroitin sulfate, and sericin to study the in vivo efficacy of second-degree burn wound model on Wistar rats. The results showed that at 21 days, wounds were contracted followed by collagen upregulation (Bhowmick et al. 2018). Thus, HA-based scaffolds play an important role in wound healing and dermal tissue-engineering applications.

7 Antimicrobial Properties of HA for Tissue-Engineering Strategies

Medical device-related infections pose a huge financial burden on healthcare services and are associated with increased patient morbidity and mortality (VanEpps and Younger 2016). For patients, infections come alongside pain, inflammation, fever, and long antimicrobial treatment regimens. Medical device-related infections can also lead to more serious conditions such as the risk of sepsis (spread of infection to blood and other organs), with this being potentially fatal. The development of tissue-engineered substitutes that possess intrinsic antimicrobial activity against the three main classes of microbes (bacteria, fungi, and virus) is vital for the success of implantable devices.

There is a dire need for new diagnostic and therapeutic strategies to combat infectious diseases. As a multidisciplinary science, tissue engineering focuses on the application of engineering principles to the biological systems for the rapid translation of technologies from the benchtop to the bedside. In the current situation we are all facing with the viral outbreak of COVID-19, tissue-engineering techniques and tools can potentially enable virologists to create infection models that combine the facile manipulation and readouts of three-dimensional tissue cultures relevant for the viral infection complexity of the animal model (Ramanan et al. 2014), as well as the development of new vaccine technologies and small-molecule drug delivery systems (Tatara 2020).

7.1 Antibacterial Activity of HA

Bacteria is a type of prokaryotic unicellular organism, and one of the world's oldest forms of life. Some bacteria live in communities embedded in a matrix consisting of extracellular polymeric substances (EPS), i.e., protein, DNA, and polysaccharides (Bhattacharya et al. 2015). Biofilm-related infections and contamination of materials are major problems encountered in medicine (Lynch and Robertson 2008). Recent studies indicate that bacterial contamination in open wounds may adversely affect the formation of bone and new connective tissue. Reduction of bacterial burden at the wound site is of importance to improve the clinical outcome of regenerative therapy. In surgical settings associated with implanted biomaterials, *Staphylococcus aureus is* a leading cause of chronic biofilm infection, and this largely impacts orthopedics, trauma, and cardiology. Treatment of these infections requires long antibiotic administration and in some cases additional surgeries, which often leads to the removal of the infected device (Ibberson et al. 2016). Several investigators have taken an interest on the influence of HA on bacteria and how it relates to invasion and virulence.

Among various polymers tested for antibacterial coatings, HA and its derivates have proved promising offering long-term safety with the ability to reduce bacterial adhesion and biofilm formation (Romanò et al. 2017). HA has been shown to exert bacteriostatic, but no bactericidal, dose-dependent effects on different microorganisms in the planktonic phase. Concerning to possible orthopedics applications, the analysis of different coatings on titanium surfaces showed that HA significantly decreased *S. aureus* adhering and density to these titanium surfaces, unveiling its potential application in osteosynthesis, orthopedics, and dental surgery (Harris and Richards 2004) as well as beyond to tissue-engineering applications.

HA-molecular weight (MW) and concentration seem to interact with bacteria and trigger various growth profiles. Three HA MW (low = 0.14 MDa, medium = 0.76 MDa, and high = 1.3 MDa) were tested on *S. aureus* ATCC 9996, *Streptococcus mutans* ATCC 10449, and *Propionibacterium acnes* UD, showing reduction of proliferation in media containing HA; moreover, the medium MW showed the highest bacterial growth inhibition (Pirnazar et al. 1999). Regardless of HA MW and concentration, no bactericidal effects were detected.

Another study evaluated the antimicrobial property of collagen, hydroxyapatite (HAp), and PLGA and HYAFF-11™ (esterified HA produced by Fidia Advanced Biopolymers) on *S. aureus* (ATCC 25923), *S. epidermidis* (ATCC 12228), and β-hemolytic *Streptococcus (*ATCC 19615) cultures (Rohr and Trepp 1996). In this study, HA suppressed bacteria growth rate in comparison to other materials. The HA effect is due to the saturation of the bacterial hyaluronidase by excess HA, which prevents the bacteria from maintaining elevated levels of tissue permeability (Carlson et al. 2004). Hyaloss ®, a hydrogel matrix composed of HYAFF™ for the treatment of periodontal defects, showed improved bone formation with no bacterial infection due to the bacteriostatic effect of the low MW HA (Ballini et al. 2009). Hyruan Plus® (linear high MW HA (3 MDa) produced by LG life sciences, Iksan, South Korea) was shown to increase wound healing exhibiting bacteriostatic effects in surgical wounds inoculated with *S. aureus* (SC 2406) (Park et al. 2017).

Seprafilm ® (high MW HA gel and carboxymethylcellulose (CMC) at a 2:1 weight ratio, Genzyme Corporation, Japan) displayed antimicrobial activity at two concentrations in *S. aureus* ATCC 27217 cultures, as the concentration of HA in the bacteria culture increased the optical density (OD) of the bacteria medium decreased, in accordance with a higher bacteriostatic effect (Uchida et al. 2011).

To penetrate the ECM, pathogenic gram-positive bacteria secrete hyaluronidase to cleave HA. *S. aureus* produces hyaluronidase, encoded in the *hysA* gene, that is conserved in all *S. aureus* lineages. *HysA* encoding is often shown as a virulence factor in a variety of infection models, which is due to its role in facilitating local spread of the infection (Ibberson et al. 2016). In a context, two strains of *S. aureus* (a wild type *hysA* positive and a mutant type *hysA* negative) were cultured in a catheter implanted in a murine model. *S. aureus* that produced *hysA* (wild type) was more invasive and increased lesion distribution and severity in comparison to the *S. aureus* that was unable to produce *hysA* (mutant type) (Ibberson et al. 2016). Additionally, biofilm formation in vitro was increased in mutant *S. aureus* when adding HA with MW above 50 kDa to the culture medium (Ibberson et al. 2016).

In addition, antimicrobial studies using other gram-positive bacteria from the streptococcus and enterococcus species show that high MW HA (1.8 MDa) against *Streptococcus mutans* ATCC 25175, *Enterococcus faecalis* ATCC 29212, and *Enterococcus hirae* ATCC 10541 possesses dose-dependent bacteriostatic effects (Ardizzoni et al. 2011). *Bacillus subtilis* ATCC 6633 and *S. aureus* ATCC 6538 also showed susceptibility to polyethyleneimine-modified HA particles, although these novel particles were more effective against *B. subtilis* (Sahiner et al. 2017).

A porous scaffold made of HA with approximately 125 kDa was produced and tested against *S. aureus* ATCC 6538 for antimicrobial properties. The results showed that HA has bacteriostatic effects by decreasing the colony formation units (CFU) from $\times 10^7$ (control) to 1.1×10^3 (HA) (Guzińska et al. 2018).

Hyabest® (S) LF-P, a food supplement containing HA with MW ranging 250–400 kDa (produced by Kewpie, Tokyo, Japan) was used to synthesize novel hydrogel for microneedle application. Antibacterial activity was observed against *S. aureus* (ATCC 6538) (Larraneta et al. 2018).

Escherichia coli is the most thoroughly studied species of bacteria, which has long been the favored organism for investigation of the basic mechanisms of molecular genetics (Blount 2015). Several *E. coli* strains are pathogenic. Seprafilm® also showed bacteriostatic effect against *E. coli* ATCC 25922 in a concentration-dependent manner (Uchida et al. 2011). A porous scaffold made of HA with approximately 125 kDa was produced and tested against *E. coli* ATCC 11229 for antimicrobial properties. The results showed that HA has bacteriostatic effects by decreasing the colony formation units (CFU) from 3.9×10^7 (control) to 50 (with HA) (Guzińska et al. 2018). Other antibacterial studies using *E. coli* also showed bacteriostatic effects of high MW HA with approximate 1 MDa (del Hoyo-Gallego et al. 2016; Pérez-Álvarez et al. 2019).

Other gram-negative bacteria (*Porphyromonas gengivalis* ATCC 33277, *Prevotella oris* ATCC 33573, and *Actinobacillus actinomycetemcomitans* Y4) were tested against three HA MWs (0.14, 0.76, or 1.3 MDa). Each bacteria showed distinct growth inhibition indexes, where *A. actino* showed higher growth inhibition in comparison to *P. oris* and *P. gingivalis*, respectively (Pirnazar et al. 1999).

Another study evaluated the antimicrobial property of collagen, hydroxyapatite (HAp), PLGA, and HYAFF-11™ on *Pseudomonas aeruginosas* (ATCC 27853), showing that HA significantly suppressed bacteria growth rate in comparison to the other materials (Carlson et al. 2004). Hyabest® (S) LF-P also showed antibacterial activity against *Proteus mirabilis* (ATCC 35508) (Larraneta et al. 2018).

Mycobacteria invade the lungs through the interaction with GAG, such as HA. Three strains of mycobacteria (*M. tuberculosis* H37Rv, *M. smegmatis* mc^2155, and *M. avium* type 4) were used to determine the influence of HA on infection and disease. Interestingly, HA promotes mycobacteria invasion and proliferation (Hirayama et al. 2009).

HA has been widely used to encapsulate a wide range of drugs, including antibiotics. Aminoglycoside antibiotics (such as streptomycin) are highly hydrophilic. This pose pharmacological challenges, particularly in the treatment of intracellular bacterial infections. Due to its high hydrophilicity, it has poor penetration within the eukaryotic cell membranes; often high doses of antibiotics still display subtherapeutic concentration inside the cell (Maurin and Raoult 2001). It was speculated that HA could be an antibiotic carrier for the treatment of intracellular bacterial infections. Antibiotics conjugated to HA were able to be phagocytize by infected eukaryotic cells through a CD44-mediated pathway, and reduce bacterial infection burden intracellularly (Qiu et al. 2017).

7.2 Antifungal Activity of HA

Fungi are a type of eukaryote. They can be single-celled, like yeast, or multicellular, like mold. Unlike the antimicrobial activity of HA in bacteria, only a few studies have been conducted to assess the effects of HA on fungi. Intrinsic antifungal properties of HMW HA (1.8 MDa) were demonstrated against *Candida glabrata* ATCC 90030 and *Candida parapsilosis* ATCC 22019. The fungistatic activity of HA was reported to be dose dependent (Ardizzoni et al. 2011).

7.3 Antiviral Activity of HA

The dilemma about viruses is if they are living organisms or not. Many feel that viruses are not live organism as they cannot replicate without other cells. Viruses target all domains (prokaryote and eukaryote cell types) containing DNA or RNA as their genetic material. They need the host cell machinery to replicate, leading to host-cellular death.

Despite so many studies on the antimicrobial activity of HA in bacteria, only a few studies have been conducted to assess the effects of HA on viruses. In vitro evidence of antiviral activity of HA has been shown with respect to herpes simplex virus (HSV-2) (Tiunnikov et al. 2002), although other reports on HSV-1 showed that HA appears to contribute to HSV-1 infection of both the brain and the skin tissues (Cohen et al. 2011).

Other studies evaluated the levels of HA produced by some rheumatoid arthritis (RA) cell lines, some of which were partially or completely resistant to infection with Newcastle disease virus (NDV), vesicular stomatitis virus (VSV), and rubella virus (RV). Normal fetal synovial cells lines were susceptible to NDV, VSV, and RV. Once infection-resistant RA cell lines were treated using hyaluronidase, viral infection became possible. Moreover, HA prevented infection of normal fetal synovial cells with VSV (Patterson et al. 1975).

Another study has shown that high MW HA (1.800 KD) demonstrated strong antiviral activity against Coxsackievirus B5 (COXB5), mumps virus (MV), and influenza virus A/H1N1; mild antiviral activity was shown against HSV-1 and porcine parvovirus (PPV), and no activity against Adenovirus 5 (ADV-5), human Herpesvirus-6 (HHV-6), and porcine reproductive and respiratory syndrome virus (PRRSV) (Fig. 26). In all cases, no virucidal activity of HA was observed (Cermelli et al. 2011). Recently, a cyclodextrin-conjugated HA was able to encapsulate acyclovir, showing good antiviral activity together with a delayed-release of acyclovir (Piperno et al. 2019).

7.3.1 Highlight on COVID-19 and Other SARS

In the first months of 2020, the world was taken by surprise by a fast spreading virus, which put most countries and economies into lockdown for a number of months. The pandemic is still far from being over, and new waves of infection in the coming months are expected. Severe acute respiratory syndrome (SARS)-CoV-2 is the virus responsible for the corona virus disease (COVID-19), which originated in Wuhan, China (Colson et al. 2020). To date, the scientific community is working relentlessly to find suitable drugs to halt the spread of the virus as well as the safe development of a new vaccine. Tissue-engineering skillsets and tools may be leveraged to have an impact on clinical practice in settings of a viral outbreak, such as in recent COVID-19 (Fig. 27).

A number of antiviral drugs such as lopinavir and ritonavir (normally used in the treatment of human immunodeficiency virus – HIV) and other antiretroviral (such as Favipiravir) and Remdesivir (originally considered for the Ebola virus, Middle East Respiratory Syndrome – MERS, and SARS), in addition to

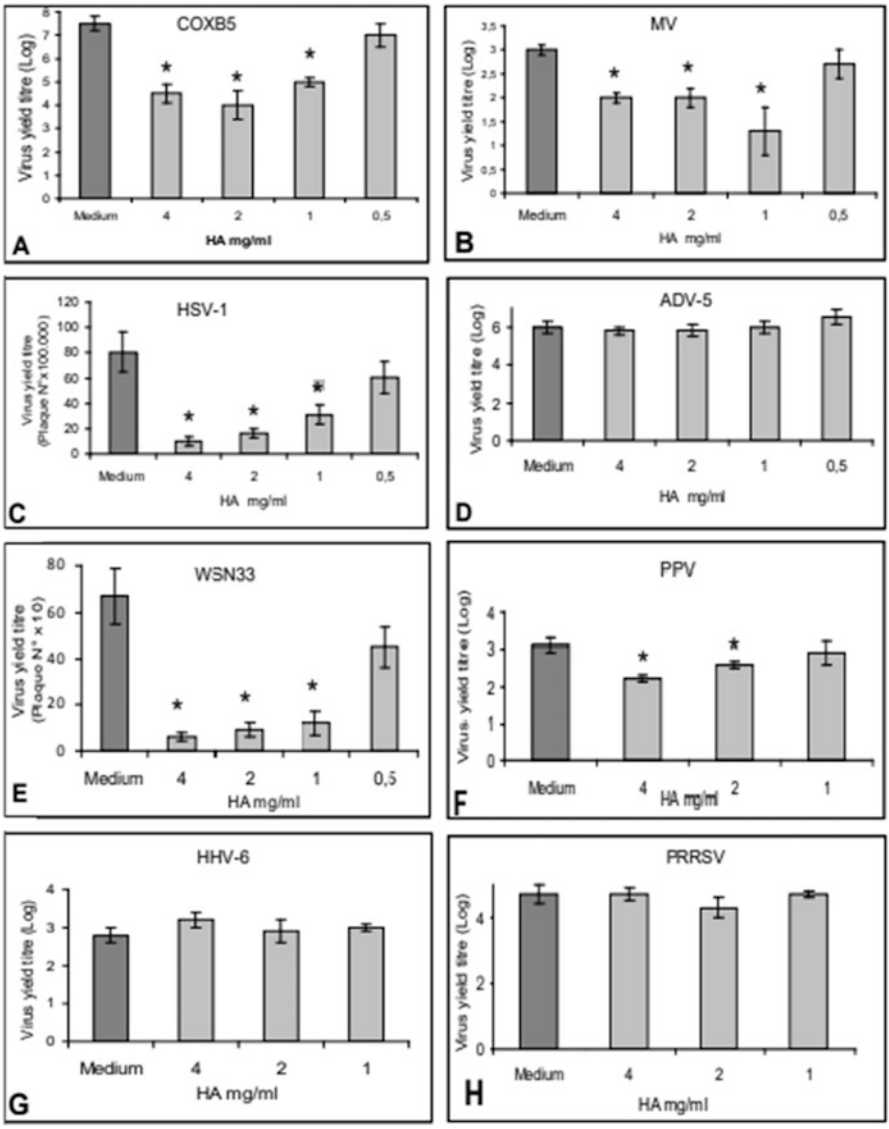


Fig. 26 Virus yield of various infected cell lines after exposure to HA in different concentrations. (**a**) VERO cells infected with COXB5, (**b**) VERO cells infected with MV, (**c**) VERO cells infected with HSV-1, (**d**) VERO cells infected with ADV-5, (**e**) WSN33, (**f**) PK15 cell line infected with PPV, (**g**) JJHAN cell line infected with HHV-6, and H) MARC145 cells infected with PRRSV. [Open access reprinting (Cermelli et al. 2011)]

hydroxychloroquine used in the treatment of malaria, show promise in the treatment of COVID-19 (Cao et al. 2020; Gautret et al. 2020). However, these kinds of drugs show systemic toxicity. Drug modification, by the conjugation of HA, seems

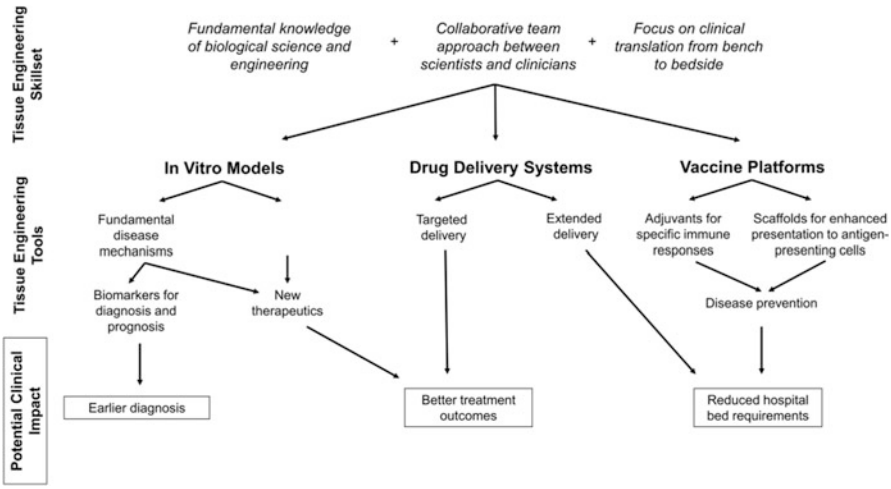


Fig. 27 Proposed examples of tissue-engineering strategies to fight viral outbreaks (Tatara 2020)

like an alternative in order to reduce toxicity and increase efficacy (Khan and Aroulmoji 2020).

Interestingly, HA has been described as a potential cause of fatalities for COVID-19 infections. This reasoning is originated from patients with severe conditions accompanied with cytokine release storm (CRS), which induces an hyperactive immune response leading to acute respiratory distress syndrome (ARDS) and lung damage (Mehta et al. 2020). The symptoms of ARDS in patients include short/rapid breathing, and cyanosis. Most of these patients are allocated into intensive care units requiring mechanical ventilators. CT images of the lungs of these patients show characteristic white patches called "ground glass." Autopsies have confirmed a clear jelly substance lining the lungs of these patients. Although the nature of this gel is not yet confirmed, it is speculated that it contains HA (Shi et al. 2020). On the mucosal surface of the airways of healthy individuals, HA retains bactericidal enzymes in order to protect the mucosa tissue from invading pathogens. However, upon infection, low-MW HA fragments are released to stimulate an immunological response and start inflammation. After resolution of inflammation, local macrophages eliminate HA via CD44-mediated receptor (Hirayama et al. 2009).

The assumption that "ground glass" contains HA is originated by the defective production and regulation of HA during COVID-19 and other SARS infections (Fig. 28). Hyaluronidase 3 (HYAL-3) gene is shown to be upregulated in human monocytic cells infected with COVID-19 (Hu et al. 2012). It is well known that the degradation of HA into small fragments induces the immune response milieu (Zamboni et al. 2018). We speculate that the inhibition of upregulated HYAL-3 could potentially improve ARDS. One well-known HYAL inhibitor is hyaluromycin (Kohi et al. 2016). Alternatively, a further potential mechanism to be exploited could be the inhibition of hyaluronic acid synthase (HAS). The production of HA can be

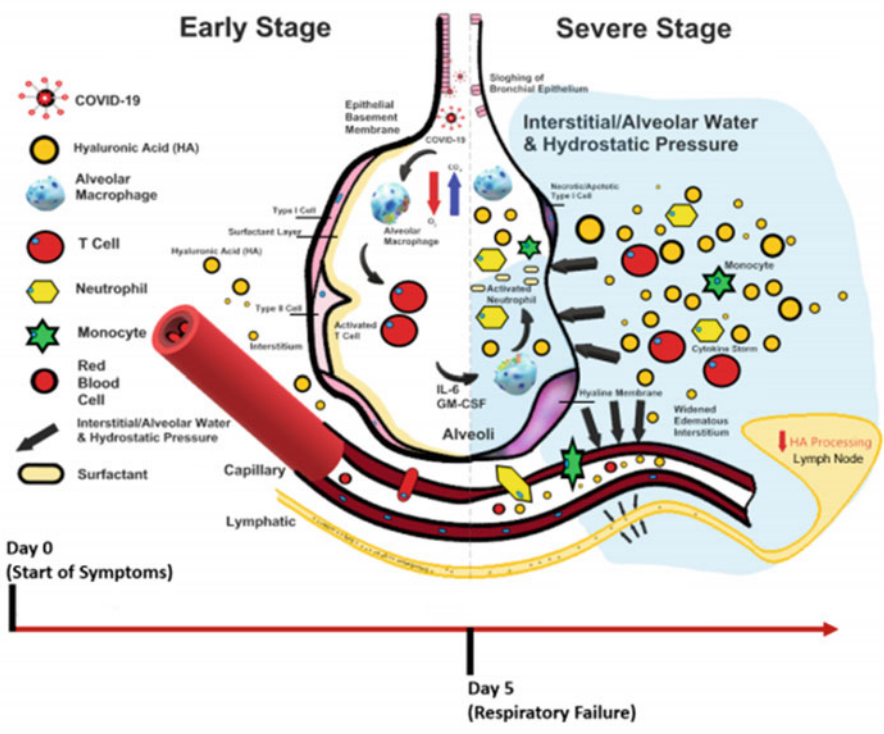


Fig. 28 Schematic of the progression of COVID-19 infection with HA accumulation in the lungs. (Modified from Mong et al. 2020)

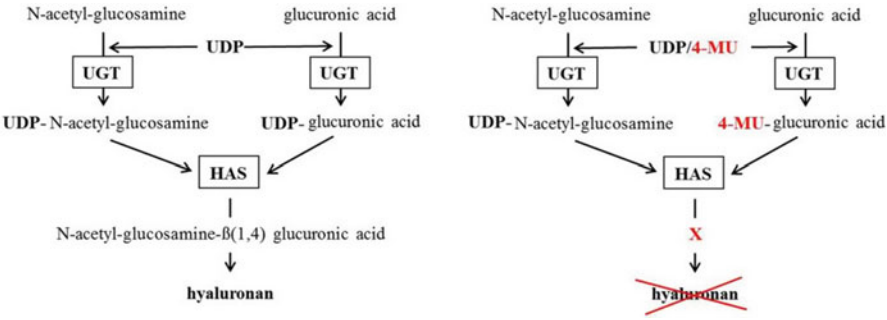


Fig. 29 Mechanism of action of 4-methylumbelliferone on HAS by inhibiting HA production (Nagy et al. 2015)

specifically inhibited by the use of 4-methylumbelliferone (4-MU) (Sukowati et al. 2019). 4-MU inhibits HA production by competing with uridine 5′-diphosphate (UDP) as the substrate for UDP-glucurosyltranferase (UGT), an enzyme involved in the HA synthesis (Fig. 29). 4-MU is a drug already in the market by the name of

hymecromone; it is a derivative of coumarin (warfarin); however, it does not possess anticoagulant properties. Its typical dose regimen for the treatment of biliary dyskinesia ranges around 300–800 mg three times/day (Nagy et al. 2015). We speculate that the development of a new topic delivery system for 4-MU, directly to the lungs by inhalers, could be of great impact in the treatment of ARDS in COVID-19 patients.

8 Conclusions and Future Outlook

The optimal bioengineered microenvironment is envisaged to mimic the native ECM and integrate different biomaterials and biofunctional molecules to display synergic anti-inflammatory effects thus enhancing cell survival and function. As we have highlighted here, HA is showing promising results as material which is both capable of protecting cells from the immune response and capable of immune modulation. Due to its intrinsic anti-inflammatory and anti-immunogenic functions, HA can protect cells from lymphocyte-mediated cell killing. This coupled with its ease of chemical modification to protract degradation, tailor mechanical response, and couple to other biomolecules or therapeutics is allowing HA to become the material of choice for enabling emerging medical technologies which depend on immune regulation such as vaccines, gene therapy, and drug and cell delivery as well as associated regenerative medicine techniques.

Acknowledgments The authors would like to thank the financial support provided by following funding bodies:

Irish Research Council (GOIPG/2015/3577); European Union's Horizon 2020 research and innovation program under the Marie Sklodowska-Curie grant agreement No. 801165, with cofunding through SSPC by its funding body, Science Foundation Ireland, grant no. 112/RC/2275_P2.

References

Abu-Hakmeh A, Kung A, Mintz BR, Kamal S, Cooper JA, Lu XL, et al. Sequential gelation of tyramine-substituted hyaluronic acid hydrogels enhances mechanical integrity and cell viability. Med Biol Eng Comput. 2016;54:1893–902.

Acharya PS, Majumdar S, Jacob M, Hayden J, Mrass P, Weninger W, et al. Fibroblast migration is mediated by CD44-dependent TGF beta activation. J Cell Sci. 2008;121(Pt 9):1393–402.

Ahmadian E, Dizaj SM, Eftekhari A, Dalir E, Vahedi P, Hasanzadeh A, et al. The potential applications of hyaluronic acid hydrogels in biomedicine. Drug Res. 2020;70(1):6–11.

Antich C, de Vicente J, Jiménez G, Chocarro C, Carrillo E, Montañez E, et al. Bio-inspired hydrogel composed of hyaluronic acid and alginate as a potential bioink for 3D bioprinting of articular cartilage engineering constructs. Acta Biomater. 2020;106:114–23.

Ardizzoni A, Neglia RG, Baschieri MC, Cermelli C, Caratozzolo M, Righi E, et al. Influence of hyaluronic acid on bacterial and fungal species, including clinically relevant opportunistic pathogens. J Mater Sci Mater Med. 2011;22(10):2329.

Asami K, Inagaki A, Imura T, Sekiguchi S, Fujimori K, Masutani H, et al. Thioredoxin-1 attenuates early graft loss after intraportal islet transplantation in mice. PLoS One. 2013;8(8):e70259.

Av T, Mohanty S, Dinda AK, Koul V. Fabrication and evaluation of gelatin/hyaluronic acid/chondroitin sulfate/asiatic acid based biopolymeric scaffold for the treatment of second degree burn wounds–Wistar rat model study. Biomed Mater. 2020;15:055016.

Bae MS, Ohe JY, Lee JB, Heo DN, Byun W, Bae H, et al. Photo-cured hyaluronic acid-based hydrogels containing growth and differentiation factor 5 (GDF-5) for bone tissue regeneration. Bone. 2014;59:189–98.

Baier Leach J, Bivens KA, Patrick CW Jr, Schmidt CE. Photocrosslinked hyaluronic acid hydrogels: natural, biodegradable tissue engineering scaffolds. Biotechnol Bioeng. 2003;82(5):578–89.

Bal T, Kepsutlu B, Kizilel S. Characterization of protein release from poly(ethylene glycol) hydrogels with crosslink density gradients. J Biomed Mater Res A. 2014;102(2):487–95.

Ballini A, Cantore S, Capodiferro S, Grassi FR. Esterified hyaluronic acid and autologous bone in the surgical correction of the infra-bone defects. Int J Med Sci. 2009;6(2):65–71.

Baranova NS, Nileback E, Haller FM, Briggs DC, Svedhem S, Day AJ, et al. The inflammation-associated protein TSG-6 cross-links hyaluronan via hyaluronan-induced TSG-6 oligomers. J Biol Chem. 2011;286(29):25675–86.

Bautista-Hernandez LA, Gomez-Olivares JL, Buentello-Volante B, Manuel Bautista-de Lucio V. Fibroblasts: the unknown sentinels eliciting immune responses against microorganisms. Eur J Microbiol Immunol. 2017;7(3):151–7.

Bencherif SA, Srinivasan A, Horkay F, Hollinger JO, Matyjaszewski K, Washburn NR. Influence of the degree of methacrylation on hyaluronic acid hydrogels properties. Biomaterials. 2008;29(12):1739–49.

Bergman K, Elvingson C, Hilborn J, Svensk G, Bowden T. Hyaluronic acid derivatives prepared in aqueous media by triazine-activated amidation. Biomacromolecules. 2007;8(7):2190–5.

Bhattacharya M, Wozniak DJ, Stoodley P, Hall-Stoodley L. Prevention and treatment of *Staphylococcus aureus* biofilms. Expert Rev Anti-Infect Ther. 2015;13(12):1499–516.

Bhowmick S, Scharnweber D, Koul V. Co-cultivation of keratinocyte-human mesenchymal stem cell (hMSC) on sericin loaded electrospun nanofibrous composite scaffold (cationic gelatin/hyaluronan/chondroitin sulfate) stimulates epithelial differentiation in hMSCs: in vitro study. Biomaterials. 2016;88:83–96.

Bhowmick S, Thanusha AV, Kumar A, Scharnweber D, Rother S, Koul V. Nanofibrous artificial skin substitute composed of mPEG-PCL grafted gelatin/hyaluronan/chondroitin sulfate/sericin for 2nd degree burn care: in vitro and in vivo study. RSC Adv. 2018;8(30):16420–32.

Blount ZD. The unexhausted potential of *E. coli*. elife. 2015;4:e05826.

Bulpitt P, Aeschlimann D. New strategy for chemical modification of hyaluronic acid: preparation of functionalized derivatives and their use in the formation of novel biocompatible hydrogels. J Biomed Mater Res. 1999;47(2):152–69.

Burdick JA, Prestwich GD. Hyaluronic acid hydrogels for biomedical applications. Adv Mater. 2011;23(12):H41–56.

Burdick JA, Chung C, Jia X, Randolph MA, Langer R. Controlled degradation and mechanical behavior of photopolymerized hyaluronic acid networks. Biomacromolecules. 2005;6(1):386–91.

Burnside ER, Bradbury EJ. Manipulating the extracellular matrix and its role in brain and spinal cord plasticity and repair. Neuropathol Appl Neurobiol. 2014;40(1):26–59.

Cahill D, Zamboni F, Collins MN. Radiological advances in pancreatic islet transplantation. Acad Radiol. 2019;26(11):1536–43.

Cankaya ZT, Gurbuz S, Bakirarar B, Kurtis B. Evaluation of the effect of hyaluronic acid application on the vascularization of free gingival graft for both donor and recipient sites with laser Doppler flowmetry: a randomized, examiner-blinded, controlled clinical trial. Int J Periodontics Restorative Dent. 2020;40(2):233–43.

Cao B, Wang Y, Wen D, Liu W, Wang J, Fan G, et al. A trial of lopinavir–ritonavir in adults hospitalized with severe covid-19. N Engl J Med. 2020;382(19):1787–99.

Carlson GA, Dragoo JL, Samimi B, Bruckner DA, Bernard GW, Hedrick M, et al. Bacteriostatic properties of biomatrices against common orthopaedic pathogens. Biochem Bioph Res Commun. 2004;321(2):472–8.
Cermelli C, Cuoghi A, Scuri M, Bettua C, Neglia RG, Ardizzoni A, et al. In vitro evaluation of antiviral and virucidal activity of a high molecular weight hyaluronic acid. Virol J. 2011;8(1):141.
Chanda A, Adhikari J, Ghosh A, Chowdhury SR, Thomas S, Datta P, et al. Electrospun chitosan/polycaprolactone-hyaluronic acid bilayered scaffold for potential wound healing applications. Int J Biol Macromol. 2018;116:774–85.
Chen JX, Cao LJ, Shi Y, Wang P, Chen JH. In situ supramolecular hydrogel based on hyaluronic acid and dextran derivatives as cell scaffold. J Biomed Mater Res A. 2016a;104(9):2263–70.
Chen SY, He ZH, Xu GJ, Xiao XF. Fabrication of nanofibrous tubular scaffolds for bone tissue engineering. Mater Lett. 2016b;182:289–93.
Choh SY, Cross D, Wang C. Facile synthesis and characterization of disulfide-cross-linked hyaluronic acid hydrogels for protein delivery and cell encapsulation. Biomacromolecules. 2011;12(4):1126–36.
Choi SC, Yoo MA, Lee SY, Lee HJ, Son DH, Jung J, et al. Modulation of biomechanical properties of hyaluronic acid hydrogels by crosslinking agents. J Biomed Mater Res A. 2015;103(9):3072–80.
Ciccone V, Zazzetta M, Morbidelli L. Comparison of the effect of two hyaluronic acid preparations on fibroblast and endothelial cell functions related to angiogenesis. Cell. 2019;8(12):1479.
Clark CC, Aleman J, Mutkus L, Skardal A. A mechanically robust thixotropic collagen and hyaluronic acid bioink supplemented with gelatin nanoparticles. Bioprinting. 2019;16:e00058.
Clegg TE, Caborn D, Mauffrey C. Viscosupplementation with hyaluronic acid in the treatment for cartilage lesions: a review of current evidence and future directions. Eur J Orthop Surg Traumatol. 2013;23:119–24.
Cohen M, Braun E, Tsalenchuck Y, Panet A, Steiner I. Restrictions that control herpes simplex virus type 1 infection in mouse brain ex vivo. J Gen Virol. 2011;92(10):2383–93.
Collins MN, Birkinshaw C. Comparison of the effectiveness of four different crosslinking agents with hyaluronic acid hydrogel films for tisslue-culture applications. J Appl Polym Sci. 2007;104(5):3183–91.
Collins MN, Birkinshaw C. Investigation of the swelling behavior of crosslinked hyaluronic acid films and hydrogels produced using homogeneous reactions. J Appl Polym Sci. 2008;109(2):923–31.
Collins MN, Birkinshaw C. Morphology of crosslinked hyaluronic acid porous hydrogels. J Appl Polym Sci. 2010;120:1040–9.
Collins MN, Birkinshaw C. Hyaluronic acid based scaffolds for tissue engineering-a review. Carbohydr Polym. 2013a;92(2):1262–79.
Collins MN, Birkinshaw C. Hyaluronic acid solutions A processing method for efficient chemical modification. J Appl Polym Sci. 2013b;130(1):145–52.
Colson P, Rolain J-M, Lagier J-C, Brouqui P, Raoult D. Chloroquine and hydroxychloroquine as available weapons to fight COVID-19. Int J Antimicrob Agents. 2020;55(4):105932.
Conrad TL, Roeder RK. Effects of porogen morphology on the architecture, permeability, and mechanical properties of hydroxyapatite whisker reinforced polyetheretherketone scaffolds. J Mech Behav Biomed Mater. 2020;106:103730.
Costantini M, Barbetta A. Gas foaming technologies for 3D scaffold engineering. In: Functional 3D tissue engineering scaffolds. Duxford: Elsevier; 2018. p. 127–49.
Credi C, De Marco C, Molena E, Nava MM, Raimondi MT, Levi M, et al. Direct photo-patterning of hyaluronic acid baits onto a fouling-release perfluoropolyether surface for selective cancer cell capture and immobilization. Mat Sci Eng C Mater. 2016;62:414–22.
Crescenzi V, Francescangeli A, Capitani D, Mannina L, Renier D, Bellini D. Hyaluronan networking via Ugi's condensation using lysine as cross-linker diamine. Carbohydr Polym. 2003a;53(3):311–6.

Crescenzi V, Francescangeli A, Taglienti A, Capitani D, Mannina L. Synthesis and partial characterization of hydrogels obtained via glutaraldehyde crosslinking of acetylated chitosan and of hyaluronan derivatives. Biomacromolecules. 2003b;4(4):1045–54.
Cui X, Xu H, Zhou S, Zhao T, Liu A, Guo X, et al. Evaluation of angiogenic activities of hyaluronan oligosaccharides of defined minimum size. Life Sci. 2009;85(15–16):573–7.
Danishefsky I, Siskovic E. Conversion of carboxyl groups of mucopolysaccharides into amides of amino acid esters. Carbohydr Res. 1971;16(1):199–205.
de Nooy AE, Capitani D, Masci G, Crescenzi V. Ionic polysaccharide hydrogels via the Passerini and Ugi multicomponent condensations: synthesis, behavior and solid-state NMR characterization. Biomacromolecules. 2000;1(2):259–67.
del Hoyo-Gallego S, Pérez-Álvarez L, Gómez-Galván F, Lizundia E, Kuritka I, Sedlarik V, et al. Construction of antibacterial poly(ethylene terephthalate) films via layer by layer assembly of chitosan and hyaluronic acid. Carbohydr Polym. 2016;143:35–43.
Della Valle F, Romeo A (1997) Crosslinked carboxy polysaccharides. Google Patents
Diaz-Gomez L, Concheiro A, Alvarez-Lorenzo C, Garcia-Gonzalez CA. Growth factors delivery from hybrid PCL-starch scaffolds processed using supercritical fluid technology. Carbohydr Polym. 2016;142:282–92.
Ding JX, Zhang J, Li JN, Li D, Xiao CS, Xiao HH, et al. Electrospun polymer biomaterials. Prog Polym Sci. 2019;90:1–34.
Drews G, Krippeit-Drews P, Dufer M. Oxidative stress and beta-cell dysfunction. Pflugers Arch - Eur J Physiol. 2010;460(4):703–18.
Eenschooten C, Guillaumie F, Kontogeorgis GM, Stenby EH, Schwach-Abdellaoui K. Preparation and structural characterisation of novel and versatile amphiphilic octenyl succinic anhydride–modified hyaluronic acid derivatives. Carbohydr Polym. 2010;79(3):597–605.
Farrell K, Joshi J, Kothapalli CR. Injectable uncrosslinked biomimetic hydrogels as candidate scaffolds for neural stem cell delivery. J Biomed Mater Res A. 2017;105(3):790–805.
Figueira DR, Miguel SP, de Sa KD, Correia IJ. Production and characterization of polycaprolactone- hyaluronic acid/chitosan- zein electrospun bilayer nanofibrous membrane for tissue regeneration. Int J Biol Macromol. 2016;93:1100–10.
Follain N, Montanari S, Jeacomine I, Gambarelli S, Vignon MR. Coupling of amines with polyglucuronic acid: evidence for amide bond formation. Carbohydr Polym. 2008;74(3):333–43.
Fuhrmann T, Obermeyer J, Tator CH, Shoichet MS. Click-crosslinked injectable hyaluronic acid hydrogel is safe and biocompatible in the intrathecal space for ultimate use in regenerative strategies of the injured spinal cord. Methods. 2015;84:60–9.
Führmann T, Tam RY, Ballarin B, Coles B, Elliott Donaghue I, Van Der Kooy D, et al. Injectable hydrogel promotes early survival of induced pluripotent stem cell-derived oligodendrocytes and attenuates longterm teratoma formation in a spinal cord injury model. Biomaterials. 2016;83:23–36.
Gao Y, Sun Y, Yang H, Qiu P, Cong Z, Zou Y, et al. A low molecular weight hyaluronic acid derivative accelerates excisional wound healing by modulating pro-inflammation, promoting epithelialization and neovascularization, and remodeling collagen. Int J Mol Sci. 2019;20(15):3722.
Gaudet AD, Popovich PG. Extracellular matrix regulation of inflammation in the healthy and injured spinal cord. Exp Neurol. 2014;258:24–34.
Gautret P, Lagier J-C, Parola P, Hoang VT, Meddeb L, Mailhe M, et al. Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial. Int J Antimicrob Agents. 2020;56:105949.
Gleadall A, Visscher D, Yang J, Thomas D, Segal J. Review of additive manufactured tissue engineering scaffolds: relationship between geometry and performance. Burns Trauma. 2018;6
Godesky MD (2020) Hyaluronic acid-based bioinks for cell-friendly bioprinting. Rutgers University-School of Graduate Studies

Gomez-Aristizabal A, Kim KP, Viswanathan S. A systematic study of the effect of different molecular weights of hyaluronic acid on mesenchymal stromal cell-mediated immunomodulation. PLoS One. 2016;11(1):e0147868.

Gupta D, Tator CH, Shoichet MS. Fast-gelling injectable blend of hyaluronan and methylcellulose for intrathecal, localized delivery to the injured spinal cord. Biomaterials. 2006;27(11):2370–9.

Guzińska K, Kaźmierczak D, Dymel M, Pabjańczyk-Wlazło E, Boguń M. Anti-bacterial materials based on hyaluronic acid: selection of research methodology and analysis of their anti-bacterial properties. Mater Sci Eng C. 2018;93:800–8.

Gwon K, Kim E, Tae G. Heparin-hyaluronic acid hydrogel in support of cellular activities of 3D encapsulated adipose derived stem cells. Acta Biomater. 2017;49:284–95.

Hallmann R, Zhang X, Di Russo J, Li L, Song J, Hannocks MJ, et al. The regulation of immune cell trafficking by the extracellular matrix. Curr Opin Cell Biol. 2015;36:54–61.

Han CZ, Luo X, Zou D, Li JA, Zhang K, Yang P, et al. Nature-inspired extracellular matrix coating produced by micro-patterned smooth muscle and endothelial cells endows cardiovascular materials with better biocompatibility. Biomater Sci. 2019;7(7):2686–701.

Harris LG, Richards RG.*Staphylococcus aureus* adhesion to different treated titanium surfaces. J Mater Sci Mater Med 2004;15(4):311–4.

He Z, Zang H, Zhu L, Huang K, Yi T, Zhang S, et al. An anti-inflammatory peptide and brain-derived neurotrophic factor-modified hyaluronan-methylcellulose hydrogel promotes nerve regeneration in rats with spinal cord injury. Int J Nanomedicine. 2019;14:721–32.

Highley CB, Prestwich GD, Burdick JA. Recent advances in hyaluronic acid hydrogels for biomedical applications. Curr Opin Biotechnol. 2016;40:35–40.

Hirano K, Sakai S, Ishikawa T, Avci FY, Linhardt RJ, Toida T. Preparation of the methyl ester of hyaluronan and its enzymatic degradation. Carbohydr Res. 2005;340(14):2297–304.

Hirayama Y, Yoshimura M, Ozeki Y, Sugawara I, Udagawa T, Mizuno S, et al. Mycobacteria exploit host hyaluronan for efficient extracellular replication. PLoS Pathog. 2009;5(10):e1000643.

Ho MT, Teal CJ, Shoichet MS. A hyaluronan/methylcellulose-based hydrogel for local cell and biomolecule delivery to the central nervous system. Brain Res Bull. 2019;148:46–54.

Hokmabad VR, Davaran S, Ramazani A, Salehi R. Design and fabrication of porous biodegradable scaffolds: a strategy for tissue engineering. J Biomat Sci Polym E. 2017;28(16):1797–825.

Hu W, Yen Y-T, Singh S, Kao C-L, Wu-Hsieh BA. SARS-CoV regulates immune function-related gene expression in human monocytic cells. Viral Immunol. 2012;25(4):277–88.

Hui E, Gimeno KI, Guan G, Caliari SR (2019) Spatial control of viscoelasticity in phototunable hyaluronic acid hydrogels. BioRxiv 646778

Huin-Amargier C, Marchal P, Payan E, Netter P, Dellacherie E. New physically and chemically crosslinked hyaluronate (HA)-based hydrogels for cartilage repair. J Biomed Mater Res A. 2006;76(2):416–24.

Ibberson CB, Parlet CP, Kwiecinski J, Crosby HA, Meyerholz DK, Horswill AR. Hyaluronan modulation impacts *Staphylococcus aureus* biofilm infection. Infect Immun. 2016;84(6):1917–29.

Ikeda K, Yamauchi D, Osamura N, Hagiwara N, Tomita K. Hyaluronic acid prevents peripheral nerve adhesion. Br J Plast Surg. 2003;56(4):342–7.

Jeon S, Karkhanechi H, Fang LF, Cheng L, Ono T, Nakamura R, et al. Novel preparation and fundamental characterization of polyamide 6 self-supporting hollow fiber membranes via thermally induced phase separation (TIPS). J Membr Sci. 2018;546:1–14.

Jha AK, Malik MS, Farach-Carson MC, Duncan RL, Jia XQ. Hierarchically structured, hyaluronic acid-based hydrogel matrices via the covalent integration of microgels into macroscopic networks. Soft Matter. 2010;6(20):5045–55.

Jha AK, Tharp KM, Browne S, Ye J, Stahl A, Yeghiazarians Y, et al. Matrix metalloproteinase-13 mediated degradation of hyaluronic acid-based matrices orchestrates stem cell engraftment through vascular integration. Biomaterials. 2016;89:136–47.

Jung JT, Kim JF, Wang HH, di Nicolo E, Drioli E, Lee YM. Understanding the non-solvent induced phase separation (NIPS) effect during the fabrication of microporous PVDF membranes via thermally induced phase separation (TIPS). J Membr Sci. 2016;514:250–63.

Kaczmarek B, Sionkowska A, Osyczka AM. The application of chitosan/collagen/hyaluronic acid sponge cross-linked by dialdehyde starch addition as a matrix for calcium phosphate in situ precipitation. Int J Biol Macromol. 2018;107:470–7.

Kalam MA. Development of chitosan nanoparticles coated with hyaluronic acid for topical ocular delivery of dexamethasone. Int J Biol Macromol. 2016;89:127–36.

Kang L, Jia W, Li M, Wang Q, Wang C, Liu Y, et al. Hyaluronic acid oligosaccharide-modified collagen nanofibers as vascular tissue-engineered scaffold for promoting endothelial cell proliferation. Carbohydr Polym. 2019;223:115106.

Kankala RK, Zhang YS, Wang SB, Lee CH, Chen AZ. Supercritical fluid technology: an emphasis on drug delivery and related biomedical applications. Adv Healthc Mater. 2017;6(16):1700433

Kenar H, Ozdogan CY, Dumlu C, Doger E, Kose GT, Hasirci V. Microfibrous scaffolds from poly (l-lactide-co-epsilon-caprolactone) blended with xeno-free collagen/hyaluronic acid for improvement of vascularization in tissue engineering applications. Mater Sci Eng C Mater Biol Appl. 2019;97:31–44.

Kendall FE, Heidelberger M, Dawson MH. A serologically inactive polysaccharide elaborated by mucoid strains of group a hemolytic streptococcus. J Biol Chem. 1937;118(1):61–9.

Kessler L, Gehrke S, Winnefeld M, Huber B, Hoch E, Walter T, et al. Methacrylated gelatin/hyaluronan-based hydrogels for soft tissue engineering. J Tissue Eng. 2017;8:2041731417744157.

Khaing ZZ, Milman BD, Vanscoy JE, Seidlits SK, Grill RJ, Schmidt CE. High molecular weight hyaluronic acid limits astrocyte activation and scar formation after spinal cord injury. J Neural Eng. 2011;8:046033.

Khan R, Aroulmoji V. Hyaluronic acid – hydroxychloroquine conjugate proposed for treatment of COVID-19. Int J Adv Sci Eng. 2020;06:1469–71.

Kheirabadi M, Shi L, Bagheri R, Kabiri K, Hilborn J, Ossipov DA. In situ forming interpenetrating hydrogels of hyaluronic acid hybridized with iron oxide nanoparticles. Biomater Sci. 2015;3(11):1466–74.

Kim JF, Kim JH, Lee YM, Drioli E. Thermally induced phase separation and electrospinning methods for emerging membrane applications: a review. AICHE J. 2016;62(2):461–90.

Kim DK, Lee JM, Jeong JY, Park HJ, Lee OJ, Chao J, et al. New fabrication method of silk fibroin plate and screw based on a centrifugal casting technique. J Tissue Eng Regen M. 2018;12(11):2221–9.

Kohi S, Sato N, Koga A, Hirata K, Harunari E, Igarashi Y. Hyaluromycin, a novel hyaluronidase inhibitor attenuates pancreatic cancer cell migration and proliferation. J Oncol. 2016;2016:9063087.

Kota DJ, Wiggins LL, Yoon N, Lee RH. TSG-6 produced by hMSCs delays the onset of autoimmune diabetes by suppressing Th1 development and enhancing Tolerogenicity. Diabetes. 2013;62(6):2048–58.

Koul V, Bhowmick S, Thanusha AV. Hydrogels for pharmaceutical applications. In: Handbook of polymers for pharmaceutical technologies, Vol 4: bioactive and compatible synthetic/hybrid polymers. Hoboken: Wiley; 2016. p. 125–44.

Kuehl C, Zhang T, Kaminskas LM, Porter CJ, Davies NM, Forrest L, et al. Hyaluronic acid molecular weight determines lung clearance and biodistribution after instillation. Mol Pharm. 2016;13(6):1904–14.

Lai JY. Relationship between structure and cytocompatibility of divinyl sulfone cross-linked hyaluronic acid. Carbohydr Polym. 2014;101:203–12.

Lai HJ, Kuan CH, Wu HC, Tsai JC, Chen TM, Hsieh DJ, et al. Tailored design of electrospun composite nanofibers with staged release of multiple angiogenic growth factors for chronic wound healing. Acta Biomater. 2014;10(10):4156–66.

Larraneta E, Henry M, Irwin NJ, Trotter J, Perminova AA, Donnelly RF. Synthesis and characterization of hyaluronic acid hydrogels crosslinked using a solvent-free process for potential biomedical applications. Carbohydr Polym. 2018;181:1194–205.

Laurent TC, Laurent UB, Fraser JRE. The structure and function of hyaluronan: an overview. Immunol Cell Biol. 1996;74:A1–7.

Lee H, Park TG. Photo-crosslinkable, biomimetic, and thermo-sensitive pluronic grafted hyaluronic acid copolymers for injectable delivery of chondrocytes. J Biomed Mater Res A. 2009;88A(3): 797–806.

Lee DW, Banquy X, Das S, Cadirov N, Jay G, Israelachvili J. Effects of molecular weight of grafted hyaluronic acid on wear initiation. Acta Biomater. 2014;10(5):1817–23.

Lee HJ, Sen A, Bae S, Lee JS, Webb K. Poly(ethylene glycol) diacrylate/hyaluronic acid semi-interpenetrating network compositions for 3-D cell spreading and migration. Acta Biomater. 2015;14:43–52.

Lee MC, Kim DK, Lee OJ, Kim JH, Ju HW, Lee JM, et al. Fabrication of silk fibroin film using centrifugal casting technique for corneal tissue engineering. J Biomed Mater Res B. 2016;104(3):508–14.

Lee S, Chung M, Lee SR, Jeon NL. 3D brain angiogenesis model to reconstitute functional human blood-brain barrier in vitro. Biotechnol Bioeng. 2020;117(3):748–62.

Lertkiatmongkol P, Liao D, Mei H, Hu Y, Newman PJ. Endothelial functions of platelet/endothelial cell adhesion molecule-1 (CD31). Curr Opin Hematol. 2016;23(3):253–9.

Levene PA, López-Suárez J. Mucins and Mucoids. J Biol Chem. 1918;36(1):105–26.

Li JG, Zhang K, Yang P, Qin W, Li GC, Zhao AS, et al. Human vascular endothelial cell morphology and functional cytokine secretion influenced by different size of HA micro-pattern on titanium substrate. Colloid Surf B. 2013;110:199–207.

Lim HL, Hwang Y, Kar M, Varghese S. Smart hydrogels as functional biomimetic systems. Biomater Sci. 2014;2(5):603–18.

Lim DK, Wylie RG, Langer R, Kohane DS. Selective binding of C-6 OH sulfated hyaluronic acid to the angiogenic isoform of VEGF(165). Biomaterials. 2016;77:130–8.

Liu Y, Ye H, Satkunendrarajah K, Yao GS, Bayon Y, Fehlings MG (2013) A self-assembling peptide reduces glial scarring, attenuates post-traumatic inflammation and promotes neurological recovery following spinal cord injury. Acta Biomater. 9. England: Acta Materialia Inc. Published by Elsevier Ltd, pp 8075–88.

Lu HD, Soranno DE, Rodell CB, Kim IL, Burdick JA. Secondary photocrosslinking of injectable shear-thinning dock-and-lock hydrogels. Adv Healthc Mater. 2013;2(7):1028–36.

Lu BT, Lu F, Zou YN, Liu JW, Rong B, Li ZQ, et al. In situ reduction of silver nanoparticles by chitosan-L-glutamic acid/hyaluronic acid: enhancing antimicrobial and wound-healing activity. Carbohydr Polym. 2017;173:556–65.

Lynch AS, Robertson GT. Bacterial and fungal biofilm infections. Annu Rev Med. 2008;59: 415–28.

Magnani A, Rappuoli R, Lamponi S, Barbucci R. Novel polysaccharide hydrogels: characterization and properties. Polym Adv Technol. 2000;11(8–12):488–95.

Mahmoodi NM, Mokhtari-Shourijeh Z. Preparation of aminated nanoporous nanofiber by solvent casting/porogen leaching technique and dye adsorption modeling. J Taiwan Inst Chem E. 2016;65:378–89.

Maleki A, Kjøniksen A-L, Nyström B. Characterization of the chemical degradation of hyaluronic acid during chemical gelation in the presence of different cross-linker agents. Carbohydr Res. 2007;342(18):2776–92.

Manavitehrani I, Le TYL, Daly S, Wang YW, Maitz PK, Schindeler A, et al. Formation of porous biodegradable scaffolds based on poly(propylene carbonate) using gas foaming technology. Mat Sci Eng C-Mater. 2019;96:824–30.

Maniglio D, Bonani W, Migliaresi C, Motta A. Silk fibroin porous scaffolds by N2O foaming. J Biomat Sci-Polym E. 2018;29(5):491–506.

Markovitsi D, Gustavsson T, Banyasz A. Absorption of UV radiation by DNA: spatial and temporal features. Mutat Res Rev Mutat. 2010;704(1–3):21–8.
Martinez-Ramos C, Doblado LR, Mocholi EL, Alastrue-Agudo A, Petidier MS, Giraldo E, et al. Biohybrids for spinal cord injury repair. J Tissue Eng Regen Med. 2019;13(3): 509–21.
Maurin M, Raoult D. Use of aminoglycosides in treatment of infections due to intracellular bacteria. Antimicrob Agents Chemother. 2001;45(11):2977–86.
Mealy JE, Rodell CB, Burdick JA. Sustained small molecule delivery from injectable hyaluronic acid hydrogels through host-guest mediated retention. J Mater Chem B Mater Biol Med. 2015;3(40):8010–9.
Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ. COVID-19: consider cytokine storm syndromes and immunosuppression. Lancet. 2020;395(10229):1033–4.
Meri S, Pangburn MK. Regulation of alternative pathway complement activation by glycosaminoglycans – specificity of the polyanion binding-site on factor-H. Biochem Biophys Res Commun. 1994;198(1):52–9.
Meyer K, Palmer JW. The polysaccharide of the vitreous humor. J Biol Chem. 1934;107(3):629–34.
Miguel SP, Figueira DR, Simoes D, Ribeiro MP, Coutinho P, Ferreira P, et al. Electrospun polymeric nanofibres as wound dressings: a review. Colloid Surf B. 2018;169:60–71.
Mong MA, Awkal JA, Marik PE (2020) Accelerated hyaluronan concentration as the primary driver of morbidity and mortality in high-risk COVID-19 patients: with therapeutic introduction of an oral hyaluronan inhibitor in the prevention of "Induced Hyaluronan Storm" Syndrome. medRxiv. 2020.04.19.20071647
Monteiro IP, Shukla A, Marques AP, Reis RL, Hammond PT. Spray-assisted layer-by-layer assembly on hyaluronic acid scaffolds for skin tissue engineering. J Biomed Mater Res A. 2015;103(1):330–40.
Mothe AJ, Tator CH. Advances in stem cell therapy for spinal cord injury. J Clin Invest. 2012;122(11):3824–34.
Mouser VHM, Levato R, Mensinga A, Dhert WJA, Gawlitta D, Malda J. Bio-ink development for three-dimensional bioprinting of hetero-cellular cartilage constructs. Connect Tissue Res. 2020;61(2):137–51.
Movahedi M, Asefnejad A, Rafienia M, Khorasani MT. Potential of novel electrospun core-shell structured polyurethane/starch (hyaluronic acid) nanofibers for skin tissue engineering: in vitro and in vivo evaluation. Int J Biol Macromol. 2020;146:627–37.
Murphy SV, Atala A. 3D bioprinting of tissues and organs. Nat Biotechnol. 2014;32(8):773–85.
Nagy N, Kuipers HF, Frymoyer AR, Ishak HD, Bollyky JB, Wight TN, et al. 4-methylumbelliferone treatment and hyaluronan inhibition as a therapeutic strategy in inflammation, autoimmunity, and cancer. Front Immunol. 2015;6:123.
Nakajima N, Ikada Y. Mechanism of amide formation by carbodiimide for bioconjugation in aqueous media. Bioconjug Chem. 1995;6(1):123–30.
Nasreen N, Mohammed KA, Hardwick J, Van Horn RD, Sanders K, Kathuria H, et al. Low molecular weight hyaluronan induces malignant mesothelioma cell (MMC) proliferation and haptotaxis: role of CD44 receptor in MMC proliferation and haptotaxis. Oncol Res. 2002;13(2): 71–8.
Natividad-Diaz SL, Browne S, Jha AK, Ma Z, Hossainy S, Kurokawa YK, et al. A combined hiPSC-derived endothelial cell and in vitro microfluidic platform for assessing biomaterial-based angiogenesis. Biomaterials. 2019;194:73–83.
Ni YL, Tang ZR, Cao WX, Lin H, Fan YJ, Guo LK, et al. Tough and elastic hydrogel of hyaluronic acid and chondroitin sulfate as potential cell scaffold materials. Int J Biol Macromol. 2015;74: 367–75.
Noh I, Kim N, Tran HN, Lee J, Lee C. 3D printable hyaluronic acid-based hydrogel for its potential application as a bioink in tissue engineering. Biomater Res. 2019;23(1):3.
O'brien FJ. Biomaterials & scaffolds for tissue engineering. Mater Today. 2011;14(3):88–95.

Oh EJ, Park K, Kim KS, Kim J, Yang J-A, Kong J-H, et al. Target specific and long-acting delivery of protein, peptide, and nucleotide therapeutics using hyaluronic acid derivatives. J Control Release. 2010;141(1):2–12.

Ozgenel GY. Effects of hyaluronic acid on peripheral nerve scarring and regeneration in rats. Microsurgery. 2003;23(6):575–81.

Palumbo FS, Di Stefano M, Piccionello AP, Fiorica C, Pitarresi G, Pibiri I, et al. Perfluorocarbon functionalized hyaluronic acid derivatives as oxygenating systems for cell culture. RSC Adv. 2014;4(44):22894–901.

Pandey MS, Weigel PH. Hyaluronic acid receptor for endocytosis (HARE)-mediated endocytosis of hyaluronan, heparin, dermatan sulfate, and acetylated low density lipoprotein (AcLDL), but not chondroitin sulfate types A, C, D, or E, activates NF-kappaB-regulated gene expression. J Biol Chem. 2014;289(3):1756–67.

Papakonstantinou E, Roth M, Karakiulakis G. Hyaluronic acid: a key molecule in skin aging. Dermato-endocrinol. 2012;4(3):253–8.

Pardue EL, Ibrahim S, Ramamurthi A. Role of hyaluronan in angiogenesis and its utility to angiogenic tissue engineering. Organogenesis. 2008;4(4):203–14.

Park KM, Yang JA, Jung H, Yeom J, Park JS, Park KH, et al. In situ supramolecular assembly and modular modification of hyaluronic acid hydrogels for 3D cellular engineering. ACS Nano. 2012a;6(4):2960–8.

Park D, Kim Y, Kim H, Kim K, Lee YS, Choe J, et al. Hyaluronic acid promotes angiogenesis by inducing RHAMM-TGFbeta receptor interaction via CD44-PKCdelta. Mol Cell. 2012b;33(6):563–74.

Park JH, Park EJ, Yi HS. Wound healing and anti-inflammatory effects of topical hyaluronic acid injection in surgical-site infection caused by *Staphylococcus aureus*. Int J Low Extrem Wounds. 2017;16(3):202–7.

Pati F, Jang J, Ha DH, Kim SW, Rhie JW, Shim JH, et al. Printing three-dimensional tissue analogues with decellularized extracellular matrix bioink. Nat Commun. 2014;5:3935.

Patterson RL, Peterson DA, Deinhardt F, Howard F. Rubella and rheumatoid arthritis: hyaluronic acid and susceptibility of cultured rheumatoid synovial cells to viruses. Proc Soc Exp Biol Med. 1975;149(3):594–8.

Pereira PAT, Bitencourt CS, Reis MB, Frantz FG, Sorgi CA, Souza COS, et al. Immunomodulatory activity of hyaluronidase is associated with metabolic adaptations during acute inflammation. Inflamm Res. 2020;69(1):105–13.

Pérez-Álvarez L, Ruiz-Rubio L, Azua I, Benito V, Bilbao A, Vilas-Vilela JL. Development of multiactive antibacterial multilayers of hyaluronic acid and chitosan onto poly(ethylene terephthalate). Eur Polym J. 2019;112:31–7.

Perng CK, Wang YJ, Tsi CH, Ma H. In vivo angiogenesis effect of porous collagen scaffold with hyaluronic acid oligosaccharides. J Surg Res. 2011;168(1):9–15.

Peroglio M, Grad S, Mortisen D, Sprecher CM, Illien-Junger S, Alini M, et al. Injectable thermoreversible hyaluronan-based hydrogels for nucleus pulposus cell encapsulation. Eur Spine J. 2012;21(Suppl 6):S839–49.

Pescosolido L, Schuurman W, Malda J, Matricardi P, Alhaique F, Coviello T, et al. Hyaluronic acid and dextran-based semi-IPN hydrogels as biomaterials for bioprinting. Biomacromolecules. 2011;12(5):1831–8.

Piperno A, Zagami R, Cordaro A, Pennisi R, Musarra-Pizzo M, Scala A, et al. Exploring the entrapment of antiviral agents in hyaluronic acid-cyclodextrin conjugates. J Incl Phenom Macrocycl Chem. 2019;93(1):33–40.

Pirnazar P, Wolinsky L, Nachnani S, Haake S, Pilloni A, Bernard GW. Bacteriostatic effects of hyaluronic acid. J Periodontol. 1999;70(4):370–4.

Poldervaart MT, Goversen B, de Ruijter M, Abbadessa A, Melchels FPW, Oner FC, et al. 3D bioprinting of methacrylated hyaluronic acid (MeHA) hydrogel with intrinsic osteogenicity. PLoS One. 2017;12(6):e0177628.

Porras AM, Sjögren F, Shi L, Ossipov DA, Tenje M (eds) (2016) Photopatterning of hyaluronic acid hydrogels for cell culture scaffolds. 27th micromechanics and microsystems Europe WORKSHOP (MME 2016), Cork, Ireland, August 28–30 2016

Portalska KJ, Teixeira LM, Leijten JC, Jin R, van Blitterswijk C, de Boer J, et al. Boosting angiogenesis and functional vascularization in injectable dextran-hyaluronic acid hydrogels by endothelial-like mesenchymal stromal cells. Tissue Eng Part A. 2014;20(3–4):819–29.

Poursamar SA, Lehner AN, Azami M, Ebrahimi-Barough S, Samadikuchaksaraei A, Antunes APM. The effects of crosslinkers on physical, mechanical, and cytotoxic properties of gelatin sponge prepared via in-situ gas foaming method as a tissue engineering scaffold. Mat Sci Eng C-Mater. 2016;63:1–9.

Prata JE, Barth TA, Bencherif SA, Washburn NR. Complex fluids based on methacrylated hyaluronic acid. Biomacromolecules. 2010;11(3):769–75.

Pravata L, Braud C, Boustta M, El Ghzaoui A, Tømmeraas K, Guillaumie F, et al. New amphiphilic lactic acid oligomer− hyaluronan conjugates: synthesis and physicochemical characterization. Biomacromolecules. 2008;9(1):340–8.

Prestwich GD, Marecak DM, Marecek JF, Vercruysse KP, Ziebell MR. Controlled chemical modification of hyaluronic acid: synthesis, applications, and biodegradation of hydrazide derivatives. J Control Release. 1998;53(1–3):93–103.

Qiu Y, Hou Y, Sun F, Chen P, Wang D, Mu H, et al. Hyaluronic acid conjugation facilitates clearance of intracellular bacterial infections by streptomycin with neglectable nephrotoxicity. Glycobiology. 2017;27(9):861–7.

Ramamurthi A, Vesely I. Smooth muscle cell adhesion on crosslinked hyaluronan gels. J Biomed Mater Res. 2002;60(1):195–205.

Ramanan V, Scull MA, Sheahan TP, Rice CM, Bhatia SN. New methods in tissue engineering: improved models for viral infection. Annu Rev Virol. 2014;1:475–99.

Ramos CM, Bargues MJM, Roca FG, Doblado LR, Sanchez TG, Mir L, et al (2018) Electrical stimulation increases schwann cells proliferation inside hyaluronic acid conduits. 2018 Emf-Med 1st World conference on biomedical applications of electromagnetic fields (Emf-Med 2018)

Rao F, Yuan ZP, Li M, Yu F, Fang XX, Jiang BG, et al. Expanded 3D nanofibre sponge scaffolds by gas-foaming technique enhance peripheral nerve regeneration. Artif Cell Nanomed B. 2019;47(1):491–500.

Rey DFV, St-Pierre J-P. Fabrication techniques of tissue engineering scaffolds. In: Handbook of tissue engineering scaffolds, vol. One. San Diego: Elsevier; 2019. p. 109–25.

Rodell CB, Kaminski AL, Burdick JA. Rational design of network properties in guest-host assembled and shear-thinning hyaluronic acid hydrogels. Biomacromolecules. 2013;14(11):4125–34.

Rodell CB, MacArthur JW, Dorsey SM, Wade RJ, Wang LL, Woo YJ, et al. Shear-thinning supramolecular hydrogels with secondary autonomous covalent crosslinking to modulate viscoelastic properties in vivo. Adv Funct Mater. 2015a;25(4):636–44.

Rodell CB, Wade RJ, Purcell BP, Dusaj NN, Burdick JA. Selective proteolytic degradation of guest-host assembled, injectable hyaluronic acid hydrogels. ACS Biomater Sci Eng. 2015b;1(4):277–86.

Rodell CB, Highley CB, Chen MH, Dusaj NN, Wang C, Han L, et al. Evolution of hierarchical porous structures in supramolecular guest-host hydrogels. Soft Matter. 2016;12(37):7839–47.

Rodriguez-Perez E, Compan AL, Pradas MM, Martinez-Ramos C. Scaffolds of hyaluronic acid-poly(ethyl acrylate) interpenetrating networks: characterization and in vitro studies. Macromol Biosci. 2016;16(8):1147–57.

Rohr RV, Trepp C (1996) High pressure chemical engineering: proceedings of the 3rd International Symposium on High Pressure Chemical Engineering, Zürich, Switzerland, October 7–9, 1996. Elsevier, Amsterdam, New York, xvii, 710 p

Romanò CL, De Vecchi E, Bortolin M, Morelli I, Drago L. Hyaluronic acid and its composites as a local antimicrobial/antiadhesive barrier. J Bone Jt Infect. 2017;2(1):63–72.

Rosales AM, Vega SL, DelRio FW, Burdick JA, Anseth KS. Hydrogels with reversible mechanics to probe dynamic cell microenvironments. Angew Chem Int Ed Eng. 2017;56(40): 12132–6.

Rosales AM, Rodell CB, Chen MH, Morrow MG, Anseth KS, Burdick JA. Reversible control of network properties in azobenzene-containing hyaluronic acid-based hydrogels. Bioconjug Chem. 2018;29:905–13.

Ruppert SM, Hawn TR, Arrigoni A, Wight TN, Bollyky PL. Tissue integrity signals communicated by high-molecular weight hyaluronan and the resolution of inflammation. Immunol Res. 2014;58(2–3):186–92.

Sahiner N, Sagbas S, Sahiner M, Ayyala RS. Polyethyleneimine modified poly(hyaluronic acid) particles with controllable antimicrobial and anticancer effects. Carbohydr Polym. 2017;159: 29–38.

Sakai S, Ohi H, Hotta T, Kamei H, Taya M. Differentiation potential of human adipose stem cells bioprinted with hyaluronic acid/gelatin-based bioink through microextrusion and visible light-initiated crosslinking. Biopolymers. 2018;109(2):e23080.

Schanté CE, Zuber G, Herlin C, Vandamme TF. Chemical modifications of hyaluronic acid for the synthesis of derivatives for a broad range of biomedical applications. Carbohydr Polym. 2011;85(3):469–89.

Schante CE, Zuber G, Herlin C, Vandamme TF. Chemical modifications of hyaluronic acid for the synthesis of derivatives for a broad range of biomedical applications. Carbohydr Polym. 2011;85(3):469–89.

Seidlits SK, Khaing ZZ, Petersen RR, Nickels JD, Vanscoy JE, Shear JB, et al. The effects of hyaluronic acid hydrogels with tunable mechanical properties on neural progenitor cell differentiation. Biomaterials. 2010;31(14):3930–40.

Seidlits SK, Liang J, Bierman RD, Sohrabi A, Karam J, Holley SM, et al. Peptide-modified, hyaluronic acid-based hydrogels as a 3D culture platform for neural stem/progenitor cell engineering. J Biomed Mater Res A. 2019;107(4):704–18.

Seon-Lutz M, Couffin AC, Vignoud S, Schlatter G, Hebraud A. Electrospinning in water and in situ crosslinking of hyaluronic acid/cyclodextrin nanofibers: towards wound dressing with controlled drug release. Carbohydr Polym. 2019;207:276–87.

Shendi D, Albrecht DR, Jain A. Anti-Fas conjugated hyaluronic acid microsphere gels for neural stem cell delivery. J Biomed Mater Res A. 2017;105(2):608–18.

Shi Y, Wang Y, Shao C, Huang J, Gan J, Huang X, et al. COVID-19 infection: the perspectives on immune responses. Cell Death Differ. 2020;27(5):1451–4.

Shimojo AA, Pires AM, Lichy R, Rodrigues AA, Santana MH. The crosslinking degree controls the mechanical, rheological, and swelling properties of hyaluronic acid microparticles. J Biomed Mater Res A. 2015;103(2):730–7.

Silva LP, Pirraco RP, Santos TC, Novoa-Carballal R, Cerqueira MT, Reis RL, et al. Neovascularization induced by the hyaluronic acid-based spongy-like hydrogels degradation products. ACS Appl Mater Interfaces. 2016;8(49):33464–74.

Singh I, Lacko CS, Zhao ZY, Schmidt CE, Rinaldi C. Preparation and evaluation of microfluidic magnetic alginate microparticles for magnetically templated hydrogels. J Colloid Interface Sci. 2020;561:647–58.

Sloane JA, Batt C, Ma Y, Harris ZM, Trapp B, Vartanian T. Hyaluronan blocks oligodendrocyte progenitor maturation and remyelination through TLR2. Proc Natl Acad Sci U S A. 2010;107(25):11555–60.

Smeds KA, Grinstaff MW. Photocrosslinkable polysaccharides for in situ hydrogel formation. J Biomed Mater Res. 2001;54(1):115–21.

Song JE, Kim MJ, Yoon H, Yoo H, Lee YJ, Kim HN, et al. Effect of hyaluronic acid (HA) in a HA/PLGA scaffold on annulus fibrosus regeneration: in vivo tests. Macromol Res. 2013;21(10): 1075–82.

Sorokin L. The impact of the extracellular matrix on inflammation. Nat Rev Immunol. 2010;10(10): 712–23.

Souness A, Zamboni F, Walker GM, Collins MN. Influence of scaffold design on 3D printed cell constructs. J Biomed Mater Res B Appl Biomater. 2018;106(2):533–45.
Stern R. Hyaluronan catabolism: a new metabolic pathway. Eur J Cell Biol. 2004;83(7):317–25.
Struve J, Maher PC, Li YQ, Kinney S, Fehlings MG, Kuntz C, et al. Disruption of the hyaluronan-based extracellular matrix in spinal cord promotes astrocyte proliferation. Glia. 2005;52(1): 16–24.
Sukowati CHC, Anfuso B, Fiore E, Ie SI, Raseni A, Vascotto F, et al. Hyaluronic acid inhibition by 4-methylumbelliferone reduces the expression of cancer stem cells markers during hepatocarcinogenesis. Sci Rep. 2019;9(1):4026.
Sun JF, Perry SL, Schiffman JD. Electrospinning nanofibers from chitosan/hyaluronic acid complex coacervates. Biomacromolecules. 2019;20(11):4191–8.
Sundaramurthi D, Rauf S, Hauser CAE. 3D bioprinting technology for regenerative medicine applications. Int J Bioprint. 2016;2(2):9–26.
Takahashi A, Suzuki Y, Suhara T, Omichi K, Shimizu A, Hasegawa K, et al. In situ cross-linkable hydrogel of hyaluronan produced via copper-free click chemistry. Biomacromolecules. 2013;14(10):3581–8.
Tatara AM. Role of tissue engineering in COVID-19 and future viral outbreaks. Tissue Eng Part A. 2020;26(9–10):468–74.
Thanusha AV, Dinda AK, Koul V. Evaluation of nano hydrogel composite based on gelatin/HA/CS suffused with Asiatic acid/ZnO and CuO nanoparticles for second degree burns. Mat Sci Eng C-Mater. 2018;89:378–86.
Theocharis AD, Skandalis SS, Gialeli C, Karamanos NK. Extracellular matrix structure. Adv Drug Deliv Rev. 2016;97:4–27.
Thomas RC, Vu P, Modi SP, Chung PE, Landis RC, Khaing ZZ, et al. Sacrificial crystal templated hyaluronic acid hydrogels as biomimetic 3D tissue scaffolds for nerve tissue regeneration. ACS Biomater Sci Eng. 2017;3(7):1451–9.
Tiunnikov G, Kostina G, Radaeva I, Bakulina L. Effects of hyaluronic acid preparation on the development of herpetic infection in cell culture. Vopr Virusol. 2002;47:37–9.
Tomihata K, Ikada Y. Crosslinking of hyaluronic acid with glutaraldehyde. J Polym Sci A Polym Chem. 1997;35(16):3553–9.
Tommeraas K, Eenschooten C. Aryl/alkyl succinic anhydride-hyaluronan derivatives. Google Patents. 2009.
Toole BP. Hyaluronan: from extracellular glue to pericellular cue. Nat Rev Cancer. 2004;4(7):528–39.
Uchida K, Otake K, Inoue M, Koike Y, Matsushita K, Tanaka K, et al. Bacteriostatic effects of hyaluronan-based bioresorsable membrane. Surg Sci. 2011;2:6.
Valachova K, Banasova M, Topol'ska D, Sasinkova V, Juranek I, Collins MN, et al. Influence of tiopronin, captopril and levamisole therapeutics on the oxidative degradation of hyaluronan. Carbohydr Polym. 2015;134:516–23.
Valachova K, Topol'ska D, Mendichi R, Collins MN, Sasinkova V, Soltes L. Hydrogen peroxide generation by the Weissberger biogenic oxidative system during hyaluronan degradation. Carbohydr Polym. 2016;148:189–93.
VanEpps JS, Younger JG. Implantable device-related infection. Shock. 2016;46(6):597–608.
Vegas AJ, Veiseh O, Doloff JC, Ma M, Tam HH, Bratlie K, et al. Combinatorial hydrogel library enables identification of materials that mitigate the foreign body response in primates. Nat Biotechnol. 2016;34(3):345–52.
Vercruysse KP, Prestwich GD. Hyaluronate derivatives in drug delivery. Crit Rev Ther Drug Carrier Syst. 1998;15(5):43.
Wang Y, Han G, Guo B, Huang J. Hyaluronan oligosaccharides promote diabetic wound healing by increasing angiogenesis. Pharmacol Rep. 2016;68(6):1126–32.
Wang HH, Jung JT, Kim JF, Kim S, Drioli E, Lee YM. A novel green solvent alternative for polymeric membrane preparation via nonsolvent-induced phase separation (NIPS). J Membr Sci. 2019a;574:44–54.

Wang N, Liu C, Wang X, He T, Li L, Liang X, et al. Hyaluronic acid oligosaccharides improve myocardial function reconstruction and angiogenesis against myocardial infarction by regulation of macrophages. Theranostics. 2019b;9(7):1980–92.
Weaver JD, Stabler CL. Antioxidant cerium oxide nanoparticle hydrogels for cellular encapsulation. Acta Biomater. 2015;16:136–44.
Weber LM, Lopez CG, Anseth KS. The effects of PEG hydrogel crosslinking density on protein diffusion and encapsulated islet survival and function. J Biomed Mater Res A. 2009;90(3):720–9.
Weng L, Gouldstone A, Wu Y, Chen W. Mechanically strong double network photocrosslinked hydrogels from N, N-dimethylacrylamide and glycidyl methacrylated hyaluronan. Biomaterials. 2008;29(14):2153–63.
West DC, Hampson IN, Arnold F, Kumar S. Angiogenesis induced by degradation products of hyaluronic acid. Science. 1985;228(4705):1324–6.
Williams CG, Malik AN, Kim TK, Manson PN, Elisseeff JH. Variable cytocompatibility of six cell lines with photoinitiators used for polymerizing hydrogels and cell encapsulation. Biomaterials. 2005;26(11):1211–8.
Wu ZL, Gong JP. Hydrogels with self-assembling ordered structures and their functions. Npg Asia Mater. 2011;3:57–64.
Xie Y, Song W, Zhao W, Gao Y, Shang J, Hao P, et al. Application of the sodium hyaluronate-CNTF scaffolds in repairing adult rat spinal cord injury and facilitating neural network formation. Sci China Life Sci. 2018;61(5):559–68.
Xu LY, Sheybani N, Yeudall WA, Yang H. The effect of photoinitiators on intracellular AKT signaling pathway in tissue engineering application. Biomater Sci. 2015a;3(2):250–5.
Xu KM, Narayanan K, Lee F, Bae KH, Gao SJ, Kurisawa M. Enzyme-mediated hyaluronic acid-tyramine hydrogels for the propagation of human embryonic stem cells in 3D. Acta Biomater. 2015b;24:159–71.
Yildirim S, Demirtas TT, Dincer CA, Yildiz N, Karakecili A. Preparation of polycaprolactone/graphene oxide scaffolds: a green route combining supercritial CO_2 technology and porogen leaching. J Supercrit Fluids. 2018;133:156–62.
Yin W, Qi X, Zhang Y et al. Advantages of pure platelet-rich plasma compared with leukocyte- and platelet-rich plasma in promoting repair of bone defects. J Transl Med. 2016;14:73. https://doi.org/10.1186/s12967-016-0825-9
Yin FQ, Lin LF, Zhan SJ. Preparation and properties of cellulose nanocrystals, gelatin, hyaluronic acid composite hydrogel as wound dressing. J Biomat Sci Polym E. 2019;30(3):190–201.
Zamboni F, Collins MN. Cell based therapeutics in type 1 diabetes mellitus. Int J Pharm. 2017;521(1–2):346–56.
Zamboni F, Keays M, Hayes S, Albadarin AB, Walker GM, Kiely PA, et al. Enhanced cell viability in hyaluronic acid coated poly(lactic-co-glycolic acid) porous scaffolds within microfluidic channels. Int J Pharm. 2017;532(1):595–602.
Zamboni F, Vieira S, Reis RL, Miguel Oliveira J, Collins MN. The potential of hyaluronic acid in immunoprotection and immunomodulation: chemistry, processing and function. Prog Mater Sci. 2018;97:97–122.
Zamboni F, Ryan E, Culebras M, Collins MN. Labile crosslinked hyaluronic acid via urethane formation using bis(β-isocyanatoethyl) disulphide with tuneable physicochemical and immunomodulatory properties. Carbohydr Polym. 2020;245:116501.
Zapotocky V, Pospisilova M, Janouchova K, Svadlak D, Batova J, Sogorkova J, et al. Fabrication of biodegradable textile scaffold based on hydrophobized hyaluronic acid. Int J Biol Macromol. 2017;95:903–9.
Zarei-Kheirabadi M, Sadrosadat H, Mohammadshirazi A, Jaberi R, Sorouri F, Khayyatan F, et al. Human embryonic stem cell-derived neural stem cells encapsulated in hyaluronic acid promotes regeneration in a contusion spinal cord injured rat. Int J Biol Macromol. 2020;148:1118–29.

Zaviskova K, Tukmachev D, Dubisova J, Vackova I, Hejcl A, Bystronova J, et al. Injectable hydroxyphenyl derivative of hyaluronic acid hydrogel modified with RGD as scaffold for spinal cord injury repair. J Biomed Mater Res A. 2018;106(4):1129–40.

Zhang H, Wei YT, Tsang KS, Sun CR, Li J, Huang H, et al. Implantation of neural stem cells embedded in hyaluronic acid and collagen composite conduit promotes regeneration in a rabbit facial nerve injury model. J Transl Med. 2008;6

Zhang Y, Heher P, Hilborn J, Redl H, Ossipov DA. Hyaluronic acid-fibrin interpenetrating double network hydrogel prepared in situ by orthogonal disulfide cross-linking reaction for biomedical applications. Acta Biomater. 2016;38:23–32.

Zhang Q, Chen S, You RC, Tariq Z, Huang JJ, Li MZ, et al. Silk fibroin/hyaluronic acid porous scaffold for dermal wound healing. Fiber Polym. 2017;18(6):1056–63.

Zhang S, Vijayavenkataraman S, Lu WF, Fuh JYH. A review on the use of computational methods to characterize, design, and optimize tissue engineering scaffolds, with a potential in 3D printing fabrication. J Biomed Mater Res B. 2019;107(5):1329–51.

Zhang Y, Li L, Mu J, Chen J, Feng S, Gao J. Implantation of a functional TEMPO-hydrogel induces recovery from rat spinal cord transection through promoting nerve regeneration and protecting bladder tissue. Biomater Sci. 2020;8(6):1695–701.

Zoratto N, Matricardi P. Semi-IPNs and IPN-based hydrogels. In: Polymeric gels. San Diego: Elsevier; 2018. p. 91–124.

Natural Polysaccharides on Wound Healing 46

Salih Maçin

Contents

Abstract

As a result of diseases and accidents, people lose their tissues and organs. Instead of difficult and troublesome methods such as tissue and organ transplantation, biocompatible, nontoxic, antitumor, antimicrobial, and wound healing natural polymers are used for the treatment of these damages. There are glycosaminoglycans as natural polysaccharides in the human body, which act as extracellular matrix and produced from fibroblasts. Wound healing is a dynamic and complex process consisting of successive periods. Tissue healing process is regular and timely in acute wounds. In chronic wounds, healing takes longer time. The use of appropriate dressings plays an important role in the wound healing process. Researches on polymeric dressings used as carriers for

S. Maçin (✉)
Department of Medical Microbiology, Selçuk University, Konya, Turkey

J. M. Oliveira et al. (eds.), *Polysaccharides of Microbial Origin*,
https://doi.org/10.1007/978-3-030-42215-8_57

local application of active ingredients to the wound surface are increasing. These polymeric systems can be natural, hydrogel-forming materials such as collagen, chitosan, and pectin, or tissue-engineered materials such as alginate. In this section, extracellular matrix, wound formation, wound healing mechanisms, and the function of natural polysaccharides which have an important role in wound healing will be examined.

Keywords

Tissue · Extracellular matrix · Glycosaminoglycan · Polymer · Wound healing

1 Introduction

Wound healing is one of the most common health problems and advanced wound management strategies are needed to achieve optimal healing. Definite times cannot be given for wound healing, but the chronic term is used for wounds that cannot heal within a reasonable time. The progression of wound healing depends on many factors, ranging from the patient's general condition to treatment, to the cause. It is necessary to act according to the characteristics of each wound. Wound healing depends on various factors such as the general health of the person and blood circulation, the reason for the formation of the wound, the location and type of wound in the body, and whether there are infections in the wound. So every wound heals according to its own characteristics (Schreiber et al. 2005).

Wounds are divided into two parts as acute and chronic wounds according to the wound healing time. Acute wounds are wounds that we encounter very often, do not cause damage after recovery, and heal quickly. Chronic wounds, on the other hand, are characterized by impaired skin integrity, functional loss, and prolonged recovery. Recovery in chronic wounds takes weeks, months, and years. In this context, appropriate wound treatment is required, which can speed up the healing process and reduce the healing time at the same time. Glycosaminoglycan derivatives, which are mostly involved in the structure of the extracellular matrix (ECM) in wound healing, have been preferred as potential treatments for many years (Tan et al. 2001).

Glycosaminoglycans (GAGs), the extracellular matrix molecule, which plays an important role in the acute or chronic wound healing process and supports wound healing, is an effective means of angiogenesis and inflammation and leads to rapid granulation, vascularization, and re-epithelization (Mitchell and Church 2002). Glycosaminoglycans are preferred in many materials due to their function in wound healing and are recommended to reduce the healing time in the chronic wound healing process. In this section, the natural polysaccharides used in wound healing and their mechanisms will be discussed in detail.

2 Extracellular Matrix and Wound

2.1 Extracellular Matrix

Connective tissue epithelium, which protects the integrity of the body and keeps all parts of the body together structurally and functionally, provides continuity between the muscle and nerve tissues. Connective tissue covers all the tissues that support the structure of the body, such as ligaments of the joints, beams, bone tissue, cartilage tissue, and adipose tissue.

The intercellular substance (extracellular matrix/extracellular matrix) created by connective tissue cells consists of two main structures:

1. Connective tissue fibers.
2. The basic substance that fills the space between connective tissue fibers and connective tissue cells.

Basic substance: It is the basic structure consisting of proteoglycan, glycoprotein, and glycosaminoglycans, and forming the extracellular space together with cell fibers. This structure forms the basic environment that meets the actual needs of the cell and tissue (Baum and Arpey 2005).

Extracellular matrix (ECM), which plays a role in the connective tissue as an extracellular matrix, is a variety of proteins and polysaccharides that are secreted by some cells in a multicellular organism, which fill cells between cells and act as binding agents in a defined area (Karabekian et al. 2009). There are two main extracellular proteins that make up the matrix. These are fibrous and proteoglycans (Ustunel et al. 2003).

Proteoglycans are peptide chains containing covalently linked glycosaminoglycans. They contain 95% carbohydrates and 5% protein in their structure. There are seven types of glycosaminoglycans (GAGs): hyaluronic acid, chondroitin sulfate, keratan sulfate I and II, heparin, heparan sulfate, and dermatan sulfate. Fibrous proteins are two types: structural proteins (collagen and elastin) and adhesive proteins (fibronectin, laminin, tenaskin, vitronectin, and integrin) (Wound repair 2005).

Glycosaminoglycans form a gelatinous and hydrated substance in the connective tissue by proteoglycans (PGs) by embedding fibrous proteins. Proteoglycans consist of a central protein called glycosaminoglycans (GAGs) that are bound to one or more polysaccharides (Pelosi et al. 2007). Glycosaminoglycans are heterogeneous polysaccharides containing long, linear, and recurrent disaccharide units. These disaccharide units are galactose, galactosamine, N-acetylgalactosamine-4-sulfate, and galacturonic acid. There are two basic types of GAG. The first one is non-sulfated GAG (hyaluronic acid), and the second is sulfated GAG (heparan sulfate, heparin, chondroitin sulfate, dermatan sulfate, and keratan sulfate). Except for hyaluronic acid, GAGs are usually covalently attached to a protein nucleus that forms a general structure called proteoglycans (Pelosi et al. 2007; Souza-Fernandes et al. 2006).

GAGs are usually large complexes consisting of small amounts of protein and negatively charged heteropolysaccharide chains. These complexes form a gel-like matrix called "ground substance" with a large amount of water binding property. They are also strong acidic biopolymers with biomedical importance that prevent the formation of factors such as virus entry into cells and angiogenesis. GAG chains fill most extracellular spaces and provide mechanical support to the tissue, as well as providing rapid diffusion of water-soluble molecules and migration of cells (Ramael et al. 1991).

2.2 Wound Formation: Acute and Chronic Wound

The wound is the disruption of the integrity of the skin or mucosa due to the effect of trauma. Factors such as shock, falling, strong impacts, atmospheric pressure, thermal effects, (burn, freezing) electric shock, and radioactivity cause injuries. As a result of acute or chronic wound, tissue integrity is impaired. Acute wounds heal without problems within the expected time. The time it takes for chronic wounds to heal usually ranges from 5, 10, or 30 days. It may occur as a result of traumatic loss of tissue or surgical procedure (Jackson et al. 1991).

Chronic wounds are often characterized by permanent injury and prolonged inflammation, high bacterial biofilm incidence, and excessive proteolysis (Robson et al. 2001). Impairment in macrophage function and angiogenic response, which is often associated with serious wound healing process, is also observed. Due to prolonged inflammation, excessive removal of inflammatory cells into the wound bed by a large number of neutrophils is required. It is known that neutrophils can remove damaged tissue from the temporary matrix of the wound site and prevent microbial infection. On the other hand, as the potential of unmanaged neutrophils to kill pathogens causes degradation of the ECM and growth factors, it can cause excessive production of protease that initiates significant tissue damage to the host that is detrimental to wound healing. In addition, inefficient cell proliferation due to the breakdown of the ECM molecule in the wound leads to angiogenesis, which indicates greater wound bed defacement and healing impairment. Therefore, prevention of prolonged inflammation is a target strategy in the treatment of severe wounds. GAG has been found to bind to neutrophils, macrophages, and lymphocytes, key cells of the inflammatory response. The effect of excessive protease production caused by too many active neutrophils in the wound area can be inhibited by electrostatic binding with some anionic polymers, such as GAG or functionalized dextrans. A high level of anionic GAG will be in ion pairing with cationic neutrophils to interfere with the activity of cationic proteins through charge interactions. Therefore, with this mechanism, it may be possible to reduce excessive neutrophil uptake and move the wound from the inflammatory stage to the next healing stage. However, after severe tissue damage, glycanases and proteases can destroy GAG (Komarcevic 2000).

In severe wounds, GAG deficiency is eliminated by adding GAG material, such as a natural polysaccharide, directly to the wound area as a wound dressing. With the rich source of GAG around the wound and a better understanding of the GAG roles in the healing processes, it was possible to formulate therapeutic strategies expected

to accelerate serious wound healing. Glycosaminoglycans (GAGs) have been shown to play important roles in cell signaling and development, angiogenesis, anti-coagulation, and co-receptors for growth factors that belong to control of all wound healing stages, both acute wounds and chronic wounds (Peplow 2005).

In chronic wounds, delays occur in the normal stages of healing, the wound cannot be repaired regularly and on time (Jackson et al. 1991). As a result of disruptions caused by various factors in one or more stages of hemostasis, inflammation, proliferation, or remodeling, the healing process cannot be completed completely. This may be caused by increased levels of infection, tissue hypoxia, necrosis, exudate, or inflammatory cytokines. Continued inflammation in the wound causes the tissue to heal response to occur in an uncoordinated and long period, resulting in frequent recurrence of wounds. Chronic wounds can be caused by various causes such as pressure, arterial and venous insufficiency, burns, continuous infection, and vasculitis (Jackson et al. 1991; Komarcevic 2000).

Among the various molecules secreted by ECM, GAG has partners that have important roles in controlling all wound healing stages, acute wounds or severe wounds. These molecules participate in cell-cell and cell-matrix interactions, cell proliferation and displacement, cytokine and growth factor signals, thereby locally modulating their biological activities. The ECM functions to guide the organized response characterized by hemostasis, inflammation, proliferation, and restructuring seen in wound healing. The effects of the various ECM components differ according to the wound stages. This dynamic and sequential order occurs as a result of the interaction of cell and growth factors (Ono et al. 1995).

3 Wound Healing

Wound healing is an extremely dynamic process and involves complex interactions of extracellular matrix molecules, soluble mediator molecules, various resident cells, and infiltrating leukocyte subtypes. The main purpose of the repair is to achieve tissue integrity and hemostasis. For this purpose, the healing process takes place in three stages (Negut et al. 2018).

1. Inflammatory phase (hemostasis and inflammation).
2. Proliferation.
3. Maturation and restructuring (remodeling).

In one of these phases, delay or negativity results in the wound not closing or healing is prolonged (Muncaster 2001).

3.1 Hemostasis

The first stage of wound healing is devoted to the formation of a hemostasis and a temporary wound matrix. The wound matrix appears immediately after the wound

and is completed a few hours later. This phase initiates the inflammatory process and is sometimes called the late phase (Robson et al. 2001).

The vital agents of hemostasis are fibrin, platelets, and blood vessels. In the first 1 or 2 h after injury, wound repair begins with the formation of a fibrin matrix through proteolytic cleavage of fibrinogen with thrombin, and fibrin binds directly to platelets to produce a clot (Muncaster 2001; Woo et al. 2004). This causes degranulation of alpha granules and dense bodies in the cytoplasm of platelets. In this way, albumin, fibrinogen, fibronectin, IgG, coagulation factor V and VIII, platelet-derived growth factor (PDGF), transforming growth factor alpha and beta, fibroblast growth factor-2 (FGF-2), platelet-derived epidermal growth factor (PDEGF), and endothelial growth factor are secreted into the environment. Among all these factors, PDGF, TGF-beta, and FGF-2 are the most important. Dense bodies, by releasing calcium, serotonin, ADP, and ATP, provide an energy source for inflammatory cells that will come to the environment (Monaco and Lawrence 2003). It also calls and activates PDGF and IGF-1 fibroblasts. GAG and collagen are synthesized to allow cells to migrate and multiply at the wound site. Fibrinolytic enzymes resist clot formation. On the other hand, sprinkles ensure that excessive fibrinolytic activity does not occur. The ECM includes a network of scaffolding proteins that are bound by GAG. GAG, especially heparin sulfate (HS), plays a key role as anticoagulants, which have important actions to manage the regulation of protein networks. HS represents 50–90% of the total GAG content and is only in contact with blood when an injury occurs. It has been determined that HS binds with more than 100 proteins involved in hemostasis, many growth factors, proteins involved in lipid metabolism, and ECM proteins. In addition, HS maintains hemostasis as an effective mediator of angiogenesis on the surface of endothelial cells (Jacob et al. 2007).

In this phase, many cells and factors are activated for the wound healing process occurring in the organism.

3.2 Inflammation

The main purpose of this phase in wound healing is to prevent infections. At this stage, the first response is to neutralize bacteria and foreign particles with phagocytosis or toxic substances released by the neutrophils coming into the scar tissue within 48 h (Lesko and Majka 2008). This phase occurs with symptoms of inflammation, such as redness, body temperature, swelling and pain around the injured place, on average, within 24–48 h. When bleeding is controlled, neutrophils, macrophages, and lymphocytes accumulate in the wound area, simultaneously releasing a large number of active mediators (cytokines and growth factors), thereby stimulating the inflammatory phase (Broughton 2nd et al. 2006; Gosain and DiPietro 2004).

Chemotactic agents such as increased vascular permeability caused by inflammation, complement factors, interleukin-1, tumor necrosis factor-alpha (TNF-α), tumor necrosis factor-beta, and platelet factor 4 (PF4) stimulate neutrophil chemotaxis (Broughton 2nd et al. 2006; van Beurden et al. 2005). Neutrophils appear in the

wound 6 h after trauma and are the cells that prevail for the first 2 days. The main task of neutrophils on the wound surface is to remove the traces of the cell that are damaged by trauma, by the phagocytosis of the foreign body, and the release of proteases (Monaco and Lawrence 2003).

Monocyte density begins on the second-third days. As the neutrophil count decreases, the number of monocytes/macrophages increases. In the third-fifth days, macrophages become 12 dominant cells in the wound (Gosain and DiPietro 2004). The presence of active macrophages in the wound area is essential for wound healing. While neutrophil absence does not disrupt the general flow of wound healing, wound healing process stops in the absence of macrophage. The main duties of macrophages in wound healing are outlined: phagocytosis and antimicrobial function, wound debridement, matrix synthesis regulation, cell activation, and angiogenesis (Broughton 2nd et al. 2006).

Macrophages not only do phagocytosis but also perform various cytokines, growth factors, and NO synthesis. They increase keratinocyte and fibroblast activation. In addition, while it catalyzes the conversion of plasminogen to plasmid with the help of neutral proteases, it also activates the complement and pre-Hageman factor (Lesko and Majka 2008). Activated macrophages are cells that play a key role in wound healing (Lesko and Majka 2008; van Beurden et al. 2005).

Hyaluronic acid (HA), a non-sulfated GAG of ECM, is involved in an important process of the inflammatory phase. At this stage, HA accumulates in the wound bed and regulates early inflammation to modulate inflammatory cell and fibroblast cell migration, pro-inflammatory cytokine synthesis, and phagocytosis of invading microbes. In addition, HA can bind and increase the effectiveness of chemokines to neutrophils. Butler et al. found that the efficiency of HA and neutrophil uptake on the endothelial surface was increased and revealed that HA revealed stimuli to neutrophils (Butler et al. 2009).

In the inflammatory phase, neutrophils collagenase and elastase eliminate damaged tissue from the temporary matrix of the wound site, while monocytes inactivate any source of microbial infections through macrophages and the activity of secreted proteases. In inflammation sites, low molecular weight HA fragments (accumulated by the degradation of high molecular weight HA) proliferate IL-6, TNF-α, and IL-1β of Toll-like receptor 2 and Toll-like receptor 4 pro-inflammatory cytokines. Also, growth factors and cytokines released by inflammatory cells induce migration and proliferation of fibroblast and keratinocyte, which synthesize HA levels (Zhong et al. 2010).

It was found that the level of HA was significantly high during the reepithelialization process, where epithelial cells migrated through the new tissue to form a barrier between the wound and the environment. The secretion of cytokines such as TGF-β, PDGF, FGF-2, IL-1, and TNF-α modulates collagen accumulation and penetration of new blood vessels into the wound area by fibroblasts. T lymphocytes (especially CD4) migrate to the wound area; it secretes IL-1, IL-2, TNF-alpha, fibroblast activating factor, EGF, and TGF-beta. Inhibition of circulating T lymphocytes delays wound healing. B lymphocytes have not been found to play a role in wound healing (Winter and Scales 1963).

In summary, in inflammation, leukocytes bind to ECM proteins through integrins. ECM proteins stimulate the activity of monocytes/macrophages and remove neutrophils and wound residues from the wound site (Miller et al. 1998).

3.3 Proliferation

This complex process consists of angiogenesis, granulation tissue formation, collagen deposition, epithelization, and wound closure, and takes about 2 days–2 weeks. A new matrix layer by fibroblasts restores tissue in the wound area. Other mesenchymal cells also enter the inflammatory region of the wound in response to growth factors necessary to stimulate cell proliferation (Lesko and Majka 2008). Also, fibroblasts, endothelial cells, and keratinocytes produce IGF-I, FGF-2, TGF-y, PDGF, and VEGF, which support cell migration and proliferation, matrix synthesis, and angiogenesis. Secreted vascular endothelial growth factor (VEGF) and other cytokines neovascularization, growth factors released from platelets; in particular, the transforming growth factor (TGF-β) and platelet-derived growth factor (PDGF) stimulate the proliferation of fibroblasts. Fibronectin and collagen production occur as the scar tissue becomes rich by fibroblasts. Fibroblasts synthesize collagen and prostaglandins (PG). Both act to create an unstructured connective tissue environment that allows new cells to migrate (Grazul-Bilska et al. 2003).

A number of PGs have been presented in the wound area, and their GAG side chains played a role in the stabilization and activation of growth factors. Sulfated PGs with chondrite sulfate (CS) and dermatan sulfate (DS) contribute to collagen polymerization. PGs provide a matrix for cellular attachment, and some PGs form triple complexes that moisten the tissue that promotes hyaluronic acid, cell survival to cover the wound site, and migration on granulation tissue (Brooks et al. 1994).

This stage takes place with a dynamic process occurring between the developing granulation tissue formation, fibroblasts, growth factors, and ECM. In this process, macrophages release growth factors that stimulate angiogenesis, collagen synthesis, and fibroblast proliferation after binding to the ECM. Endothelial cells bind ECM proteins through integrins, helping to advance the angiogenesis process (Arora et al. 1999).

3.4 Maturation and Restructuring

Remodeling is the stage characterized by the rearrangement of synthesized collagen up to a year after the initial wound formation. There is a balance between collagen production and destruction, characterized by the wound surface, it is the last stage of wound healing (Miller et al. 2003).

At this stage, with the transition of granulation tissue to a mature scar, a new epithelium is formed. This process is accompanied by high mechanical strength of the formed tissue, reduction of capillary amounts by mixing with larger blood vessels, decreasing cell density and metabolic activity of tissue, and lowering GAG content. Inflammatory cells gradually decrease. The early matrix skeleton consists of Type 3 collagen and fibronectin, while the final matrix skeleton is created

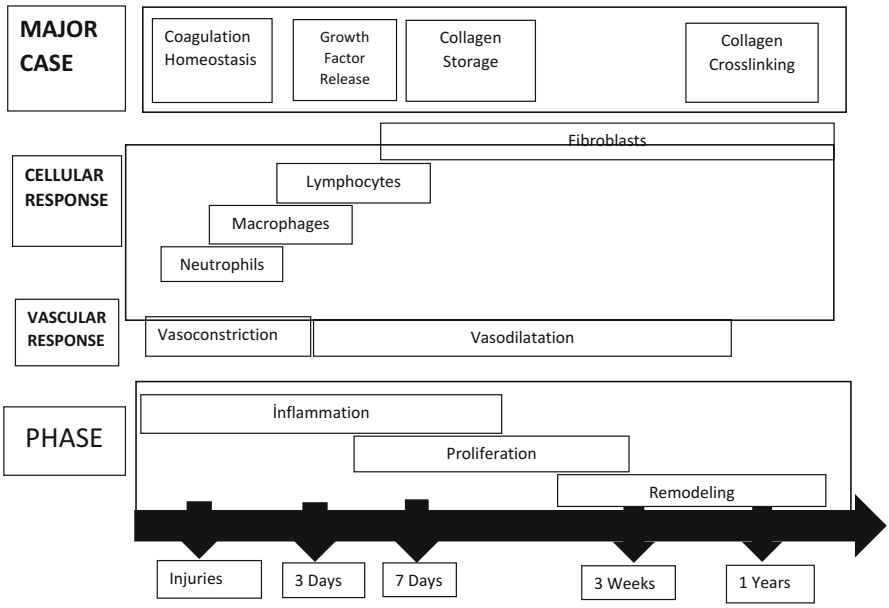


Fig. 1 Wound healing process Inflammation; the first response is the phase in which bacteria and foreign particles of neutrophils that come to the scar tissue within 48 h are inactivated by phagocytosis or toxic substances secreted. The phase in which proliferation angiogenesis, granulation tissue formation, collagen deposition, epithelization, and wound closure lasting approximately 2 days to 2 weeks, and a new matrix layer by fibroblasts, tissue in the wound area is restored. The remodeling phase creates a balance between collagen production and destruction, characterized by the wound surface, and wound healing takes place in three phases

by Type 1 collagen. The resistance of the scar tissue reaches 80% of the original tissue. The remodeling process continues for 21 days–2 years. In order to achieve optimum wound healing, good wound nutrition should be provided, pain should be reduced, clean wound and wound surface should be created, wounds should be protected from trauma and infection, systemic conditions should be corrected or improved, and expenses should be minimized (Ueno et al. 2001).

The mechanical strength of the formed tissue is equal to 25% in relation to the dermis and 80% with unchanged tissue after reconstruction for months (Ko et al. 2014). Considering that GAG activities can reduce inflammatory responses and ECM accumulation in the early stages of wound healing, an appropriate wound treatment at the start of injury of a GAG-rich material is expected to heal closer to normal skin (Mantle et al. 2001) (Fig. 1).

4 The Role of Extracellular Matrix in Wound Healing

Among the various molecules secreted by ECM, GAG has partners that have important roles in controlling all wound healing stages, acute wounds or severe wounds. These molecules participate in cell-cell and cell-matrix interactions, cell

proliferation and displacement, cytokine and growth factor signals. Thus, it modulates its biological activities locally. The ECM functions to guide the organized response characterized by hemostasis, inflammation, proliferation, and restructuring seen in wound healing. The effects of the various ECM components differ according to the wound stages. This dynamic and sequential order occurs as a result of the interaction of cell and growth factors (Ono et al. 1995).

ECM components are involved in every phase of wound healing. By interacting with cells and growth factors, they play a role in a dynamic shopping process that ultimately causes wound closure. More specifically, ECM components play a key role in stimulating cell proliferation and differentiation, directing cell migration, and modulating cellular responses. When the ECM loses its function (for example, in difficult to heal or chronic wounds), wound healing is slowed or stopped (Midwood et al. 2004).

5 Uses of Natural Polymers in Wound Healing

In addition to its presence in bacteria, hyaluronic acid, which is found in extracellular matrix and joint fluids in animal tissues, is important in terms of ensuring the elasticity of cartilage and tendons. However, it has played an important role in synovial fluid, in the vitreous of the eye, cell adhesion, cell mobility, embryo development, and in the living organism as a growth hormone promoter. It is also frequently used in wound healing, cancer metastases, and treatment of diseases such as nervous disorders and arthritis. Since hyaluronic acid polymers are organic substances that are capable of dissolving in water, they are also important because they act as viscosity increasing agents in the liquids they contain. Hyaluronic acid in the skin affects the passage rate of various substances through the skin. Hyaluronidase, which breaks down hyaluronic acid, facilitates the entry of various substances into the tissue. This enzyme, which is found in some microorganisms that cause pathogenic diseases, causes some pathogens to spread in the organism. It is known that the enzyme is also effective during fertilization (Wagener et al. 2017).

Heparin is the only GAG with anticoagulant properties. In other words, it inhibits the prothrombin-thrombin conversion and the effect of prothrombin on fibrinogen and prevents blood clotting. It is known to be used in the treatment of many diseases such as allergic rhinitis, asthma, and cancer. It is applied parenterally for the treatment of thrombosis, phlebitis, and embolism. Heparan sulfate oligosaccharide production is associated with the secondary accumulation of GM2 and GM3 ganglia in the brain, the formation of large cytoplasmic content in various brain cell types, in the accumulation of the C subunit of mitochondrial ATP synthesis, and the irregularity of GAP43 mRNA expression in brain tissues. It leads to very serious advanced mental retardation and premature deaths in the early onset of neurological diseases in children (Celebi and Onat 2006).

Chondroitin sulfate is involved in adults, part of learning and memory, and the neurohypophysis system in the hypothalamus. It also plays an important role in the damages and diseases in MSS. Chondroitin sulfate is the main stopper in the

component of glial wounds after damage in CNS. Surgical chondroitin sulfate regulates axonal regeneration and functional gains. It may also affect pathological stages in diseases such as epilepsy and Alzheimer's (Walker et al. 2005).

Dermatan sulfate is also found in the skin, blood vessels, heart valves, tendons, and lungs. It has a heparin-like antithrombic effect. But there are minimal whole blood anticoagulant and blood lipid cleansing activities. Dermatan sulfate is known to show therapeutic properties in coagulation, cardiovascular diseases, infection, recurrent wounds, and fibrosis (Kobayashi et al. 1997).

5.1 Drawbacks

While natural polymers used in wound healing provide advantages for patients, they also create disadvantages. Based on the wound type, healing time and effectiveness of the material used in patients are not the same. According to the researches, it is stated that the traditional cotton gauze allows moisture to evaporate from the wound surface, adheres to the wound bed and causes pain during removal, and therefore it is emphasized that the gauze dressing should be changed frequently. In order to overcome this problem, wound dressings produced with different features, especially modern dressings, meet the need in the medical field to a large extent. However, there are some problems with the dressings used in the treatment of wounds and burns. For example, the fluid amounts of different wound types should vary, and ideal dressings suitable for different wound types should be developed. Therefore, multidisciplinary studies are needed to further improve the existing dressings (Sidhu et al. 1998).

Natural polysaccharides have shown significant success in the treatment of chronic wounds for their ability to maintain anti-inflammatory and moist wound environment. However, in people with an allergic disease, natural polysaccharides cause excessive reaction and irritation due to the complex structure of the immune system. Therefore, control of the molecular weight of natural polysaccharides is expected to overcome this limitation. By selecting the desired molecular weight, the portion of the natural polysaccharide that can cause a hypersensitivity reaction should be simplified or removed. In addition, these properties in dry wound can also lead to inefficiency of wound healing process. By causing dehydration of the dry wound, it reduces blood flow and migration ability of epithelial cells around the wound area, thereby interrupting the formation of new tissue (Kumar et al. 1993).

Natural polysaccharides have been shown to be a great potential for medical, pharmaceutical, and biomedical applications, including wound dressings, biomaterials, and tissue regeneration, due to their economic, less toxic, and appropriate compatibility profiles. However, these polysaccharides have a lack of protein structures, very poor bio-stability, and difficulty in forming a "matrix" to bridge damaged tissue in the wound healing process, thus facilitating wound contraction and leading to scar formation (Yang et al. 2005). In the light of this information, the most reasonable product should be preferred in natural polysaccharide wound healing.

6 In Vivo and In Vitro Studies

From the polysaccharides, nanofibrillar structures, which are used in the treatment of wounds and burns, are obtained by the electrostatic spinning method. Among the homoglycans, cellulose, chitin, chitosan, dextran, alpha and beta glucan; among the heteroglycans, alginate, agar, agarose, carrageenan, pectin, gum and glycosaminoglycans (hyaluronic acid, heparin, chondroid sulfate, etc.) are polysaccharides frequently used in wound and burn dressings. Experimental animal wound models are still the most preferred models used to examine wound healing as they provide complex conditions that can best mimic wound formation and tissue repair. In these studies, the effectiveness of many natural polysaccharides has been evaluated. Alginate, chitosan, and hyaluronic acid from natural polysaccharides have been accepted as good candidates for the treatment of wounds for years. Its natural polysaccharide ability reduces scar formation in severe wound injuries due to its rich GAG content, which is known to support wound healing and leads to rapid granulation, vascularization, and reepithelialization, thereby providing absolutely minimal scar formation. Also, when the dressing is combined with the wound, an ion exchange reaction occurs between calcium in alginate and sodium in exudate, which produces a soluble gel that helps maintain a moist wound environment (Beer et al. 1997).

Regarding immune system activation, the release of pro-inflammatory cytokines such as IL-1β, IL-6, IL-8, IFN-γ, and TNF-α after wound injury also plays an important role in the wound healing process. Various important processes have been addressed with these cytokines, such as stimulation of keratinocyte and fibroblast proliferation in the wound site, synthesis and breakdown of ECM proteins, and regulation of the immune response. Expressions during the inflammatory phase of expressions have been shown to be intensely upregulated and strongly reduced after impairment of wound healing. Natural polysaccharide can stimulate human cells to produce cytokines with oligosaccharides (glu-glucan, xyloglucan, chitin, pectin, d-mannuronic, and l-guluronic). In particular, the β-glucan mechanism is mediated by many receptors, including dectin-1 receptor, Toll-like receptors complement receptor 3, scavenger receptor, and lactosylceramide. After binding to dectin-1 as the most important receptor, p-glucan stimulates the production of many cytokines or activates other immune and nonimmune reaction mechanisms (Iwamoto et al. 2005).

Chitosan is β-(1–4)-D glycosamino-N-acetyl-D-glycosamine, obtained by deacetylation of chitin. It has very low toxicity after biological degradation. Chitosan is widely used in the treatment of burns and traumatic wounds (33, 102). Chitosan and chitin accelerate wound healing by showing a chemoattractant feature for neutrophils in the early period of wound healing (Ishihara et al. 2006).

Ueno et al. (Ueno et al. 2001) and Ishihara et al. (Ishihara et al. 2006) found that chitosan increased the functions of polymorph nuclear neutrophils (PMNs) and macrophages in their studies. In addition to these features, it provides the proliferation and migration of vascular endothelial cells together with fibroblasts, and also prevents the secretion of interleukin-8 (IL-8) from fibroblasts (Ishihara et al. 2006).

Cross-linked chitosan containing fibroblast growth factor-2 (FGF-2) has been found to accelerate wound healing in diabetic mice. In the research conducted in second-degree burn injuries with chitosan gel containing epithelial growth factor

(EGF), it was emphasized that a faster epithelization was achieved compared to the control group. Chitosan stimulates fibroblast proliferation, the collagen needed, and natural hyaluronic acid synthesis from the wound edge (Kim et al. 2002).

Chitin and chitosan have been shown to stimulate canine PMNs in vitro to release leukotriene (LTB4), in vivo either directly or through complement activation, affecting canine PMNs by the production of arachidonic acid or cytokine. Glucuronic acid N-acetylglycosamine is one of the most hygroscopic molecules in nature, with a disaccharide structure. This hygroscopic feature of hyaluronan helps weaken the bond between the ECM and the cells, thereby dividing the cells by migration. Due to its high viscous feature, it prevents the contact of viral and bacterial passages into cells by creating pericellular region rich in hyaluronan. Hyaluronan also has antioxidant action as a free radical scavenger. Hyaluronan and derivatives have been used for wound healing, and it has been reported that both hyaluronan and derivatives show a bacteriostatic effect and protect the wound area against microorganisms (Presti and Scott 1994).

Martins et al. in their study, they found that a polysaccharide-rich fraction of *Agaricus brasiliensis* can regulate host response by activating both pro- and anti-inflammatory mechanisms, thereby increasing TNF-a and IL-1β production by human monocytes through modulation of Toll-like receptor 4 (Presti and Scott 1994). In addition, even after TLR blockade, these polysaccharides still activate monocytes to produce sufficient levels of IFN-γ, IL-1, and IL-10. TNF-a and IFN-γ are considered important agents of antimycobacterial cytokine cascade, and IL-10 is considered an inhibitory cytokine that is important for adequate balance between inflammatory and immunopathological responses. On the other hand, IL-1β is known as a critical inflammatory agent that plays a role in neutrophil mobilization, endothelial cellular adhesion, and white blood cell infiltration (Dinarello 1996).

Zhao et al. in their study, they determined the wound healing effect and mechanism of Astragalus membranaceus polysaccharide treatment through in vitro and in vivo studies. The results showed that this polysaccharide can promote fibroblast spread in the human skin and accelerate the progression of the cell cycle, and also significantly confirms the secretion of TGF-β1, bFGF, and EGF, which substantially confirms accelerated wound closure in the mouse wound. TGF-β1 is an important promoter in fibroblast proliferation and ECM secretion, and while preventing its deterioration, EGF and bFGF are important stimulants in the formation of reepithelialization and keratinocyte migration in wound healing. In addition, pain and mechanism of pain signals, including peripheral and central processing, are related to the modulation of keloids and TGF-mod involved in hypertrophic pathogenesis (Zhu et al. 2012).

7 Conclusion

It is very important for the materials used in wound healing and wound management. Natural polysaccharides used in the treatment of chronic or acute wounds provide many advantages in terms of wound repair and time. Natural polysaccharides are

more preferred than difficult and laborious methods with their biocompatible, biodegradable, nontoxic, antitumor, antimicrobial, and wound healing properties.

With the developing technology, the use of natural polysaccharide should be increased in wound healing and the product with this feature should be developed. Studies have increased the variety of products that protect the wound surface and speed up the healing and reveal new treatment options. However, more studies are needed to create effective evidence.

References

Arora PD, Narani N, McCulloch CA. The compliance of collagen gels regulates transforming growth factor-beta induction of alpha-smooth muscle actin in fibroblasts. Am J Pathol. 1999;154(3):871–82.

Baum CL, Arpey CJ. Normal cutaneous wound healing: clinical correlation with cellular and molecular events. Dermatol Surg. 2005;31(6):674–86. discussion 686

Beer HD, Longaker MT, Werner S. Reduced expression of PDGF and PDGF receptors during impaired wound healing. J Invest Dermatol. 1997;109(2):132–8.

Brooks PC, Clark RA, Cheresh DA. Requirement of vascular integrin alpha v beta 3 for angiogenesis. Science. 1994;264(5158):569–71.

Broughton G 2nd, Janis JE, Attinger CE. The basic science of wound healing. Plast Reconstr Surg. 2006;117(7 Suppl):12S–34S.

Butler LM, Rainger GE, Nash GB. A role for the endothelial glycosaminoglycan hyaluronan in neutrophil recruitment by endothelial cells cultured for prolonged periods. Exp Cell Res. 2009;315(19):3433–41.

Celebi A, Onat T. Echocardiographic study on the origin of the innocent flow murmurs. Pediatr Cardiol. 2006;27(1):19–24.

Dinarello CA. Biologic basis for interleukin-1 in disease. Blood. 1996;87(6):2095–147.

Gosain A, DiPietro LA. Aging and wound healing. World J Surg. 2004;28(3):321–6.

Grazul-Bilska AT, et al. Wound healing: the role of growth factors. Drugs Today (Barc). 2003;39(10):787–800.

Ishihara M, et al. Controlled releases of FGF-2 and paclitaxel from chitosan hydrogels and their subsequent effects on wound repair, angiogenesis, and tumor growth. Curr Drug Deliv. 2006;3(4):351–8.

Iwamoto M, et al. Structure-activity relationship of alginate oligosaccharides in the induction of cytokine production from RAW264.7 cells. FEBS Lett. 2005;579(20):4423–9.

Jackson RL, Busch SJ, Cardin AD. Glycosaminoglycans: molecular properties, protein interactions, and role in physiological processes. Physiol Rev. 1991;71(2):481–539.

Jacob M, et al. The endothelial glycocalyx affords compatibility of Starling's principle and high cardiac interstitial albumin levels. Cardiovasc Res. 2007;73(3):575–86.

Karabekian Z, et al. Effects of N-cadherin overexpression on the adhesion properties of embryonic stem cells. Cell Adhes Migr. 2009;3(3):305–10.

Kim MS, et al. Water-soluble chitosan inhibits the production of pro-inflammatory cytokine in human astrocytoma cells activated by amyloid beta peptide and interleukin-1beta. Neurosci Lett. 2002;321(1–2):105–9.

Ko CH, et al. Healing effect of a two-herb recipe (NF3) on foot ulcers in Chinese patients with diabetes: a randomized double-blind placebo-controlled study. J Diabetes. 2014;6(4):323–34.

Kobayashi T, Yamasaki Y, Watanabe T. Diabetic scleredema: a case report and biochemical analysis for glycosaminoglycans. J Dermatol. 1997;24(2):100–3.

Komarcevic A. The modern approach to wound treatment. Med Pregl. 2000;53(7–8):363–8.

Kumar AS, et al. Right thoracotomy revisited. Tex Heart Inst J. 1993;20(1):40–2.

Lesko E, Majka M. The biological role of HGF-MET axis in tumor growth and development of metastasis. Front Biosci. 2008;13:1271–80.
Mantle D, Gok MA, Lennard TW. Adverse and beneficial effects of plant extracts on skin and skin disorders. Adverse Drug React Toxicol Rev. 2001;20(2):89–103.
Midwood KS, Williams LV, Schwarzbauer JE. Tissue repair and the dynamics of the extracellular matrix. Int J Biochem Cell Biol. 2004;36(6):1031–7.
Miller SJ, et al. Re-epithelialization of porcine skin by the sweat apparatus. J Invest Dermatol. 1998;110(1):13–9.
Miller RS, et al. The clinical effects of hyaluronic acid ester nasal dressing (Merogel) on intranasal wound healing after functional endoscopic sinus surgery. Otolaryngol Head Neck Surg. 2003;128(6):862–9.
Mitchell JW, Church FC. Aspartic acid residues 72 and 75 and tyrosine-sulfate 73 of heparin cofactor II promote intramolecular interactions during glycosaminoglycan binding and thrombin inhibition. J Biol Chem. 2002;277(22):19823–30.
Monaco JL, Lawrence WT. Acute wound healing an overview. Clin Plast Surg. 2003;30(1):1–12.
Muncaster D. The physiology of wound healing and wound assessment. Br J Perioper Nurs. 2001;11(8):362–70.
Negut I, Grumezescu V, Grumezescu AM. Treatment strategies for infected wounds. Molecules. 2018;23(9):2392.
Ono I, et al. Studies on cytokines related to wound healing in donor site wound fluid. J Dermatol Sci. 1995;10(3):241–5.
Pelosi P, et al. The extracellular matrix of the lung and its role in edema formation. An Acad Bras Cienc. 2007;79(2):285–97.
Peplow PV. Glycosaminoglycan: a candidate to stimulate the repair of chronic wounds. Thromb Haemost. 2005;94(1):4–16.
Presti D, Scott JE. Hyaluronan-mediated protective effect against cell damage caused by enzymatically produced hydroxyl (OH.) radicals is dependent on hyaluronan molecular mass. Cell Biochem Funct. 1994;12(4):281–8.
Ramael M, et al. Immunohistochemical distribution patterns of epidermal growth factor receptor in malignant mesothelioma and non-neoplastic mesothelium. Virchows Arch A Pathol Anat Histopathol. 1991;419(3):171–5.
Robson MC, Steed DL, Franz MG. Wound healing: biologic features and approaches to maximize healing trajectories. Curr Probl Surg. 2001;38(2):72–140.
Schreiber J, et al. Adenoviral gene transfer of an NF-kappaB super-repressor increases collagen deposition in rodent cutaneous wound healing. Surgery. 2005;138(5):940–6.
Sidhu GS, et al. Enhancement of wound healing by curcumin in animals. Wound Repair Regen. 1998;6(2):167–77.
Souza-Fernandes AB, Pelosi P, Rocco PR. Bench-to-bedside review: the role of glycosaminoglycans in respiratory disease. Crit Care. 2006;10(6):237.
Tan W, Krishnaraj R, Desai TA. Evaluation of nanostructured composite collagen–chitosan matrices for tissue engineering. Tissue Eng. 2001;7(2):203–10.
Ueno H, Mori T, Fujinaga T. Topical formulations and wound healing applications of chitosan. Adv Drug Deliv Rev. 2001;52(2):105–15.
Ustunel I, et al. Immunohistochemical distribution patterns of collagen type II, chondroitin 4-sulfate, laminin and fibronectin in human nasal septal cartilage. Acta Histochem. 2003;105(2):109–14.
van Beurden HE, et al. Myofibroblasts in palatal wound healing: prospects for the reduction of wound contraction after cleft palate repair. J Dent Res. 2005;84(10):871–80.
Wagener J, et al. Candida albicans chitin increases Arginase-1 activity in human macrophages, with an impact on macrophage antimicrobial functions. MBio. 2017;8(1):e01820–16.
Walker S, et al. Chemistry and biology of ramoplanin: a lipoglycodepsipeptide with potent antibiotic activity. Chem Rev. 2005;105(2):449–76.
Winter GD, Scales JT. Effect of air drying and dressings on the surface of a wound. Nature. 1963;197:91–2.

Woo YC, et al. Changes in tissue pH and temperature after incision indicate acidosis may contribute to postoperative pain. Anesthesiology. 2004;101(2):468–75.
Wound repair. The remarkable healing power of the human body. Mayo Clin Womens Healthsource. 2005;9(12):4–5.
Yang F, et al. Electrospinning of nano/micro scale poly(L-lactic acid) aligned fibers and their potential in neural tissue engineering. Biomaterials. 2005;26(15):2603–10.
Zhong SP, Zhang YZ, Lim CT. Tissue scaffolds for skin wound healing and dermal reconstruction. Wiley Interdiscip Rev Nanomed Nanobiotechnol. 2010;2(5):510–25.
Zhu Y, et al. Transforming growth factor beta induces sensory neuronal hyperexcitability, and contributes to pancreatic pain and hyperalgesia in rats with chronic pancreatitis. Mol Pain. 2012;8:65.

Xanthan Gum for Regenerative Medicine

47

Renata Francielle Bombaldi de Souza, Fernanda Carla Bombaldi de Souza, Cecília Buzatto Westin, Rafael Maza Barbosa, and Ângela Maria Moraes

Contents

R. F. Bombaldi de Souza · C. B. Westin · Â. M. Moraes (✉)
Department of Engineering of Materials and of Bioprocesses, School of Chemical Engineering, University of Campinas, Campinas, Brazil
e-mail: ammoraes@unicamp.br

F. C. Bombaldi de Souza
R-Crio Cryogenics S.A., Campinas, Brazil

R. M. Barbosa
Department of Engineering of Materials and of Bioprocesses, School of Chemical Engineering, University of Campinas, Campinas, Brazil

R-Crio Cryogenics S.A., Campinas, Brazil

J. M. Oliveira et al. (eds.), *Polysaccharides of Microbial Origin*,
https://doi.org/10.1007/978-3-030-42215-8_59

Abstract

Xanthan gum, a branched polysaccharide produced by *Xanthomonas* bacteria, is traditionally used as an additive in several industrial applications, from food to cosmetics and petroleum, due to its rheological behavior and stability in a wide range of temperature, pH, and ionic strength. These characteristics, along with properties such as biocompatibility and biodegradability, also make this polysaccharide a very attractive material for biomedical applications, including drug delivery and regenerative medicine. The great potential of xanthan gum in tissue engineering and cell therapy fields has been evidenced in the recent years through many studies that show its ability to modulate the release profile of bioactive agents, such as drugs, growth factors, antibacterial agents and cells, and also to tune physicochemical and mechanical properties of biomaterials able to support cell growth. In this chapter, an overview of the microbial polysaccharide production is provided, from the fermentation process to polymer recovery and purification. The structure and conformation of xanthan gum molecule in different conditions is described, as well as its main functional properties, such as viscoelasticity, pH-dependent polyanionic behavior, and gelation capacity. Moreover, methods of functionalization and modification of xanthan gum structure are discussed, including physical, chemical, and chemo-enzymatic treatments to improve polymer processing and properties, such as mechanical performance and bioactivity. Furthermore, examples of the use of xanthan gum-based biomaterials for several targeted applications in soft or hard tissue repair are provided. Finally, current trends are identified and directions on future developments are presented.

Keywords

Xanthan gum · Biomaterials · Polysaccharide · Fermentation · Regenerative medicine

1 Introduction

Xanthan gum (XG) is a branched extracellular polysaccharide produced by *Xanthomonas* bacteria. Since its approval by FDA as a food additive, in 1969, XG has been extensively used in the food industry mostly due to its rheological properties. XG is very efficient as a thickener, shows high viscosity in aqueous solutions even at low concentration, exhibits pseudoplastic behavior in aqueous solutions, is nontoxic, and highly stable in a wide range of pH, temperature, and ionic strength conditions (Kumar et al. 2018).

These properties, along with other superior characteristics of this polysaccharide, such as shear resistance, ability to form both physical and chemical networks, as well as biocompatibility, biodegradability, intrinsic activity as an immunological agent, and capacity to mimic the extracellular matrix, make XG very attractive for biomedical applications (tissue engineering, cell therapy, drug delivery) apart from its

already consolidated uses in industrial applications (e.g., food, cosmetics, petroleum recovery) (Petri 2015; Kumar et al. 2018).

XG and its derivatives, in combination or not with other macromolecules and bioactive agents, have been extensively used to produce different types of biomaterials, including micro/nanoparticles, nanofibrous films, foams, and hydrogels, depending on the specific application desired (Kumar et al. 2018).

In this chapter, the production process, structure, properties, and methods for the modification of XG with the purpose of further improving its properties are presented. Moreover, several applications that demonstrate the promising future of this polysaccharide in tissue engineering and regenerative medicine, including its use in drug delivery systems related to these fields of application, are discussed.

2 Xanthan Gum Production

Xanthan gum is produced in large scale by fermentation of carbohydrates using bacterial cells belonging to the genus *Xanthomonas*. Figure 1 depicts the usual process flowchart of XG production, from inoculum build-up to polymer recovery, purification, and packing.

XG yield and characteristics, such as acetate and pyruvate content, conformational structure, and molar mass, are affected by several factors related to the fermentation process, e.g., bacterial strain, carbon (C) and nitrogen (N) sources, bioreactor type and operation conditions (e.g., batch or fed-batch), pH, temperature, inoculum concentration, airflow rate, mixing rate, and fermentation duration (Kreyenschulte et al. 2014).

Regarding cell strain, despite *Xanthomonas campestris* being the most used for XG industrial manufacture (Petri 2015), xanthan gum can also be produced by other Gram-negative bacteria from the *Xanthomonas* genus, such as *X. arboricola, X. axonopodis, X. campestris, X. citri, X. fragaria, X. gummisudans, X. juglandis, X. phaseoli,* and *X. vasculorium*.

The maximum efficiency of XG production is achieved when glucose, sucrose, maltose, or starch are used as carbon sources. Glucose is the most frequently used carbon source for commercial purposes and product yield using this substrate may reach 2–5%, leading to final XG concentrations of 15 g/L or even higher. The most effective N source for XG production is yeast extract, and by controlling the C:N ratio it is possible to improve XG concentration. However, culture medium formulation issues are normally combined with the choice of fermentation mode (batch, fed-batch, or continuous) to further increase xanthan gum production. Fed-batch fermentation is often more adequate to obtain high XG concentrations. The production of XG during the exponential phase of *X. campestris* is limited, and higher yields are attained in the stationary phase, which is normally reached after 40 h of fermentation (Kreyenschulte et al. 2014).

Not only carbon and nitrogen, but also phosphorus, and sulfur contents in the culture medium affects XG production, while bacterial growth is more influenced by the amount of carbon, nitrogen, phosphorus, and magnesium. An effective medium

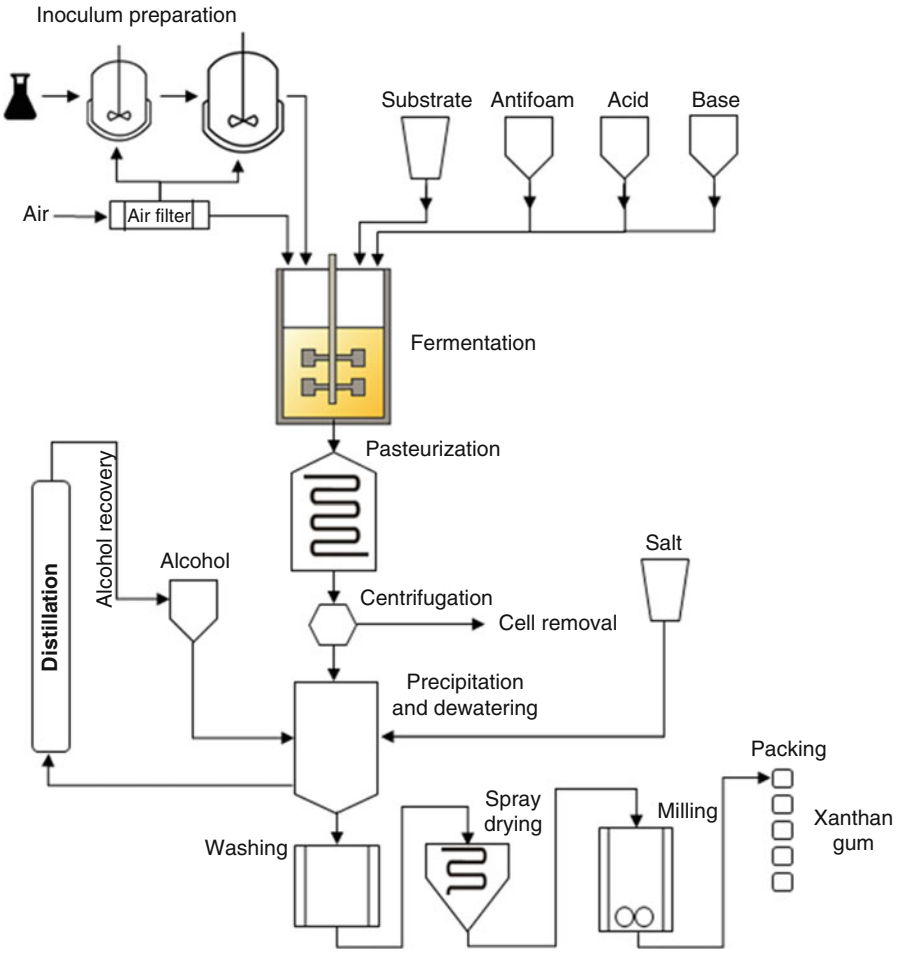


Fig. 1 Typical steps observed in processes of xanthan gum production in industrial scale. (Adapted from Kreyenschulte et al. 2014)

formulation combines, for instance, sucrose (40 g/L), citric acid (2.1 g/L), NH_4NO_3 (1.1 g/L), KH_2PO_4 (2.9 g/L), $MgCl_2$ (0.507 g/L), Na_2SO_4 (0.089 g/L), H_3BO_3 (0.006 g/L), ZnO (0.006 g/L, $FeCl_3.6H_2O$ (0.020 g/L), $CaCO_3$ (0.020 g/L), and concentrated HCl (0.13 mL/L) (García-Ochoa et al. 2000). However, the formulation can be varied with time to better fulfill process demands. Usually, keeping a low C:N ratio in the cell growth phase and a high C:N ratio in the XG production phase enhances xanthan gum production (Kreyenschulte et al. 2014). The fermentation can then be initiated, as an example, with a medium containing 40 g/L of glucose, and afterward (from 30 to 82 h), continuous supplementation of this carbon source at a flow rate of 1.3 g/(L.h) can be implemented to increase XG concentration (Kreyenschulte et al. 2014). Since the cost of the fermentation broth is a major

concern in the industrial production of XG, the use of several low-cost raw materials has been considered to provide nutrients to the cells (Kreyenschulte et al. 2014), e.g., sugar beet pulp, olive mill wastewaters, agricultural wastes, unmodified starch, cheese whey, sugarcane molasses, and grape juice concentrate.

X. campestris can be successfully cultured at different temperatures, ranging from 25 °C to 30 °C; however, the optimal growth temperature is usually around 28 °C (García-Ochoa et al. 2000). The pH decreases from neutral conditions to values around 5 when XG production starts, due to the acid groups present in the polymer. pH control in the neutral range enhances bacterial growth; however, in this situation, XG production stops once the stationary growth phase is reached. Hence, pH control at such ranges is not recommended (García-Ochoa et al. 2000).

XG recovery from the fermentation broth consists in a multistep process and represents up to 70% of all production costs. After fermentation, XG is available in the broth as an extracellular product. This can be considered as an advantage, since there is no need to lyse the cells to access the product. The broth is usually pasteurized to inactivate all microorganisms, the cells are removed by centrifugation, and the polysaccharide is obtained by a precipitation step, followed by washing and drying operations. The pasteurization treatment enhances XG recovery from the cells and decreases the viscosity of the broth, but it should be performed in conditions in which product thermal degradation is avoided. The most used pasteurization conditions are between 80 °C and 130 °C, for 10–20 min, at a pH of 6.3–6.9 (García-Ochoa et al. 2000). The precipitation of the polysaccharide can be done with several water-miscible non-solvents, such as ethanol, isopropanol, and acetone, by the addition of certain salts and by changes in pH. Considering the purification cost, the required volume of alcohol as a precipitating agent is an important parameter. To minimize its need, electrolytes such as potassium or sodium chloride can be combined to the precipitating agent, reducing the amount of alcohol required to about half when compared to the use of alcohol alone (García-Ochoa et al. 2000; Kreyenschulte et al. 2014).

The exact sequence of steps for XG recovery and purification depends on the intended use of the purified polysaccharide (García-Ochoa et al. 2000). For various applications, e.g., for uses in the food industry, the steps mentioned above are sufficient to result in a product with adequate quality. Nevertheless, for applications in the biopharmaceutical area, further purification steps are required. In general, the accepted levels of DNA contents derived from bacterial cells in this type of product are extremely low, in the range of 10–100 pg of residual DNA per dose of a parenterally administered product. The processes used to achieve this high degree of purification can include adsorption by diatomite, cake filtration, enzymolysis by addition of alkaline protease, adsorption by active carbon, and a secondary filtration step. In addition, XG can be repeatedly precipitated with alcohol for further purification (Han et al. 2012; Petri 2015).

After the polymer is obtained as a wet precipitate, it is dried, milled to a predetermined mesh size, and packed (García-Ochoa et al. 2000). Prior to the use in regenerative medicine purposes, XG sterilization is also required. This can be performed by steam sterilization or by exposure to ethylene or propylene oxide and

ionizing radiation. Steam sterilization can affect significantly its rheological behavior due to effects on molecular conformation and molar weight distribution, mostly if performed with XG solutions. Furthermore, it is possible to perform filtration sterilization of its dilute solutions. In this case, after the sterilization process, the polymer solution is concentrated by ultrafiltration to obtain the final concentration desired for the product.

3 Properties of Xanthan Gum

As already mentioned, xanthan gum is a branched polysaccharide in which the backbone consists of repeated pentasaccharide blocks. The backbone of XG contains ß-D-glucose units linked at the 1 and 4 positions and the side chain consists of a (1,3) α-D-mannose, a (1,2) ß-D-glucuronic acid, and a terminal (1,4) ß-D-mannose. The side residues are mostly acetylated at position O-6 of the internal α-D-mannose and approximately half of the terminal ß-D-mannose residues are pyruvated, as shown in Fig. 2 (Petri 2015). The trisaccharide branches can be closely associated to the backbone, originating a stiff chain, which can exist as single, double, or triple helices capable of interacting with other polymer molecules and forming complexes (García-Ochoa et al. 2000). The size and content of acetate and pyruvate groups of

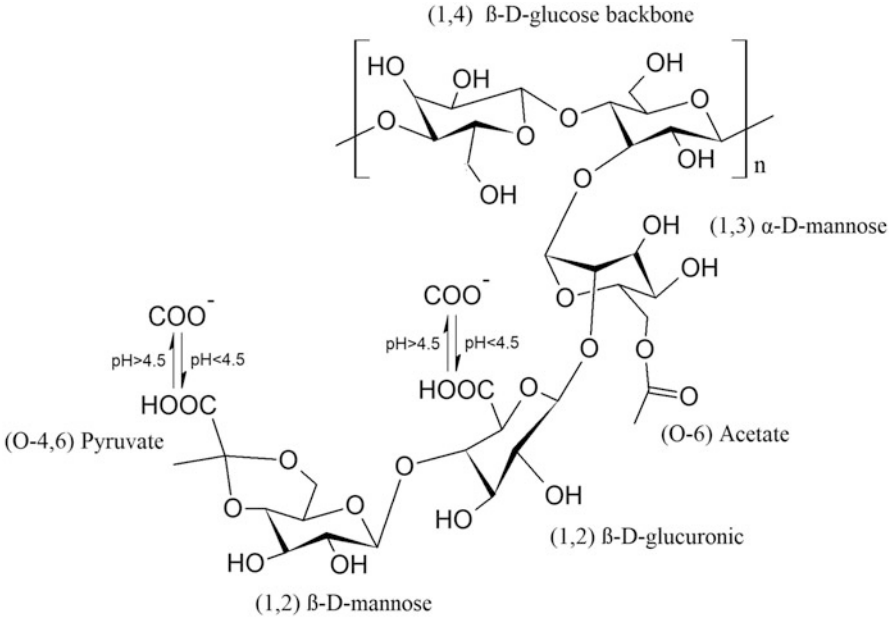


Fig. 2 Chemical structure of xanthan gum. Although the presence of pyruvate coupled to the terminal mannose of the side chain is indicated, alternatively this group can be replaced by acetate, since these two molecules compete for the same carbon atom during biosynthesis (Schmid and Sieber 2015)

XG chains may vary according to the cell strain and process conditions used during the fermentation, as mentioned in the previous section.

The structural conformation of XG in aqueous solution can be tuned mainly by changes in temperature, ionic strength, and polymer concentration. Depending on the specific condition, XG chains can be arranged in an ordered or disordered state, represented by helicoidal or coiled conformations, respectively (Milas and Rinaudo 1979; García-Ochoa et al. 2000). The ordered helix conformation of XG chain remains stable due to hydrogen bonds within the molecule. The polysaccharide undergoes conformational transition from ordered to disordered state when the ionic strength is decreased and the temperature is increased. A change from helix to coil occurs as a consequence of chain destabilization due to electrostatic repulsion between charged carboxylic groups (Petri 2015; Kumar et al. 2018). Conformational alterations in the structure of XG caused by changes in environmental conditions may affect the rheological behavior of the polysaccharide (García-Ochoa et al. 2000).

The viscosity of a XG solution depends on both the temperature at which the polymer is dissolved and the temperature at which the property is measured (García-Ochoa et al. 2000). Usually, the viscosity decreases as the temperature increases, and this behavior is reversible at temperatures between 10 °C and 80 °C. However, when dissolving XG, the viscosity may increase as the temperature increases from 40 °C to 60 °C (Milas and Rinaudo 1979; García-Ochoa et al. 2000). This behavior can be associated with conformational changes in the XG molecule that shifts from an ordered state at lower-dissolution temperatures to a disordered conformation when dissolution temperature is higher (García-Ochoa et al. 2000). This transition corresponds to the spreading of the side chains, i.e., they project away from the backbone of the molecule and, as a consequence, the rigidity of the ß-D-glucose main chain is decreased. The spreading of the side chains leads to an increase in the hydrodynamic volume of the molecule and also to an increase in the swelling and hydration of the polymer chain (Milas and Rinaudo 1979; García-Ochoa et al. 2000). The content of acetate and pyruvate in the side chains can also influence the rheological behavior of a XG solution. Usually, high pyruvate content promotes an increased viscosity and gel behavior due to macromolecular interaction within charged carboxylic groups. In turn, high acetyl content decreases solution viscosity and inhibit gel behavior (Petri 2015; Kumar et al. 2018). XG solutions act as non-Newtonian fluids and present a pseudoplastic or shear-thinning behavior, which means that the conformation of polysaccharide chains can be altered with time and shear rate. The apparent viscosity of XG decreases as the shear rate increases (Kumar et al. 2018). This rheological behavior is an important characteristic of XG solutions that make them very attractive for several industrial applications.

Despite temperature changes lead to denaturation of the native XG double helix structure, depending on xanthan gum concentration, renaturation may occur by cooling or adding salt to the solution. However, the viscosity of the renatured XG solution is different from the native one, as the global conformation of the chains are no longer the same (Matsuda et al. 2009). As indicated in Fig. 3, in a diluted solution heated up to 80 °C, the XG double helix structure can dissociate into two individual

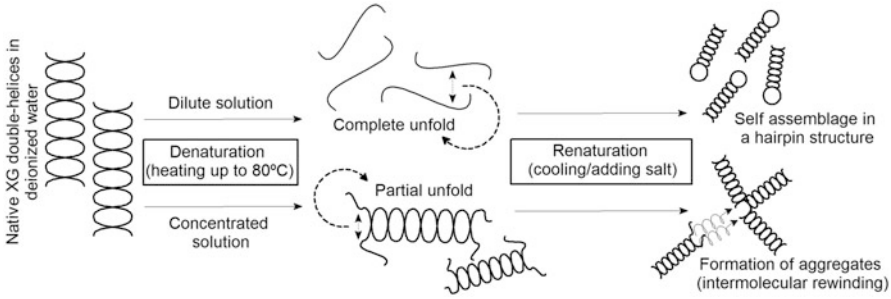


Fig. 3 Denaturation and renaturation models for dilute and concentrated XG solutions. (Based on Matsuda et al. 2015)

chains and, after a renaturation process, individual helicoidal chains are observed. On the other hand, when concentrated XG solutions are heated, only the final part of the chain is denatured, while the central region remains as a double helix due to interactions of the charged residues of the molecule. In this case, the renaturation process creates complex structures with the denatured portions of other molecules (Matsuda et al. 2009, 2015). When XG concentration is sufficiently high, the renatured polymer solution forms a gel, which is another important property of xanthan in industrial applications (Matsuda et al. 2009). An additional factor that can influence the renaturation process is the molar mass of XG, which can vary between 100 and 20,000 kDa (Matsuda et al. 2015; Petri 2015; Kumar et al. 2018).

The stabilization of the ordered structure of XG can originate gels with good mechanical responses, depending on the polymer concentration. The ordered double helix shape (Fig. 3) favors ionic interaction with bivalent cations, resulting in gels with different mechanical properties, such as thermally induced shape-memory and elastic behavior under pressure and tensile modes (Milas and Rinaudo 1979; Petri 2015). The ability of XG to build up physical networks is directly linked to the polymer net charge and mainly to the proportion of acetyl and pyruvil residues. When the pH is above its pKa (4.5), XG assumes an anionic characteristic due to the deprotonation of carboxyl residues. In this case, XG is able to bind to cations, such as Ca^{2+} and Mg^{2+}, as well as to other positively charged macromolecules, such as proteins below their pKa value (Petri 2015; Kumar et al. 2018).

Biodegradability is another important property of XG for application in tissue engineering and regenerative medicine. The main chain of the polysaccharide, comprised of ß-D-glucose, is similar to cellulose and can undergo enzymatic hydrolysis by cellulase. However, it occurs only when the XG molecule presents a disordered conformation, since the side saccharides pose a hysterical hindrance that can block the cleavage of the structure when in ordered form (Milas and Rinaudo 1979; Petri 2015; Kumar et al. 2018). In acidic and neutral conditions, XG hydrogels are stable, but in alkaline conditions, its ester bonds can be hydrolyzed (Petri 2015). In addition, microbial enzymes can also degrade xanthan gum. For these reasons, applications of this polymer in intestine tissues could be of particular interest.

Several and *in vitro* studies carried out with XG-based biomaterials with tissue regeneration purposes confirm its non-cytotoxicity (Fabela-Sánchez et al. 2009; Han et al. 2012; Bellini et al. 2015; Liu and Yao 2015; Elizalde-Peña et al. 2017).

Besides XG biocompatibility and biodegradability, characteristics such as capacity to biologically mimic the composition and architecture of the extracellular matrix, stability at a wide range of temperatures, resistance to acidic environments, and ability to build up networks via intra- and intermolecular bonds make XG a very versatile and attractive polysaccharide to be used in the biomedical and tissue engineering fields (Kumar et al. 2018).

4 Modification of Xanthan Gum

The use of XG in biomedical or other technological applications often depends on polymer structural modifications and fitting of its physicochemical properties. Indeed, the use of unmodified XG in biomedical formulations presents some drawbacks, such as possibility of microbial contamination, poor bioactivity, and uncontrolled rate of hydration and degradation of XG-based biomaterials. Using the chemically modified form of the polysaccharide can minimize the aforementioned drawbacks and favor processing and mechanical performance, as well as improve the bioactivity of XG-based biomaterials for several targeted applications in tissue engineering and drug delivery (Petri 2015; Kumar et al. 2018).

The modification of XG, similarly to what is performed with other polysaccharides, can be carried out using different strategies: (i) by applying metabolic engineering and knowledge about polymer biosynthesis pathways toward improved production of a tailor-made polysaccharide during the fermentation process; or (ii) by *in vitro* or post-process modification, in which the isolated biopolymer can be modified by subjecting it to a specific treatment.

In this section, different approaches for post-process modification of XG are discussed. Due to its branched chain and to the presence of a great number of functional groups, such as hydroxyl and carboxyl, a variety of modification and functionalization methods may be employed to alter XG structure and its ability to build up physical and chemical networks (Petri 2015; Kumar et al. 2018), as mentioned in the previous section.

To improve the properties of XG aiming at better biomaterials, the most commonly used methods of functionalization and modification include physical, chemical, and chemo-enzymatic treatments; plasma-assisted chemical grafting; and their combinations (Petri 2015; Kumar et al. 2018).

4.1 Physical Methods

The rheological property of XG solutions correlates with their gel-like behavior and is crucial in the design of biomaterials such as injectable hydrogels used in tissue engineering. The rheological behavior of XG may be altered by physical means,

such as mechanical treatment, intermolecular cross-linking and network formation, or by blending with other natural polymers (Petri 2015; Kumar et al. 2018).

Different methods based on high-pressure processes, such as microfluidization and high-pressure homogenization, represent alternative approaches to control XG rheological behavior without affecting the polymer chemical composition. Mechanical degradation of the polysaccharide occurs when a physical stress is applied, being associated with reduction of its molar weight and increase in polydispersity. It leads to the disorganization of XG structured network and, as consequence, to rheological changes such as reduced viscosity and pseudoplastic behavior.

In the previous section, the ability of XG to form physical networks in the presence of specific bivalent cations was discussed. However, several trivalent metallic ions, such as Cr^{3+}, Al^{3+}, Fe^{3+}, have also been used to modify the gelation behavior of XG, improving its rheological properties (Kumar et al. 2018). Electrostatic interactions lead not only to the formation of networks between xanthan carboxylate groups and these ions, but also between XG and positively charged macromolecules, such as proteins or other biopolymers (Petri 2015). Polyelectrolyte complexes resulting from interactions between XG and oppositely charged polymers as well as blends resulting from the physical mixture with other materials may lead to products with enhanced physicochemical properties, such as improved swelling capacity, and biological performance (Bellini et al. 2015; Liu and Yao 2015). Besides, the development of composites by the combination of XG with reinforcement agents or bioactive components, such as hydroxyapatite or magnetite nanoparticles, can be a useful strategy to modulate the characteristics of the biomaterial and tune its mechanical, physicochemical, and biological properties (Bueno et al. 2014; Glaser et al. 2015).

4.2 Chemical Methods

Chemical modification is the most commonly used method to change the structure of polysaccharides, either by removal of an existing functionality or by addition of substituent groups, originating new properties (Kumar et al. 2018).

The addition of new functionalities can be carried out by modifying either carboxyl or hydroxyl groups of the XG structure (Kumar et al. 2018). Grafting of molecules on to the polymer chain by covalent bonds to modify the molecular structure of XG is performed through different approaches, such as carboxymethylation, thiolation, esterification, or introduction of aldehyde groups. Table 1 shows how the grafting of different types of molecules on to XG structure affects its properties.

The chemical modification of XG by graft copolymerization has also been extensively reported. A graft copolymer is the combination of a macromolecular main chain with a side chain with a different structure. Graft copolymerization of 2-hydroxyethyl methacrylate (HEMA) and acrylic acid (AA) on to XG was performed to fabricate a XG-g-poly(HEMA-co-AA)-based superporous hydrogel system (Gils et al. 2009). Moreover, graft copolymerization of acrylamide on to XG and carboxymethyl-XG using different methods was also investigated. Using this approach, it is possible to

Table 1 Effects on XG properties of chemical modification by grafting different types of molecules on to the polysaccharide molecule

Modification	Effect	References
Introduction of aldehyde groups by periodate-mediated oxidation	Schiff base bonding between oxidized XG and amine groups of other molecules (e.g., chitosan) originate self-healing hydrogels or materials that respond to physical, chemical, and biological stimuli	Salazar et al. (2018)
Carboxymethylation	Decrease in viscosity; faster release of drug (sodium diclofenac)	Ahuja et al. (2012)
	Better perspective for long-term cell therapies	Mendes et al. (2012)
Thiol derivatization via esterification with mercaptopropionic acid and thioglycolic acid	Improved mucoadhesive property; sustained release of drug (metronidazole) over a prolonged period	Bhatia et al. (2015)
Thiolation via amide bond formation (covalent attachment of L-cysteine)	Higher water uptake capacity and stability against erosion; enhanced mucoadhesive strength; faster release of drug (tannic acid)	Laffleur and Michalek (2017)
Succinic anhydride (SA) functionalization	Anionization due to increased presence of carboxylic groups as a strategy to incorporate small cationic molecules (e.g., gentamicin) in XG-based hydrogels; improved elasticity	Wang et al. (2016a)
Esterification with poly (maleic anhydride/1-octadecene)	Better performance regarding salt tolerance and temperature resistance; higher viscosity, improved resistance to shear force, and enhanced viscoelastic behavior	Wang et al. (2016b)

obtain materials with increased swelling capacity, faster release of loaded drugs, and improved thermal stability (Badwaik et al. 2013). Further studies also report the graft copolymerization of XG with ethyl acrylate, 2-acrylamidoglycolic acid, and 2-acrylamido-2-methyl-1-propane sulfonic acid aiming to obtain more thermostable polysaccharide-based hydrogels, with improved swelling capacity, metal ion sorption, and resistance to biodegradation (Badwaik et al. 2013).

Besides polymer functionalization, cross-linking of functional groups via covalent interactions using chemical agents can be performed to control the kinetics of hydrogel formation, rheological performance, and mechanical stability of XG networks (Kumar et al. 2018). The use of sodium trimetaphosphate (STMP) for the fabrication of XG hydrogels by chemical cross-linking was already demonstrated (Tao et al. 2016). Several other molecules can be used as cross-linkers, such as citric acid, epichlorohydrin, glutaraldehyde, N,N-methylenebisacrylamide, glicerol, polypropylene diglycidyl ether, adipic acid dihydrazide, and adipoyl chloride (Petri 2015).

As mentioned before, the removal of an existing functional group from the polymer chain represents another way of modifying the polysaccharide structure.

The removal of acetyl groups from XG side chains can be performed to improve the viscosity and stability of xanthan solutions. Deacetylation may be performed under alkaline condition or by enzymatic treatment. In the last case, the use of enzymes enables to eliminate targeted acetyl groups, contrary to random removal of the groups and backbone degradation that occurs when the chemical treatment is used (Kool et al. 2014). Other enzymatic treatments are discussed below.

4.3 Enzymatic Treatment and Plasma Irradiation

Besides allowing the targeted elimination of functional groups, the enzymatic treatment may be used for other purposes. Xanthan lyases, for instance, are well characterized xanthan modifying enzymes able to remove the terminal mannosyl residue. In turn, XG molecular weight reduction can be performed via cellulase-mediated hydrolysis (Kool et al. 2014). These treatments can alter the rheological response of XG, as the amount and proportion of acetate and pyruvate groups in the polysaccharide structure, as well as the size of its chain, pose a large influence on the viscosity of XG solutions. Enzymatic treatments affect also the stability of XG solutions toward the addition of salts and changes in temperature and pH conditions (Kool et al. 2014).

Finally, the use of plasma irradiation is also described as an efficient route to modify XG structure. Cold-plasma is a nonthermal process used for microbial inactivation and modification of polysaccharides. It can be used to alter the rheological properties of XG solutions by grafting primary amines on to XG molecules, which are, afterward, cross-linked with glutaraldehyde (Kumar et al. 2018).

5 Application of Xanthan Gum in Regenerative Medicine

XG and its derivatives, in combination or not with other macromolecules, bioactive agents, and different types of mammalian cells, have been extensively used to produce materials for biomedical applications with outstanding physical and chemical properties. As already discussed, materials with very different morphologies and purposes can be obtained by the combination of xanthan gum with other compounds, as shown in Figs. 4 and 5.

The biocompatibility and biodegradability of XG enable the exploitation of these materials in regenerative medicine and tissue engineering applications as scaffolds for cell adhesion, proliferation, and differentiation. In this section, several examples of the use of XG-based biomaterials for application in soft or hard tissue repair are discussed.

5.1 Skin

Tissue engineered skin substitutes have been developed as alternatives to traditional wound healing strategies, especially in cases of deep injuries and burns. These

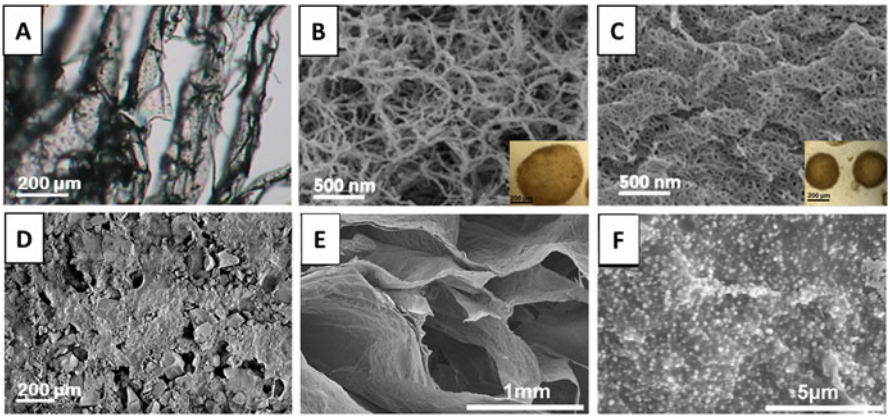


Fig. 4 Different types of XG-based biomaterials. (**a**) photograph of a XG-chitosan **sponge** (polymer ratio 1:1); (**b**) surface morphology of **microcapsules** constituted of phospholipid (1,2-dioleoyl-sn-glycerophosphoetilamine, DOPE) conjugated with XG, and (**c**) XG-DOPE **microcapsules** coated with poly-L-lysine (embedded pictures show the microcapsules); (**d**) micrograph of the surface of a XG **membrane** containing 5% (w/w) of hydroxyapatite; (**e**) cross-sectional morphology of a **hydrogel** constituted XG and gellan, and (**f**) the same **hydrogel** containing chitosan nanoparticles loaded with dexamethasone disodium phosphate. ((**a**) Reproduced from Ibrahim and Fahmy 2016 by permission of Taylor & Francis Ltd.; (**b** and **c**) Reproduced from Mendes et al. 2013 by permission of Elsevier; (**d**) Kindly provided by M.Sc. Rafael Maza Barbosa; (**e** and **f**) Reproduced from Sehgal et al. 2017 by permission of John Wiley & Sons)

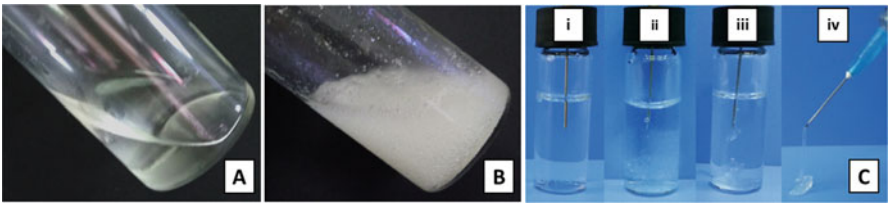


Fig. 5 XG-based thermoresponsive hydrogels. (**a**) 8% (w/v) methylcellulose (MC) hydrogel without XG and (**b**) 8% (w/v) MC hydrogel with 1% (w/v) XG; (**c**) photos of the injections of hydrogels containing different proportions of XG and MC into 37 °C phosphate-buffered solution: (*i*) 23 °C (MC 10 wt.% solution), (*ii*) 23 °C (XG 2wt.% weak gel) and (*iii*) 23 °C (XG 2wt.%/MC 10wt.% blend solution), as well as (*iv*) injection of 37 °C XG 2wt.%/MC 10wt.% hydrogel using 23G needle. ((**a** and **b**) Kindly provided by Dr. Cecília Buzatto Westin; (**c**) Reproduced from Liu and Yao 2015 by permission of Elsevier)

materials can be either acellular or contain cells such as fibroblasts or stem cells to improve their bioactivity. They are able to provide components from both the dermis and the epidermis that are required for effective wound closure and tissue functions recovery. Most importantly, these materials can act as a protection barrier and are also capable of reducing pain and promoting healing by tissue regeneration (Juris et al. 2011; Bellini et al. 2015).

XG has been employed in tissue engineered skin substitute's formulations in the form of solid membranes and, most commonly, topical hydrogels. The topical application of XG-based hydrogels is favored by its high ability to adhere to the lesion bed (Petri 2015).

Dense and porous chitosan-xanthan gum membranes can be successfully used to culture multipotent mesenchymal stromal cells for application as bioactive dressings for dermo-epidermal wounds. The membranes are not mutagenic and allow efficient adhesion and proliferation of the cells *in vitro*. The nonporous membranes, when in combination with the mesenchymal stromal cells, lead to a significant improvement in the healing of skin wounds on Wistar rats (Bellini et al. 2015).

Antibacterial polyelectrolyte hydrogels produced by combining XG and chitosan and incorporating silver nanoparticles have demonstrated to be cytocompatible with 3T3 fibroblast cells and hence are also promising for the application as skin substitutes (Rao et al. 2016).

XG has been combined with other polysaccharides to prepare hydrogels with a range of mechanical and degradation properties, as well as good biocompatibility with human fibroblast (MeWo) cells. The combination of XG with konjac gum, iota-carrageenan, and kappa-carrageenan originates strong hydrogels that could be explored as epidermis skin scaffolds. While XG and konjac gum may produce amorphous matrices, iota and kappa carrageenans can function as fiber reinforcements for the composite hydrogels (Juris et al. 2011). The hydrogels can be prepared with different elasticity degrees, depending on the softness required (Almeida et al. 2014).

5.2 Articular Cartilage

Joint surfaces are covered with a specialized type of cartilage, called hyaline or articular cartilage (Han et al. 2017). Because the inner cartilage tissues within joints are characterized by the absence of lymphatic vascular system and nerve endings, the treatment of diseases that affect this tissue is difficult. Thus, the use of tissue engineering approaches can represent an attractive solution.

XG-based materials have potential to aid in this purpose in different forms, e.g., as solutions, membranes, and hydrogels. XG solutions, for instance, are reported not to cause adverse effects to chondrocytes (the cartilage cell type) *in vitro* in a wide range of concentrations (from 10 to 2000 μg/mL) (Han et al. 2012). When chondrocytes are cultivated in the presence of XG and interleukin-1β, an inductor of degradation, XG is capable of protecting the cells in a dose-dependent manner, stimulating their growth. A solution of XG (10 g/L in phosphate-buffered saline, molar mass from 5100 to 5400 kDa) was reported to be useful for the treatment of joints of Wistar rats with osteoarthritis, a disease that affects the cartilage tissue (Han et al. 2017). The solution was injected into the animal's joints at a ratio of 0.2 mL/kg and was helpful to reduce the pain caused by the disease, also decreasing cartilage degradation.

Porous membranes with high culture medium absorption capacity formulated by combining XG and chitosan (Westin et al. 2017) are also promising for the treatment

of this condition. This formulation is useful to culture mesenchymal stem cells and if cultivation is performed in the presence of kartogenin, the cells can be successfully differentiated into chondrocytes, what supports the feasibility of this approach for application in cartilage tissue engineering.

Different proportions of XG, methylcellulose, and carboxymethyl chitosan can be combined to produce thermoresponsive hydrogels with potential to be used in injectable formulations for noninvasive treatment of cartilage lesions (Westin et al. 2020). These formulations are stable, have high medium absorption capacity, interconnected porous structure, and adequate compressive mechanical characteristics to be used in joint regions. The drugs dexamethasone, diclofenac sodium, and gallic acid, used to treat osteoarthritic joints, can also be incorporated in the XG-based hydrogels, further improving the bioactivity of the formulation.

5.3 Tendons

Tendon tissue engineering has stood out lately in regenerative medicine as an alternative to autograft and allograft treatments, as it overcomes common problems of the grafting techniques, such as pain and donor site morbidity. Besides that, after common tendon-repair surgery, adhesion between the healing tendon and the synovial membrane can occur, resulting in poor functional repair of this tissue.

This could be prevented by the use of a simple mechanical barrier implant such as the membranes developed by Kuo et al. (2014), who studied four distinct formulations constituted by different amounts of XG, gellan gum (GG), and hyaluronic acid (HA). The membranes can be wrapped around surgically repaired rat tendons. Evaluation after 3 weeks showed that all formulations of XG/GG/HA hydrogel membranes reduced tendon adhesion with efficacy equivalent to that of Seprafilm®, a commercially available HA and carboxymethyl cellulose membrane. The developed membranes are capable to quickly swell and can cover the tendon more easily and in closer proximity than Seprafilm®. These membranes are slowly degraded, which allows them to work as barriers for longer periods.

5.4 Neuronal Tissue

Neurodegenerative diseases and spinal cord injury are the most recurrent conditions that affect the nervous system. Nerve tissue regeneration is a complex biological process (Elizalde-Peña et al. 2017). Depending on the type and site of injury, tissue regeneration does not occur and tissue engineering is an approach that may be adopted.

In this sense, biomaterials with composition also based on XG could be promising. Fabela-Sánchez et al. (2009), for instance, combined XG with methacrylated chitosan to produce hydrogels applicable as scaffolds for neural cells from BALB/c mice. These hydrogels were able to provide a favorable environment for cell proliferation. A similar hydrogel, also consisting of (chitosan-g-glycidyl methacrylate)–xanthan gum,

was introduced into a spinal cord lesion of Wistar rats (Elizalde-Peña et al. 2017). A recovery five times faster was observed when using this biomaterial in comparison to the control. The XG-based hydrogel proved to be biodegradable by enzymatic hydrolysis, which allows the material to act as a scaffold during spinal cord recovery.

In the same line, Glaser et al. (2015) demonstrated that hybrid scaffolds made of XG and magnetite nanoparticles at different concentrations and with distinct magnetization profiles are able to promote adhesion and proliferation of neural cells. Cell adhesion is more pronounced on XG-containing magnetic particles than on neat XG scaffolds. However, proliferation is independent from the scaffold type. In turn, the differentiation of embryonic stem cells into neural cells is more expressive when neat XG is used, which was associated to the high density of negative charge present in this case.

5.5 Bone and Periosteal Tissue

Critical-sized bone defects caused by fractures and tumor resections, for example, present very restricted spontaneous regeneration capacity. Such lesions cannot be treated by traditional methods that involve surgical reconstruction with autografts, allografts, or metal implants, since these treatments offer complications as insufficient osseointegration and risk of infection. Bone tissue engineering with polymer-ceramic composite scaffolds is a promising alternative approach, allowing the modulation of the mechanical and degradation properties of materials to meet the requirements of the defective site (Dyondi et al. 2013; Izawa et al. 2014).

XG has been explored for the production of biomaterials in the form of sponges or hydrogels to be used for bone regeneration. XG hydrogels can serve as an organic template for biomimetic mineralization since carboxyl groups in the polymer chain may interact with calcium ions in ceramic materials such as hydroxyapatite (Hap) and other phases of calcium phosphate, the main inorganic component of bone tissue (Dyondi et al. 2013; Izawa et al. 2014).

The mineralization of Hap upon a XG hydrogel has already been demonstrated (Izawa et al. 2014), by using an alternate soaking process. The mineralization induces a microstructure change in the XG-matrix from a layered to a porous structure with unique mechanical properties. Xanthan gum chains can also coat the surface of strontium-substituted Hap nanoparticles, significantly increasing colloidal stability (Bueno et al. 2014). These biomaterials are suitable for osteoblasts growth and can induce high alkaline phosphatase activity.

A successful approach to enhance the mechanical stability and cytocompatibility of XG scaffolds is the incorporation of silica glass and cellulose nanocrystals to the formulation (Kumar et al. 2017). Through this approach it is possible to produce hybrid scaffolds with good compatibility to osteoblastic MC3T3-E1 cells and mechanical properties appropriate for low load-bearing bone tissue engineering applications.

XG has also been explored for the engineering of periosteum, a dense and highly vascularized membrane that covers most of bone surfaces. The periosteum is

essential for bone healing, as it provides key components for tissue repair, such as osteogenic progenitor cells. Bioactive, porous, and flexible scaffolds can be easily produced by combining XG to other polymeric compounds for periosteum repair. The introduction in the formulation of phosphorylated chitosan, a chemically modified form of chitosan that shows osteoconductive properties, can further improve the bioactivity of these XG-based scaffolds (Bombaldi de Souza et al. 2020). Osteogenic differentiation of adipose-derived stem cells and mineralization on the surface of the materials confirms their potential for osseointegration and bone regeneration. Figure 6 shows the visual aspect and morphology of these scaffolds, constituted by the combination of XG and chitosan or phosphorylated chitosan as dense or porous membranes, and Fig. 7 shows the potential of such material to promote cell attachment and mineralization.

5.6 Periodontal Tissue

Different biomaterials containing XG can be used to aid in the treatment of oral diseases such as periodontitis and loss of alveolar bone. The early detection of the conditions that negatively affect the oral cavity is crucial to choose the adequate

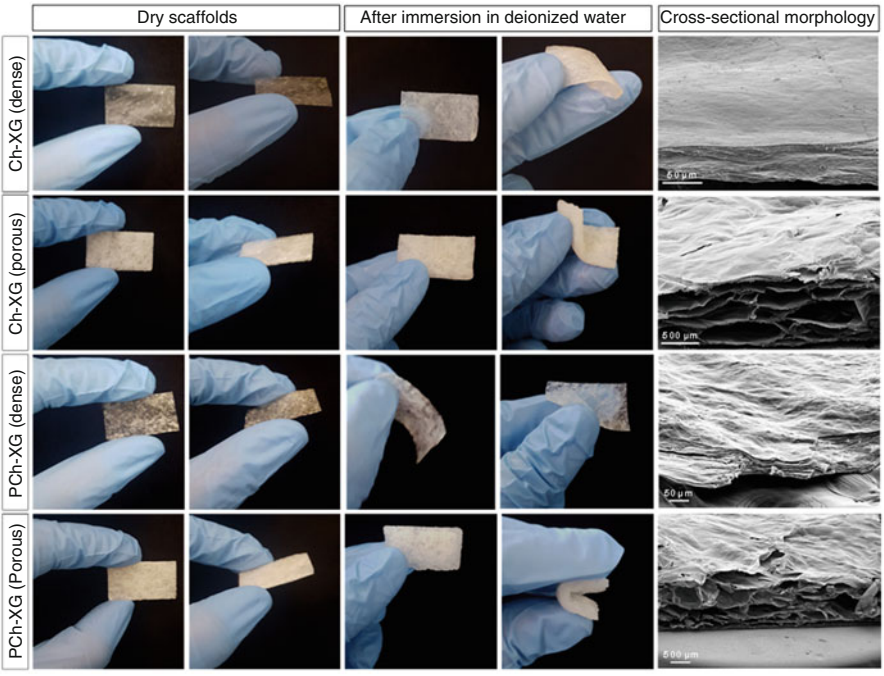


Fig. 6 Visual aspect (materials before and after immersion in water) and cross-sectional morphology of dense or porous scaffolds constituted by XG combined with chitosan (Ch) or phosphorylated chitosan (PCh). (Image kindly provided by Dr. Renata F. Bombaldi de Souza)

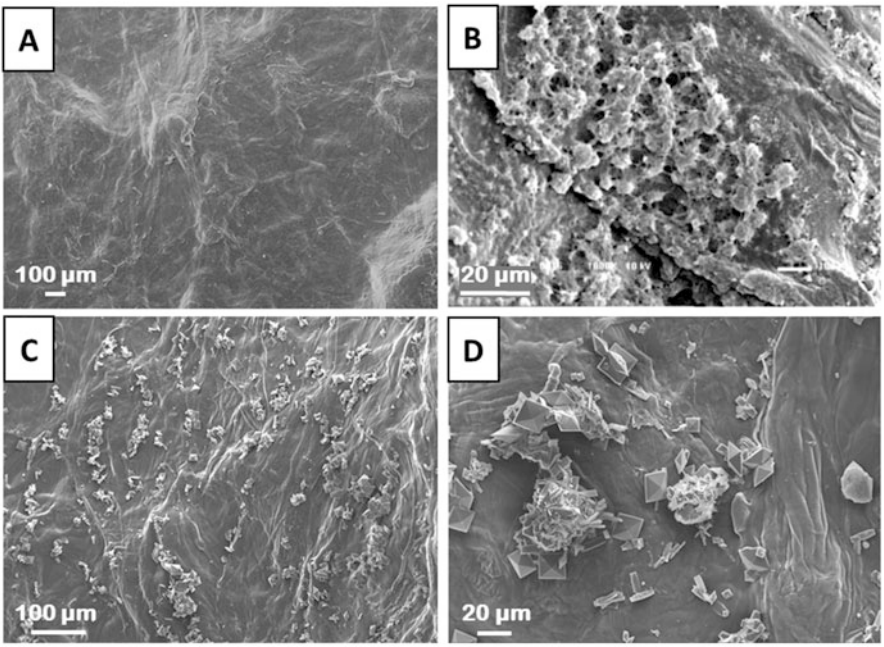


Fig. 7 XG-chitosan (Ch) dense scaffolds (membranes). (**a**) morphology of the surface before cell seeding; (**b**) surface 96 h after inoculation of mesenchymal stromal cells (from bone marrow of rats); (**c** and **d**) surfaces after 3 weeks of adipose-derived stem cells culture in the presence of differentiation medium containing dexamethasone and ascorbic acid. Mineralization is observed due to the presence of calcium phosphate and oxalate crystals. ((**a**, **c**, and **d**) Kindly provided by Dr. Renata F. Bombaldi de Souza; (**b**) Reproduced from Bellini et al. 2015 with permission from SAGE journals)

technique to address the problem. Depending on the severity of the disease or the tissue affected, different types of biomaterials, in the form of hydrogels or membranes, can be selected for the treatment.

The periodontium is the structure responsible for supporting dental elements, being formed by gingiva, ligaments, cementum, and alveolar bone. As the oral cavity supports a heterogeneous microbiota that can exceed 700 different species of microorganisms (Hashim 2018), the periodontal pocket can be particularly vulnerable to microbial attack. In this case, inflammation and degeneration of the tissue adjacent to the dental element are often observed, and, in severe situations, loss of the dental element can occur.

XG can be formulated to provide gel-like biomaterials relevant as drug delivery systems for the treatment of diseases that affect the oral cavity. For instance, hydrogel formulations capable to adhere to the oral mucosa can be of particular interest in this sense, since they could increase the retention time of bioactive agents incorporated in their formulations in the affected periodontal pocket. The period in which the biomaterial remains in the oral cavity has a great importance for the effectiveness of the treatment, since an adequate time-response is expected for local drug delivery. With that purpose, Needleman et al. (1997) proposed three

formulations consisting of chitosan, poly(ethylene oxide), and XG. The XG formulation presented the most prolonged adhesion time in the oral mucosa, approximately 1.7 and 3.6 times higher than the retention time observed for poly(ethylene oxide) and chitosan formulations, respectively. Polyelectrolyte complexes consisting of a XG hydrogel combined with chitosan microspheres are also of relevance concerning this goal, being attractive both for the treatment of acute and chronic periodontitis (Kim et al. 2017). The concentration of XG in the hydrogels can be tuned to control the release of antiseptic molecules. The biomaterial has also shown to be nontoxic to human fibroblasts and able to inhibit the growth of *Pseudomonas gingivalis*, one of the main causes of periodontitis.

The use of XG gels seem indeed to be attractive also as an adjunct therapy with scaling and root planning procedures for the treatment of chronic periodontitis. The clinical effectiveness of a commercial product (Chlosite®) consisting of a XG hydrogel containing chlorhexidine for the treatment of chronic periodontitis was analyzed in 30 patients, with positive results (Jain et al. 2013). Significant decreases in gingival and plaque index were reported (these dental indices indicate, respectively, the severity of gingiva inflammation and the formation of films or deposits on the edges of the tissues adjacent to the tooth). Moreover, a decrease in bleeding on probing and also on clinical attachment, both considered as criteria to diagnose gingival inflammation loss, were also observed.

In severe cases of periodontitis, in which partial or complete absorption of the alveolar bone are detected, guided regeneration techniques that include the use of membranes to aid tissue regeneration can be applied. There are several types of membranes available for the use in guided bone regeneration, most of them constituted by collagen. XG has not yet been exploited for this application; however, materials such as the membranes produced by Bombaldi de Souza et al. (2020), described in the previous section, with intended use in periosteum reconstruction, could be attractive candidates for this purpose.

5.7 Delivery of Bioactive Agents

Delivery of biochemical cues to the body is one of the fundamental pillars of regenerative medicine. Bioactive agents such as growth factors and small molecules can modulate the microenvironment of regenerating tissues, stimulating exogenously added cells or recruiting endogenous cells to act on tissue repair. Cell growth and differentiation, as well as angiogenesis or cicatrization, can be triggered, controlled, or improved. Delivery of drugs with antioxidant, anti-inflammatory, antibacterial, or anticancer properties are also of interest, as they can improve the prognosis of many degenerative diseases.

Scaffolds with a variety of morphologies and properties have been used to achieve sustained release of biomolecules and bioactive components. The incorporated bioactive agents can be released at particular phases of tissue regeneration to match specific processes. This controlled release is often achieved by fabricating either multicomponent or core-shell scaffolds. Besides, smart drug delivery systems that respond to external stimuli such as temperature, pH, and physical fields have

Table 2 Selected examples of XG-based materials for the delivery of bioactive agents

Formulation		Form	Bioactive agent	Targeted application	Remarks	References
Polymer matrix	Other components					
XG/Gellan gum	Chitosan nanoparticles	Injectable hydrogel	Basic fibroblast growth factor (bFGF), and bone morphogenetic protein 7 (BMP7)	Bone tissue engineering	Injectable system with dual growth factors allows bone cells to differentiate	Dyondi et al. (2013)
XG/Chitosan	–	Sponge	Rosuvastatin (promotes BMP-2 expression and osteoblast differentiation *in vitro*)	Bone tissue engineering	Biodegradable sponges bring drug into immediate contact with the fractured bone	Ibrahim and Fahmy (2016)
XG/Gellan gum (coating)	Drug loaded chitosan nanoparticles	Coating for ceramic scaffolds	Dexamethasone (osteoinductive drug)	Bone tissue engineering	Sustained delivered of the drug for 5 days improves the potential for osteogenic differentiation	Sehgal et al. (2017)
XG/ Methylcellulose	–	Injectable thermo-responsive hydrogel	Doxorubicin (anticancer drug)	Drug release and tissue engineering	The hydrogel is biocompatible and biodegradable in rat body and releases the drug in a sustained way	Liu and Yao (2015)
XG modified with succinic anhydride	–	Hydrogel	Gentamicin (antibacterial agent)	Drug release and tissue engineering	Antibacterial hydrogels can be used to modify implants and other biomedical devices	Wang et al. (2016a)

Aldehyde-modifiedXG/ Carboxymethyl-modified chitosan	–	Injectable hydrogel	Vascular endothelial growth factor, VEGF (angiogenic factor)	Abdominal wall reconstruction	Controlled drug release in tissues with much excretion or exudation such as digestive tracts and open wounds	Huang et al. (2018a)
XG/Gellan gum	Polymethyl methacrylate (PMMA) particles	pH-responsive hydrogel conduits	Bovine serum albumin (BSA) and diclofenac sodium (model compounds)	Peripheral nerve repair	XG/gellan ratio variability and pore-inducing effects of intercalated PMMA yielded a means for fine tuning matrix rigidity and flexibility	Ramburrun et al. (2017)
XG/ Galactomannan (coating)	Dioctadecyldimethylammonium bromide(DODAB) cationic liposomes	Coating for liposomes	Epidermal growth factor (EGF)	Wound treatment/ Skin reconstruction	Association of anionic XG and neutral galactomannan for liposomes coating resulted in nanoparticles capable of improving the release profile of EGF, a low-stability drug	Kaminski et al. (2016)

also been developed with this purpose. These stimulus-responsive materials allow to set off or to modulate the delivery of the bioactive molecules using external signals.

XG has been employed in the formulation of numerous biomaterials for the delivery of bioactive agents, such as drugs, growth factors, antibacterials, and also cells. These delivery systems are often produced in the form of injectable or topical hydrogels, sponges, pastes, particles, and others. XG is typically combined with other materials, including polymers, ceramics, and metallic particles, to tune the desired properties according to the intended application (Petri 2015).

Table 2 comprises examples of XG-based materials developed for the delivery of bioactive molecules in different tissues. The examples show that XG can be successfully used in the formulation of platforms for drug delivery owing to its characteristic functional properties such as viscoelasticity, pH-dependent polyanionic behavior, as well as pH, temperature, and ionic strength-dependent gelation capacity, previously discussed in this chapter.

Figure 8 illustrates a drug delivery system developed by Huang et al. (2018a). An injectable hydrogel was formulated by combining solutions of aldehyde-modified xanthan (Xan-CHO) and carboxymethyl-modified chitosan (NOCC), which are

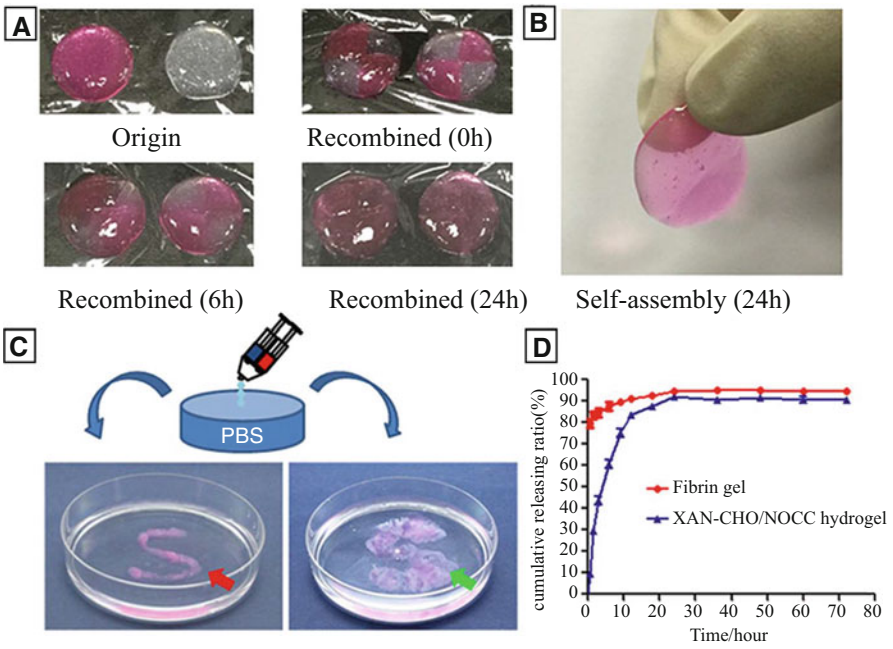


Fig. 8 Polysaccharide-based injectable hydrogels via self-cross-linking of aldehyde-modified xanthan (Xan-CHO) and carboxymethyl-modified chitosan (NOCC). (**a**) recombination process of different parts of the hydrogel; (**b**) lifting up the hydrogel after recombination; (**c**) local injection of the hydrogel and fibrin gel in phosphate-buffered saline (PBS) (red arrow: Xan-CHO/NOCC hydrogel, green arrow: fibrin gel); (**d**) cumulative releasing ratio of protein after local injection in PBS. (Reproduced from Huang et al. 2018a by permission of Elsevier)

Table 3 Other applications of xanthan gum in regenerative medicine

Formulation		Targeted application	Role of XG in the formulation	Cells used	Remarks	References
Polymer matrix	Other components					
XG/PEGDA	–	Bioink for the fabrication of 3D scaffolds for tissue engineering	Prevents cell sedimentation within the bioink	3T3 fibroblasts	XG + PEGDA behaves as a weak gel due to polymer entanglements. It results in more homogenous cell distribution compared to PEGDA + Alginate	Dubbin et al. (2017)
XG/Chitosan	Iron oxide magnetic nanoparticles	Magnetically stimulated system for tissue engineering	Facilitates gelation and improves water retention, mimicking the natural extracellular matrix	3T3 fibroblasts	The polyelectrolyte complex promotes stabilization of the magnetic nanoparticles. The particles favors cell adhesion and proliferation in a magnetic field	Rao et al. (2018)
XG/Chitosan	Kolliphor® P188 (surfactant)/ Silpuran® 2130A/B (elastomer)	Soft tissue engineering	Improves water retention/modulates degradation and mechanical properties	Human dermal fibroblasts/ Adipose-derived stem cells	Dense and porous XG-based scaffolds present appropriate porosity, liquid uptake capacity, stability, mechanical properties, thrombogenicity and cytotoxicity for applications in soft tissue engineering	Bombaldi de Souza et al. (2019)

(continued)

Table 3 (continued)

Formulation		Targeted application	Role of XG in the formulation	Cells used	Remarks	References
Glycidyl methacrylate-modified XG (photopolymerizable)	–	Gut repair/ Gastrointestinal fistula closing	Performs as an anti-digestive polymer due to structural characteristics	Intestinal epithelial cells-6 (IEC-6)	IECs-6 cells growth on the surface of the hydrogel and achieved their gut barrier functions. Calcium ions induced a swelling-shrinking behavior of the material, resulting in a practical method for removal from the gut wall as tissue regeneration is achieved	Huang et al. (2018b)
XG/DOPE(1,2-dioleoyl-sn-glycerophosphoetilamine)	–	Cell-based transplantation therapies	When conjugated to DOPE phospholipid, originates an amphiphilic polymer able to form capsular structures by self-assembly	Murine ATDC5 chondrocyte cells	By using a microfluidic device, stable and size-controlled spherical microcapsules were obtained. Cells were encapsulatedwithin the self-assembled XG/DOPE microcapsules and presented enhanced metabolic activity for a prolonged time	Mendes et al. (2013)

chemically cross-linked between the two reactant groups via Schiff's base reaction. A re-gelifying strategy of controlled drug release in liquids was proposed, in which the injected hydrogel is capable of avoiding the influence of tissue exudates so that a loaded drug can be released in a controlled manner. An angiogenic factor (VEGF) was loaded to the hydrogels to improve the effects on abdominal wall reconstruction. The biomaterial had the advantage of providing controlled drug release, especially in tissues with much excretion or exudation such as those of the digestive tract and open wounds. The hydrogel loaded with VEGF resulted in accelerated angiogenesis and was capable of regenerating the abdominal wall tissue.

5.8 Other Applications

XG has been explored for additional biomedical applications. Table 3 shows some examples of the use of XG for regenerative medicine applications not yet discussed in the previous sections. These alternative uses include XG combination with inorganic particles to create smart composite hydrogels for tissue engineering, incorporation in bioinks for the 3D printing of tissues and organs with complex geometries, complexation and blending with other polymers to produce scaffold materials with tunable properties to match the native characteristics of many native tissues, among others.

6 Conclusions

Despite all the recent advances observed in the area of regenerative medicine, defining, designing, and producing biomaterials suitable for this purpose is still a major challenge. Natural polymers, such as xanthan gum, a high molar exopolysaccharide produced by bacteria of the genus *Xanthomonas*, continue to attract attention due to its structural similarity to the extracellular matrix, its biocompatibility, high availability, and competitive price. The fact that xanthan gum can be obtained on a large scale from fermentative processes increases its attractiveness, since the control of several of the variables associated with the production process makes it possible to obtain a biopolymer with a quality more appropriate for clinical applications than those extracted directly from algae, plants, or animals. The control of the process allows obtaining a more homogeneous product concerning the composition and size of the chains from batch to batch, aspects of paramount importance in the development of clinical and pharmacological biomaterials.

Xanthan gum, in addition to not being cytotoxic even at relatively high concentrations, is biodegradable and stable over a wide range of temperature and pH conditions. Besides, XG has peculiar rheological properties and is capable of forming structures in the form of networks stabilized by intra- and intermolecular bonds with a high capacity to absorb physiological solutions. Altogether, these properties make it possible to use xanthan gum to obtain a variety of products applicable in various fields of regenerative medicine.

Even in situations where xanthan gum alone cannot meet all requirements for a given type of application, this polysaccharide can be successfully combined with other natural or synthetic polymers. Moreover, XG can also have its chemical structure modified by chemical, physical and enzymatic means to better meet specific demands for functionality. The literature already records a vast list of strategies for modifying and combining this molecule with other compounds, not only of the category of polymers, but also of diverse bioactive agents, such as drugs, growth factors, and products with antimicrobial activity. Many other new approaches can still be proposed and explored with the purpose of further expanding the scope of molecular architecture, functionality, and applications of xanthan gum.

Another advantage of xanthan gum is its high processability, which makes it possible to obtain biomaterials from it in the form of solutions, hydrogels, particles, films, membranes, and porous monolithic devices, among others. These biomaterials, associated or not with cells, mainly stem cells, have proven to be effective, exhibiting excellent biological response in a wide range of applications in regenerative medicine, both in soft and hard tissues. Examples of its use in this regard include the therapy of lesions occurring in skin, articular cartilage, tendons, bones, neural, periosteal, periodontal, and gastrointestinal tissues. Furthermore, XG can be also used in the release of bioactive agents and to produce bioinks for tissue engineering approaches based on additive manufacturing.

Xanthan gum has proved to be much more than a thickening agent, a stabilizer, or an emulsifier additive useful in the food industry. The knowledge acquired in the last decades regarding this polysaccharide shows that it also has solid perspectives of use in tissue repair and regeneration.

Acknowledgments The authors would like to acknowledge the financial support by the National Council for Scientific and Technological Development (Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq, Brazil – Grant #307829/2018-9), and the Coordination for the Improvement of Higher Educational Personnel (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES, Brazil – Finance Code 001).

References

Ahuja M, Kumar A, Singh K. Synthesis, characterization and in vitro release behavior of carboxymethyl xanthan. Int J Biol Macromol. 2012;51:1086–90.

Almeida N, Mueller A, Hirschi S, Rakesh L. Rheological studies of polysaccharides for skin scaffolds. J Biomed Mater Res A. 2014;102:1510–7.

Badwaik HR, Giri TK, Nakhate KT, et al. Xanthan gum and its derivatives as a potential bio-polymeric carrier for drug delivery system. Curr Drug Deliv. 2013;10:587–600.

Bellini MZ, Caliari-Oliveira C, Mizukami A, et al. Combining xanthan and chitosan membranes to multipotent mesenchymal stromal cells as bioactive dressings for dermo-epidermal wounds. J Biomater Appl. 2015;29:1155–66.

Bhatia M, Ahuja M, Mehta H. Thiol derivatization of xanthan gum and its evaluation as a mucoadhesive polymer. Carbohydr Polym. 2015;131:119–24.

Bombaldi de Souza RF, Bombaldi de Souza FC, Rodrigues C, et al. Mechanically-enhanced polysaccharide-based scaffolds for tissue engineering of soft tissues. Mater Sci Eng C Mater Biol Appl. 2019;94:364–75.

Bombaldi de Souza RF, Bombaldi de Souza FC, Thorpe A, et al. Phosphorylation of chitosan to improve osteoinduction of chitosan/xanthan-based scaffolds for periosteal tissue engineering. Int J Biol Macromol. 2020;143:619–32.

Bueno VB, Bentini R, Catalani LH, et al. Synthesis and characterization of xanthan–hydroxyapatite nanocomposites for cellular uptake. Mater Sci Eng C. 2014;37:195–203.

Dubbin K, Tabet A, Heilshorn SC. Quantitative criteria to benchmark new and existing bio-inks for cell compatibility. Biofabrication. 2017;9:044102.

Dyondi D, Webster TJ, Banerjee R. A nanoparticulate injectable hydrogel as a tissue engineering scaffold for multiple growth factor delivery for bone regeneration. Int J Nanomedicine. 2013;8: 47–59.

Elizalde-Peña EA, Quintero-Ortega IA, Zárate-Triviño DG, et al. (Chitosan-g-glycidyl methacrylate)-xanthan hydrogel implant in Wistar rats for spinal cord regeneration. Mater Sci Eng C Mater Biol Appl. 2017;78:892–900.

Fabela-Sánchez O, Zarate-Triviño DG, Elizalde-Peña EA, et al. Mammalian cell culture on a novel chitosan-based biomaterial crosslinked with gluteraldehyde. Macromol Symp. 2009;283–284: 181–90.

García-Ochoa F, Santos VE, Casas JA, Gómez E. Xanthan gum: production, recovery, and properties. Biotechnol Adv. 2000;18:549–79.

Gils PS, Ray D, Sahoo PK. Characteristics of xanthan gum-based biodegradable superporous hydrogel. Int J Biol Macromol. 2009;45:364–71.

Glaser T, Bueno VB, Cornejo DR, et al. Neuronal adhesion, proliferation and differentiation of embryonic stem cells on hybrid scaffolds made of xanthan and magnetite nanoparticles. Biomed Mater. 2015;10:045002.

Han G, Shao H, Zhu X, et al. The protective effect of xanthan gum on interleukin-1β induced rabbit chondrocytes. Carbohydr Polym. 2012;89:870–5.

Han G, Chen Q, Liu F, et al. Low molecular weight xanthan gum for treating osteoarthritis. Carbohydr Polym. 2017;164:386–95.

Hashim NT. Oral microbiology in periodontal health and disease. Oral microbiology in periodontitis; 2018.

Huang J, Deng Y, Ren J, et al. Novel in situ forming hydrogel based on xanthan and chitosan re-gelifying in liquids for local drug delivery. Carbohydr Polym. 2018a;186:54–63.

Huang J, Li Z, Hu Q, et al. Bioinspired anti-digestive hydrogels selected by a simulated gut microfluidic chip for closing gastrointestinal fistula. iScience. 2018b;8:40–8.

Ibrahim HK, Fahmy RH. Localized rosuvastatin via implantable bioerodible sponge and its potential role in augmenting bone healing and regeneration. Drug Deliv. 2016;23:3181–92.

Izawa H, Nishino S, Maeda H, et al. Mineralization of hydroxyapatite upon a unique xanthan gum hydrogel by an alternate soaking process. Carbohydr Polym. 2014;102:846–51.

Jain M, Dave D, Jain P, et al. Efficacy of xanthan based chlorhexidine gel as an adjunct to scaling and root planing in treatment of the chronic periodontitis. J Indian Soc Periodontol. 2013;17:439.

Juris S, Mueller A, Smith B, et al. Biodegradable polysaccharide gels for skin scaffolds. J Biomater Nanobiotechnol. 2011;2:216–25.

Kaminski GAT, Sierakowski MR, Pontarolo R, et al. Layer-by-layer polysaccharide-coated liposomes for sustained delivery of epidermal growth factor. Carbohydr Polym. 2016;140:129–35.

Kim J, Hwang J, Seo Y, et al. Engineered chitosan–xanthan gum biopolymers effectively adhere to cells and readily release incorporated antiseptic molecules in a sustained manner. J Ind Eng Chem. 2017;46:68–79.

Kool MM, Schols HA, Wagenknecht M, et al. Characterization of an acetyl esterase from Myceliophthora thermophila C1 able to deacetylate xanthan. Carbohydr Polym. 2014;111:222–9.

Kreyenschulte D, Krull R, Margaritis A. Recent advances in microbial biopolymer production and purification. Crit Rev Biotechnol. 2014;34:1–15.

Kumar A, Rao KM, Kwon SE, et al. Xanthan gum/bioactive silica glass hybrid scaffolds reinforced with cellulose nanocrystals: morphological, mechanical and in vitro cytocompatibility study. Mater Lett. 2017;193:274–8.

Kumar A, Rao KM, Han SS. Application of xanthan gum as polysaccharide in tissue engineering: a review. Carbohydr Polym. 2018;180:128–44.
Kuo SM, Chang SJ, Wang H-Y, et al. Evaluation of the ability of xanthan gum/gellan gum/hyaluronan hydrogel membranes to prevent the adhesion of postrepaired tendons. Carbohydr Polym. 2014;114:230–7.
Laffleur F, Michalek M. Modified xanthan gum for buccal delivery – a promising approach in treating sialorrhea. Int J Biol Macromol. 2017;102:1250–6.
Liu Z, Yao P. Injectable thermo-responsive hydrogel composed of xanthan gum and methylcellulose double networks with shear-thinning property. Carbohydr Polym. 2015;132:490–8.
Matsuda Y, Biyajima Y, Sato T. Thermal denaturation, renaturation, and aggregation of a double-helical polysaccharide xanthan in aqueous solution. Polym J. 2009;41:526–32.
Matsuda Y, Sugiura F, Mays JW, Tasaka S. Atomic force microscopy of thermally renatured xanthan with low molar mass. Polym J. 2015;47:282–5.
Mendes AC, Baran ET, Pereira RC, et al. Encapsulation and survival of a chondrocyte cell line within xanthan gum derivative. Macromol Biosci. 2012;12:350–9.
Mendes AC, Baran ET, Reis RL, Azevedo HS. Fabrication of phospholipid–xanthan microcapsules by combining microfluidics with self-assembly. Acta Biomater. 2013;9:6675–85.
Milas M, Rinaudo M. Conformational investigation on the bacterial polysaccharide xanthan. Carbohydr Res. 1979;76:189–96.
Needleman IG, Smales FC, Martin GP. An investigation of bioadhesion for periodontal and oral mucosal drug delivery. J Clin Periodontol. 1997;24:394–400.
Petri DFS. Xanthan gum: a versatile biopolymer for biomedical and technological applications. J Appl Polym Sci. 2015;132.
Ramburrun P, Kumar P, Choonara YE, et al. Design and characterization of neurodurable gellan-xanthan pH-responsive hydrogels for controlled drug delivery. Expert Opin Drug Deliv. 2017;14:291–306.
Rao KM, Kumar A, Haider A, Han SS. Polysaccharides based antibacterial polyelectrolyte hydrogels with silver nanoparticles. Mater Lett. 2016;184:189–92.
Rao KM, Kumar A, Han SS. Polysaccharide-based magnetically responsive polyelectrolyte hydrogels for tissue engineering applications. J Mater Sci Technol. 2018;34:1371–7.
Salazar GM, Sanoh NC, Pernaloza DP Jr. Synthesis and characterization of a novel polysaccharide-based self-healing hydrogel. KIMIKA. 2018;29:44–8.
Schmid J, Sieber V. Enzymatic transformations involved in the biosynthesis of microbial exo-polysaccharides based on the assembly of repeat units. Chembiochem. 2015;16:1141–7.
Sehgal RR, Roohani-Esfahani SI, Zreiqat H, Banerjee R. Nanostructured gellan and xanthan hydrogel depot integrated within a baghdadite scaffold augments bone regeneration. J Tissue Eng Regen Med. 2017;11:1195–211.
Tao Y, Zhang R, Xu W, et al. Rheological behavior and microstructure of release-controlled hydrogels based on xanthan gum crosslinked with sodium trimetaphosphate. Food Hydrocoll. 2016;52:923–33.
Wang B, Han Y, Lin Q, et al. In vitro and in vivo evaluation of xanthan gum–succinic anhydride hydrogels for the ionic strength-sensitive release of antibacterial agents. J Mater Chem B. 2016a;4:1853–61.
Wang X, Xin H, Zhu Y, et al. Synthesis and characterization of modified xanthan gum using poly (maleic anhydride/1-octadecene). Colloid Polym Sci. 2016b;294:1333–41.
Westin CB, Trinca RB, Zuliani C, et al. Differentiation of dental pulp stem cells into chondrocytes upon culture on porous chitosan-xanthan scaffolds in the presence of kartogenin. Mater Sci Eng C Mater Biol Appl. 2017;80:594–602.
Westin CB, Nagahara MHT, Decarli MC, et al. Development and characterization of carbohydrate-based thermosensitive hydrogels for cartilage tissue engineering. Eur Polym J. 2020;129:109637.

Cellulose and Tissue Engineering

48

Paula Cristina de Sousa Faria-Tischer

Contents

Abstract

The field of tissue engineering is growing fast and at the same time the use of cellulose also has aroused interest of several groups of researchers and companies. The wide possibility of cellulose modification (physical and/or chemical) combined with its biological properties makes this biopolymer an important candidate to produce tissue engineering templates designed to replicate the niche, or microenvironment, of the target cells to produce fully functional tissues. The structural organization and nanofiber three-dimensional (3D) network of this polymer (isolated or biocomposite) have demonstrated fruitful outcome and challenges too. One important factor, of several, to achieve the promising results is to use scientific rationale in each step of development coupling engineering and biology systems. Regulatory aspects, partners (scientific and commercial), ethics, bioprocessing, and financial investment are some of the challenges, in my point of view, that represent opportunities driving the tissue engineering, ensuring the progress toward realizing the clinical and commercial endpoints.

P. C. de Sousa Faria-Tischer (✉)
Glucom Biotech Ltda., Londrina, Brazil
e-mail: paula.tischer@glucombiotech.com

J. M. Oliveira et al. (eds.), *Polysaccharides of Microbial Origin*,
https://doi.org/10.1007/978-3-030-42215-8_62

Keywords

Nanocellulose · Tissue engineering · 3D printing · Wound healing

1 Tissue Engineering: Definition and Requirements

Tissue engineering employs a combination of engineering, biology, and bioactive constructs to improve function by repairing, replacing, or regenerating tissue. The expanded concept of regenerative medicine includes tissue engineering but also incorporates research on the regeneration of tissue directly in vivo, where the body uses its own systems to repair, replace, or regenerate function in damaged or diseased tissue with the help of exogenous cells, scaffolds, or biological factors (Dzobo et al. 2018). The regenerative medicine relies on harnessing the body's natural ability to self-heal. However, tissue engineering evolved from the field of biomaterials development and refers to the practice of combining scaffolds, cells, and biologically active molecules into functional tissues (Brien 2011).

Actually, there are six basic requirements widely accepted for designing polymer scaffolds: high porosity and proper pore size; high surface area; biodegradability and a proper degradation rate to match the rate of neotissue formation; mechanical integrity; not be toxic to cells (i.e., biocompatible); and finally, bioactivity which means interaction with cells, including enhanced cell adhesion, growth, migration, and differentiated function (Ma 2004).

The success of the development of tissue engineering is closely related to the conditions for tissue culture that involves the in vitro maintenance and propagation of cells in optimal conditions. The comprehension of interaction between the ligands present in the extracellular matrix and the receptors of the cell allows multiple intracellular signaling processes that can result in the alteration of cellular behaviors, such as growth, migration, and differentiation and several natural biomaterials have been explored recently. Three-dimensional biomaterials with large pore size (greater than 100 μm) carry a high number of functional units essential for the regeneration of various tissues. Pore size greater than 100 μm is essential for the cell adhesion and proliferation. However, to design functional units of tissue, not only are the subcellular and cellular scales required, but also nanostructures, 1–100 nm in size. This type of structural arrangement is essential to control cell behavior, in particular cell–cell interactions, cell–molecular interactions, and the cellular environment (Bhatia and Goli 2018).

Cell adhesion, mitosis, and growth are often caused by proteins that have been attached to scaffold material, so bioactive molecules like growth factors or proteins of the extracellular matrix (ECM) have been included in the polymers to support these functions. Bifunctional groups can also be added to the polymer materials for improved spreading of cells. These include glycolipids, oligopeptides, and oligosaccharides. Fibronectin, collagen, laminin, tenascin, vitronectin, and thrombospondin, a glycoprotein that mediates cell-to-cell and cell-to-matrix interactions, are some examples that increase the spreading of cells (Ogueri et al. 2019).

Particularly the "RGD" arginine-glycine-aspartate sequence in fibronectin is responsible to stimulate the cellular response, in this way several researcher groups are testing only this tripeptide RGD anchored in the polymer surface, instead of the whole molecule (Ruoslahti 1996). Scaffolds coated with polylysine, polyornithine, or lactose and N-acetlyglucosamine and micropaths have been created and a positive effect in the cell attachment and spreading have been observed (Sigma-Aldrich 2008; Lam and Longaker 2012).

Actually, the researcher's centers are looking for materials that mimic natural ECMs in terms of their composition, structural characteristics, and mechanical properties. To find scaffolds capable to transmit a signal to actively construct and degrade their microenvironment, providing cellular adhesion, proteolytic degradation, and growth factor (GF)-binding, as well as space-filing mechanical support capable to behavior as bioactive and dynamic environment to mediate cellular functions is a challenge nowadays and 3D scaffolding materials can represent a new choice to get these properties (Dutta et al. 2019).

Cellulose has been widely applied in engineering of blood vessels, reconstruction of urethra and dura mater, liver and adipose tissue, neural tissue, bone, cartilage, repairing connective tissue and congenital heart defects, and constructing protective barriers and contact lenses.

This chapter reviewed some of the newest researches in the last 5 years, reporting the development of scaffolds based in cellulose for tissue engineering (bone, cartilage, and skin). The intention is to highlight cellulose structural characteristics that make their application more attractive while tailoring them to tissue regeneration demands improving the methods of repair, replacement, or regeneration of damaged tissues and organs.

2 Cellulose: Function, Structure, and Properties

Structure–property and structure–function relationships have long been considered important explanatory concepts at hierarchical levels and provide insight to design new mimetic tissues able to be employed into tissue engineering.

Cellulose is a semicrystalline polymer formed by (1-4)-linked betha-D-glucosyl residues that are alternately rotated by 180° along the polymer axis to form flat ribbon-like chains. Each glucosyl unit bears three hydroxyl groups, one on hydroxymethyl group. It has been long recognized that these hydroxyl groups and their ability to bond via hydrogen bonding not only play a major role in directing how the crystal structure of cellulose forms but also in governing important physical properties of cellulose materials (Brown and Saxena 2000).

Cellulose is a polycrystalline material and the crystals are aligned along the microfibrils and present a polymorphism (Delmer and Amor 1995; Atalla and VanderHart 1984). As a result, cellulose has several polymorphs, namely cellulose I, II, III, and IV and their varieties Iα, Ibetha, III_I, IV_I, III_{II}, and IV_{II}. Most of these polymorphs result from chemical treatments of polymorph (Šturcova et al. 2004; VanderHart and Atalla 1984). The degree of crystallinity, i.e., the quality of the

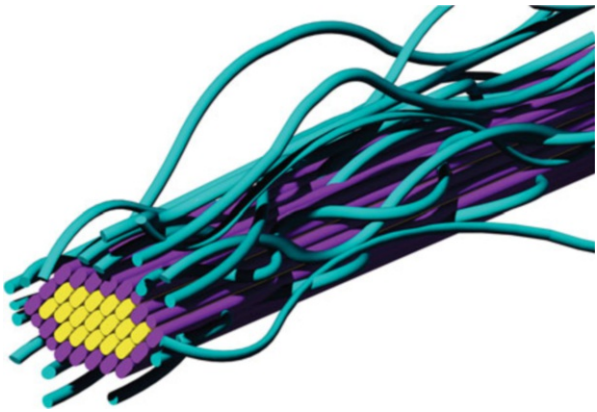


Fig. 1 Layers of cellulose microfibril composed by crystalline and amorphous regions surrounded by hemicellulose

cellulose crystal, is another important factor that varies extensively from one cellulosic material to another (Revol 1985).

The interaction between water and cellulose is of utmost importance in order to understand and control the properties of cellulosic materials (Sugiyama 1984). Indeed, the unusual physical and chemical properties of cellulose such as highly hydrophilic nature, good mechanical properties, low density and thermal conductivity, and good thermal stability arise from its structural architecture (Chami Khazraji and Robert 2013). Therefore, it is possible to get more assertive manipulation process of cellulose when we have knowledge of its structural crystalline organization.

In nature cellulose occurs as a slender rodlike or threadlike entity, called microfibril (collection of cellulose chains); this entity forms the basic structural unit of any "cellulose" independent of its origin (Fig. 1). Each microfibril can be considered as a string of cellulose crystals linked along the chain axis by amorphous domains. The outer regions of wood microfibrils are strongly disordered, mostly due to the direct contact with hemicellulose, which is largely amorphous in nature. These regions can show the form of paracrystalline or fully amorphous cellulose (Fig. 2) (Dufresne 2019; Nishiyama 2009).

Not only the microfibril diameter (nm) is different but also the DP (degree of polymerization). Values of DP ranging from hundreds and several tens of thousands have been reported and the DP is heavily dependent on the source of the original cellulose (Habibi et al. 2010).

Different protocols focusing in chemical and physical modifications have been applied to cellulose with the purpose to obtain particular morphologies and structures seeking to meet specific properties capable to stimulate specific tissue regeneration.

3 Cellulose: Derivatives and Cellulose Nanostructures

As mentioned above, our body is formed by different tissues and they possess distinct characteristics raised from its structural organization. In this way cellulose usually is obtained and modified in order to provide the specific characteristics

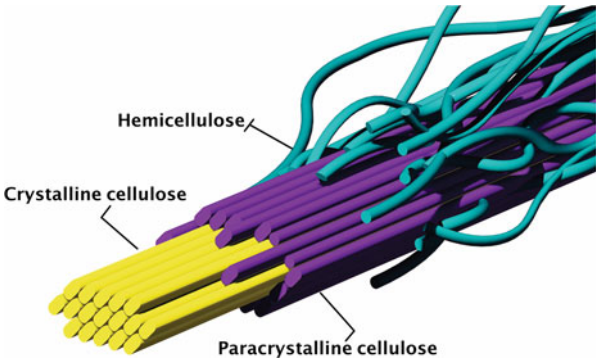


Fig. 2 Crystalline and paracrystalline regions

necessary to distinct functions. Cellulose can be tailored to exhibit particular physical and chemical properties using different methodologies, one of them is by varying the pattern and degrees of substitution within the cellulose backbone.

There is a large distribution in the worldwide market of cellulose ethers and esters, in biomedicine cellulose ethers derivatives are more used (e.g., methylcellulose [MC], ethyl cellulose [EC], hydroxyethylcellulose [HEC], hydroxypropyl cellulose [HPC], hydroxypropyl methyl cellulose [HPMC], and carboxymethyl cellulose [CMC]). Recently, hydroxypropyl methyl cellulose (HPMC) have been evaluated in association with other polymers for tissue engineering, e.g., for cell-based cartilage engineering (Rederstorff et al. 2017), corneal regeneration (Long et al. 2018), and filler material for use in oral and craniofacial fields (Huh et al. 2015), and methylcellulose (MC) was crosslinked to form hydrogels (Niemczyk-Soczynska et al. 2019) for bone regeneration (Kim et al. 2018), thermally reversible hydrogels (Kummala et al. 2020), and more recently aroused interest as a versatile printing material for bio-fabrication of tissues (Law et al. 2018; Ahlfeld et al. 2020; Roushangar Zineh et al. 2018). Carboxymethyl cellulose has been intensively researched for bone regeneration (Singh and Pramanik 2018; Hasan et al. 2018; Matinfar et al. 2019).

Due to the reduced structure of the cellulose chain arrangement in the disordered region, it has a lower density and thus exhibits more free volume than the crystalline region (De Souza Lima and Borsali 2004). Therefore, several researches have been focusing to obtain nanostructures of cellulose through preferentially acid hydrolyses which remove the disordered amorphous region leaving the crystalline region largely intact due to their tight packing. The correct timing and hydrolysis conditions enabling the obtention of CNC (cellulose nanocrystals) produced as individualized particles (De Souza Lima and Borsali 2004; Anglès and Dufresne 2001). These nanoscale crystalline structures are isolated from native cellulose mostly via an acid hydrolysis, but enzymatic hydrolysis with cellulases (Siqueira et al. 2010; Henriksson et al. 2007) TEMPO ((2,2,6,6-tetramethylpiperidin-1-oxyl)-mediated oxidation (Saito et al. 2007), and ionic liquids has also been reported to prepare cellulosic nanoparticles (Man et al. 2011).

Typically, nanocellulose can be categorized into two major classes: (1) nanostructured materials (cellulose microcrystals and cellulose microfibrils) and (2) nanofibers

(cellulose nanofibrils, cellulose nanocrystals, and bacterial cellulose) (Trache et al. 2017).

Nanocellulose market increased a lot in the last years and the employment of new applications has driven the researchers and the industry to exploit even more its employment. Therefore, International Organization for Standardization (ISO), Technical Association of the Pulp and Paper Industry (TAPPI), and Canadian Standards Association (CSA) standards on cellulose nanocrystals (CNCSs) are being developed and published. In 2011, TAPPI proposed international standards for cellulose-based nanomaterials that could remove the trade barriers and harmonize research and development in nanocellulose-based products (Reid et al. 2017).

These standards help to categorize nanocellulose avoiding overcoming the problems related to multiple definitions and ambiguity. The physicochemical and structural properties of nanocellulose are dependent of initial biomass type or microbial source selected (Trache et al. 2017), cellulose polymorphs (Gong et al. 2018), pretreatment process of cellulose extraction and acid hydrolysis, or enzymatic treatment (Mondal 2017), followed for nanocellulose fabrication.

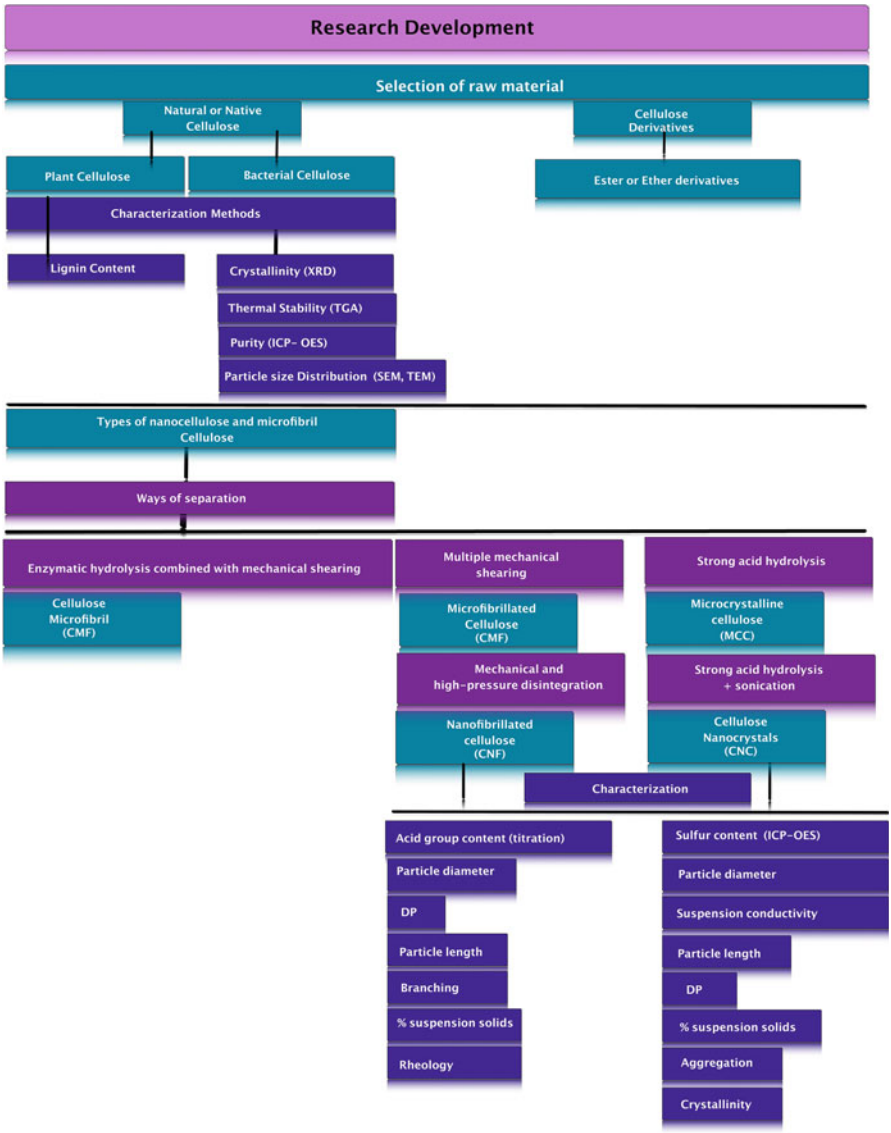
Among the standards are ISO/TC 229 – TS 20477:2017: Standard terms and their definition for cellulose nanomaterial and some terms and abbreviations of cellulose nanomaterials were established, e.g., nanocrystalline cellulose (NCC), nanofibrillar cellulose (NFC), cellulose nanocrystals (CNC), cellulose nanowhiskers (CNW), cellulose nanofibrils (CNF), bacterial cellulose (BC), and tempo-oxidized cellulose nanofibrils (TOCN) (Reid et al. 2017). The good choice of biomaterial is the first step to design of scaffold. Scheme 1 shows some important points related to selection and characterization of cellulose materials.

4 Cellulose Applied to Bone Tissue Engineering

Bone acts as the supportive structure of the body, functions as a mineral reservoir, guards vital organs, is the site of blood cell production, and helps maintain acid–base balance in the body (De Witte et al. 2018). The aim of bone tissue engineering is to develop 3D scaffolds that mimic the extracellular matrix (ECM) and provide mechanical support, thereby aiding in the formation of new bone.

Scaffolds provide a template for cell attachment and stimulate functional bone tissue formation in vivo through tailored biophysical cues to direct the organization and behavior of cell (Bose et al. 2013). Bone tissue scaffolds present some specific biological requirements between them which are as follows (De Witte et al. 2018):

(a) Biodegradable, nontoxic, osteogenic, presence of GFs (growth factors)
(b) Mechanical requirements: mechanical properties, compressive strength ~2–12 MPa, and Young's modulus ~0.1–5 GPa
(c) Structural requirements: interconnected porosity, average pores size 300 nm, and nanotopography



Scheme 1 Steps for selection and characterization of cellulose

Bone tissue engineering requires the presence of a bioactive component like hydroxyapatite $Ca_5(PO_4)_3OH$ (HAp) and tricalcium phosphate (TCP) $Ca_3(PO_4)_2$. Several researches report different methodologies to achieve the formation of these biocomposites (cellulose and Hap or TCP). Scaffolds were fabricated with silk fibroin (SF) and carboxymethyl chitosan (CMCS) incorporated with strontium

substituted hydroxyapatite (Sr-HAp) and there were enhanced protein adsorption and ALP (alkaline phosphatase) activity.

The expression of osteogenic gene markers such as RUNX2 (Runt-related factor-2), ALP, OCN (osteocalcin), OPN (Osteopotin), BSP (Bone Sialoprotein), and COL-1 was also stimulated (Zhang et al. 2019a).

A smoother scaffold with better distribution of HA with good interconnectivity, hardness range of scaffolds of 550–640 MPa, compression strength range of 110–180 MPa, an elastic modulus of ~5 GPa, and a fracture toughness value of ~6 $MPa^{1/2}$ in the range of cortical bone was obtained using the association of (TEMPO)-oxidized cellulose nanofibrils (TCNF) and cellulose nanocrystals (CNC) with hydroxyapatite (HA) (Ingole et al. 2020).

The alignment of cellulose nanofibers hydrogels appeared to be a key structural feature in the successful and thorough infiltration of minerals as observed in a study where mineralized hydrogels were fabricated with TEMPO-oxidized cellulose and well-aligned nanofibers using a biomimetic method. The scaffold presented roughly 70 wt.% mineral content in the mineralized cellulose scaffold comparable with the mineral content in natural hard tissues (ranging ∼70–85 wt.%) (Qi et al. 2019).

Biomimetic growth of biphasic ceramics (HA/β-TCP) was used also to produce mineralized tissue with nanocellulose obtained from açaí integument (Euterpe Oleracea Mart.) (HA/β-TCP) (Valentim et al. 2018).

Bacterial cellulose (BC) has been an important choice to fabricate mineralized tissues proving to be a template for the ordered formation of calcium-deficient hydroxyapatite (CDHAP) (Hutchens et al. 2006). BC associated to hydroxyapatite (HA) and anti-bone morphogenetic protein antibody (anti-BMP-2) produced a noncytotoxic, genotoxic, and mutagenic biomaterial in MC3T3-E1 cells with increased mineralization nodules and the levels of ALP activity (Coelho et al. 2019).

Aligned scaffolds using cellulose nanocrystals loaded with bone morphogenic protein-2 (BMP-2) were produced by electrospinning and cellulose seems aligned human mesenchymal stem cells (BMSCs) growth and mineralized nodules formation in vitro (Zhang et al. 2019b). Cryogels formed upon contact with body fluids, with high porosity and high specific surface area, a rough hydroxyapatite layers and release of ions (Si, Ca, P, and Na) that were produced because there is a synergy between cellulose nanofibrils/bioactive (organic and inorganic) materials. So the cell differentiation is directly affected by the combination of high porosity, hydroxy apatite formation, and ion release and these set of events and conditions increases the release of BMP-2 greatly improving bone formation (Ferreira et al. 2019).

Nanocellulose (NC) containing BMP2-VEGF (BNBV) was loaded in porous sponge biphasic calcium phosphate (BCP) scaffold produced by replica method and bone marrow mesenchymal stem cells (RBMSCs) were seeded in these scaffolds. Bone formation increased because BMP2 stimulated the differentiation of stem cells to osteoblasts and the angiogenesis was facilitated by VEGF drawing the attention to the important role of nanocellulose as carrier for growth factors (Sukul et al. 2015).

The cellulose modification followed by the incorporation of bioactive molecules expand the possibilities to change its properties and reactivity; it can be achieved

using ex situ chemical (e.g., periodate oxidation and grafting (Leguy et al. 2018) functionalization through linker (Ribeiro-Viana et al. 2016), or crosslinking reactions (Kirdponpattara et al. 2015)) or physical modifications (physical absorption from solutions or particle suspensions, the homogenization, or dissolving BC mixing with additive material or yet to add the additive material in the culture medium) (Lopes et al. 2014).

Recently through controlled nucleation, HA nanocrystals were produced in CNCs functionalized with sulphonic groups and aerogels obtained from nanocellulose with incorporated sulfate and phosphate groups crosslinked with hydrazine increased the cell metabolism of Saos-2 cells on the porous scaffolds. Twelve weeks after implantation in rats the osteoconductivity and bone volume were increased (Osorio et al. 2019).

Considering the importance of biodegradability and functionality to regulate the bone regeneration process, injectable bone composed of bisphosphonate-modified nanocellulose (pNC) was prepared with bisphosphonate groups on nanocellulose. The results demonstrated that pNC released under osteoclast microenvironment can control the osteoclast activity and, moreover, pNC-α/β-TCP composites promoted osteoblast differentiation (Nishiguchi and Taguchi 2019).

3D printing has been a recent trend to get the production of hydrogels with biomimetic structures for tissue regeneration and organ reconstruction. For this purpose, the development of bioinks capable to mimetic the properties and morphologies of tissues is fundamental to achieve the success. Bacterial cellulose nanofibers demonstrated a benefic effect improving the shape fidelity and mechanical properties of the 3D printed scaffolds composing silk fibroin and gelatin (Huang et al. 2019). Scaffolds hydrogels were produced using 3D printing through partial crosslinking of TEMPO-oxidized cellulose with alginate and biomimetic mineralization using simulated body fluid was the method used to get hydroxyapatite nucleation using calcium ions (Abouzeid et al. 2018).

Other approaches have demonstrated that scaffolds derived from apple hypanthium tissue can act as a bioactive biomaterial (Modulevsky et al. 2014, 2016; Hickey et al. 2018). After removing the native cellular components, the reminiscent structure presented pore size (100 and 200 μm) which was shown to be the optimal pore size for biomaterials used for bone tissue engineering. Additionally, pre-osteoblasts were seeded (MC3T3-E1) and the localized mineralization was proved by the presence of calcium deposits after 4 weeks, specifically on the edge of the pores (Karageorgiou and Kaplan 2005).

Table 1 summarizes some representative researches realized in the last 5 years focusing cellulose modification to use in bone tissue engineering.

5 Cellulose Applied to Cartilage Tissue Engineering

Cartilage is a connective tissue which does not spontaneously heal, it is a tough, semitransparent, elastic, flexible connective tissue consisting of cartilage cells scattered through a glucoprotein material that is strengthened by collagen fibers.

Table 1 Examples of researches with cellulose applied for bone tissue engineering

Bone tissue engineering			
Source of cellulose	Technique applicated	Achievements	References
Silk fibroin (SF) and carboxymethyl chitosan (CMCS) incorporated with strontium	Freeze drying	Stimulus of osteogenesis Interconnect porous Mechanical properties	Zhang et al. (2019a)
Cellulose nanofibers loaded with bone morphogenic protein-2 (BMP-2)	Electrospinning	Growth of aligned human mesenchymal stem cells (BMSCs) Porous 100–600 nm Stimulus of osteogenesis In vivo formed aligned collagen fibers	Zhang et al. (2019b)
TCNF and CNC with HA	Prepared suspensions Ultrasonication Drying overnight in an oven at 60 °C	Hardness range of 550–640 MPa Compression strength range of 110–180 MPa Elastic modulus of ~5 GPa Stimulus of HA crystallites formation	Ingole et al. (2000)
Nanofibrils (CNF) from *Eucalyptus grandis and* bioctive glass	Freeze-casting	High porosity Hight specific surface area Release of ions (Si, Ca, P, and Na) by mineral phase Increase differentiation of BMP-2	Gadim et al. (2014)
Calcium phosphate (BCP) with BMP2-VEGF incorporated nanocellulose	Sponge replica	Hight cell attachment Hight proliferation No bone formation in ectopic sites BMP2 and VEGF stimulate new bone formation in the orthotopic defects	Sukul et al. (2015)
TOCN from softwood pulp $CaCl_2$, K_2HPO_4 and PAA	Biomimetic mineralization process	Oriented hybrid nanostructure Cellulose scaffold mineralized with HAp crystals Hard tissues 70 wt.%	Qi et al. (2019)
Bacterial cellulose incubated in solutions of calcium chloride followed by sodium phosphate dibasic	Incubation cycle	Incorporated substantial amount of apatite (50–90% of total dry weight) BC provides a template for the ordered formation of CdHAP Composite similar to the physiological biomineralization of bone	Hutchens et al. (2006)

(continued)

Table 1 (continued)

Bone tissue engineering			
Source of cellulose	Technique applicated	Achievements	References
		producing apatite crystals of comparable shape and size	
CNC from Açai Synthesis of HA and β-TCP	Biomimetic growth	X-ray diffraction prove nucleation of HA and β-TCP Particle size gauge range of 164.2×10^{-9}–4748×10^{-9} m Composite with tendencies to flocculation	Valentim et al. (2018)
TOCN from Whatman cotton ashless filter aid	Surface functionalization methods	Macropores in the range of 10–950 mm Increased ALP activity over 7 days Nucleation of HA over S-CNC Osteoconductive properties	Osorio et al. (2019)
CNCs from spruce and pine 5–10 nm wide 100–300 nm long	Controlled nucleation with sulphonic and carboxylate groups TEMPO oxidation Casting	Transparent thin coatings successfully fabricated via evaporation-induced assembly of CNC–HA nanocomposites The c-axes of the CNC and HA in the nanorods aligned parallel to the surface Water-resistant transparent coatings 2–4 μm thick	Ishikawa et al. (2015)
TOCN-bleached fibers from Bagasse	Biomimetic mineralization 3D printing	Pastes show highly thixotropic behavior Hydroxyapatite in the mineralized scaffolds was estimated to be 20.1% Best hydrogel with 50% T-CNF and 50% alginate hydrogel (CNF50) Compressive strength of 455 MPa (CNF50)	Abouzeid et al. (2018)
McIntosh apples	Decellularization Scaffolds untreated Scaffolds coated with collagen solution Pre-osteoblasts	Pre-osteoblasts adhered and proliferated in both scaffolds with and without collagen Mineralization occurred particularly in the edges Increase in the Young's	Leblanc Latour et al. (2020)

(continued)

Table 1 (continued)

Bone tissue engineering			
Source of cellulose	Technique applicated	Achievements	References
	MC3T3-E1 Subclone 4 cells seeded	modulus after mineralization Pore size (100 and 200 μm) Limitation Low Young's modulus	

The main purpose of cartilage is to provide a framework on which bone deposition may begin. Another important purpose of cartilage is to cover the surfaces of joints, allowing bones to slide over one another, thus reducing friction and preventing damage; it also acts as a shock absorber (Zhang et al. 2009). Engineered cartilage regeneration in gels necessitate the control of mechanical properties (strength, rigidity, and elongation) and easy processing into complex shapes (Fu et al. 2017). Hydrogels with nanocellulose also have been extensively explored for cartilage scaffolds, however, to match these requirements. Cellulose-based gels are often combined to other materials and are produced by different methodologies in order to achieve efficient and bioactive scaffolds.

As already mentioned, 3D bioprinting is a powerful tool emerged in the last years for the production of highly structured tissue engineering scaffolds, allowing to dispense hydrogels in three dimensions with precision and high resolution (Kang et al. 2016; Mandrycky et al. 2016). The printed material gel is prepared with cells encapsulated with homogeneous density, which permits homogeneous cell distribution and the scaffold can be fully colonized and the combination of crosslinked sodium alginate and NC has been recently explored for cartilage tissue engineering, for articular and nasal reconstruction (Puelacher et al. 1994; Nguyen et al. 2017; Engineering and Wood 2017).

Not only for bone tissue but also for human cartilage nanocellulose–alginate hydrogels is a promising combination to obtain scaffolds with 3D printed (Table 2). Recently one of the bionks developed is composed by the induction of pluripotent stem cells (iPSCs) and human chondrocytes printed together with the hydrogel matrix, e.g., nanofibrillar cellulose/alginate (NCF/A) bionk was embedded with human bone marrow–derived stem cells (hBMSCs) and human nasal chondrocytes (hNC) (Möller et al. 2017). The coculture enhanced chondrogenesis and after 60 days chondrocyte cell clusters indicated the ability of embedded cells to proliferate and the formed tissue presented all qualitative features of proper cartilage. Furthermore, cell clusters contained human chromosomes proving their human origin (de Windt et al. 2014). Additionally was observed high cell viabilities of 73% and 86% after 1 and 7 days of 3D culture, respectively, in coculture NFC/A bioinks for bioprinting iPSCs to support cartilage production and formation of cartilaginous tissue by expression of collagen II was observed after 5 weeks (Nguyen et al. 2017). Similarly, human auricular cartilage was obtained from cells cultured for 28 days inside a 3D printed scaffold with 75% of cells were still viable and increased

Table 2 Examples of researches with cellulose applied for cartilage tissue engineering

Cartilage tissue engineering			
Type of cellulose	Technique applied	Achievements	References
Bacterial nanocellulose Alginate	Homogeneous BNC/alginate mixture Phase separation BNC/alginate comoposite scaffolds Freeze-drying process Ionic liquid EMIMAc to join the layers Heating plate at 80 °C for 2 min The bilayer BNC scaffolds stabilized in 100 mM $CaCl_2$ in ethanol to precipitate the dissolved cellulose between the layers Simultaneously crosslinking the alginate to bind the BNC in the porous layer Culture-expanded human nasoseptal chondrocytes (NC) combined with human mononuclear cells (MNC) to form cartilage in vitro and in vivo	Bilayer BNC scaffolds Porosity of 75% and mean pore size of 50 ± 25 μm Nonpyrogenic BNC will provide long-term structural integrity after implantation The ability of scaffolds to attract and trap water was enhanced through the production and accumulation of proteoglycans and glycosaminoglycans in the bilayer BNC scaffolds Some limitations: Collagen matrix was not effectively produced in the porous layer Higher cell density is needed to benefit from increased cell–cell contacts signaling chondrogenic	Martínez Ávila et al. (2015)
Nanofibrillated cellulose (CELLINK AB, Gothenburg, Sweden) Alginate Cellulose/alginate (NFC-A) bioink	3D bioprinting technology Combination of nanofibrillated cellulose/alginate hydrogel, hNCs, and hBMSCs for 3D bioprinting Crosslinked with 100 mM $CaCl_2$ solution for 5 min at 37 °C Human nasal chondrocytes (hNCs) Human bone marrow derived stem cells (hBMSCs) Coculture of hNCs and hBMSCs Subcutaneous implantation of scaffolds in mice	Good mechanical properties Keep their structural integrity after 60 days of implantation Increase of GAG deposition The presence of human type II collagen Some limitations: Compression analysis: Difficulties in area determination of the measured constructs Mechanical properties of the neocartilage of this study with native human cartilage was not able to be correlated	Möller et al. (2017)

(continued)

Table 2 (continued)

Cartilage tissue engineering			
Type of cellulose	Technique applied	Achievements	References
Nanocellulose from CELLINK AB (Gothenburg, Sweden) Alginate sulfate	Alginate sulfate synthesis Nanocellulose water dispersion (1.9% dry content) Mixed with either alginate or alginate sulfate	Chondrocytes in alginate sulfate–nanocellulose gel discs were viable, mitogenic and synthesized collagen II Printing conditions greatly affected the behavior of the cells Wide diameter, conical needles providing the best preservation of cell function Biological performance of the cells was highly dependent on the nozzle geometry	Engineering and Wood (2017)
Nanocellulose-based hydrogels combined with sodium alginate Plant-derived nanocellulose (hydrophilic Bioplus® cellulose nanofibrils gel, hydrophilic Bioplus® cellulose nanocrystals gel, and hydro philic Bioplus® blend gel – a blend of fibrils and crystal) provided by American Process, Inc. (Georgia, USA) Produced via the AVAP® technology	NC-based hydrogels were crosslinked with varying concentrations of $CaCl_2$ Blend nanocellulose produced in situ Hydrogels prepared by mixing nanocellulose with 2.5% (w/v) sodium alginate (80,000–120,000 Da), medium viscosity (2% at 25 °C), 1.56 mannuronate/guluronate ratio) Evaluate effect of crosslinking – using calcium chloride ($CaCl_2$) – on the structural and mechanical properties And Exposure of the NC-based hydrogels to different sterilization methods: exposure to ultraviolet (UV) light, autoclaving, and ethanol immersion	Increasing concentrations of the crosslinker $CaCl_2$ yielded visible changes architecture, pore size, and porosity Pore size was the most affected by the sterilization method CNC was more affected by the crosslinker concentrations CNF and CNC were affected by all sterilization methods NCB was more resilient to changes when exposed to different sterilization methods and crosslinker concentrations	Al-Sabah et al. (2019)

by 20% the expression of ECM proteins (Martínez Ávila et al. 2016). In another study the viability of human chondrocytes within an ear-like structure was 86% after 7 days (Markstedt et al. 2015).

Bacterial NFC or named BCN (bacterial nanocellulose) was also applied to design layered scaffolds and the cellulose solvent system "ionic liquid EMIMAc," was used to bond tightly the two layers. Particularly the scaffolds were able to produce and accumulate cartilage-specific ECM components and the chondrogenesis shown by upregulation of the expression of chondrogenic markers. Bone marrow mononuclear cells (MNC) was also loaded and 8 weeks post-implantation had a macroscopically cartilage-like appearance (Martínez Ávila et al. 2015). Alginate acts as a crosslinker to increase gel viscosity. It improves the scaffold structure and guarantees shape to be maintained during the process (Engineering and Wood 2017).

Taking into account the importance and potential application of sodium alginate with nanocellulose as cartilage tissue scaffold (nasal and articular cartilage), one of new challenges is to understand how crosslinking and sterilization methods can affect structural and mechanical properties of nanocellulose-based hydrogels, contemplating cellulose nanofibrils, cellulose nanocrystals, or a blend of the two. So, recently the effect of crosslinking – using calcium chloride ($CaCl_2$) – on the structural and mechanical properties of AVAP® produced was evaluated (Al-Sabah et al. 2019).

American Process Inc.'s patented AVAP® technology produces cellulose nanocrystals (CNC); cellulose nanofibrils (CNF); and hydrophobic, lignin-coated varieties of CNC and CNF directly from woody and nonwoody biomass (Nelson 2014). The mechanical properties of the hydrogels were mildly affected by the sterilization method, apart from the chemical sterilization using ethanol that yielded significantly stronger hydrogels, possibly due to the dehydration. Whereas UV and ethanol sterilization have shown roughly similar pore sizes in all NC-based hydrogels not affecting the porosity. All sterilization methods did not significantly affect the stiffness of NCB hydrogels, but in contrast the stiffness of CNC was affected independent of the sterilization method used (Sulaeva et al. 2015).

In 2001 bacterial cellulose (BC) was molded into tubular form with diameter <6 mm and tubes having 1 mm diameter and 5 mm length with a wall thickness of 0.7 mm was obtained. The normal blood presents a tensile strength (800 mN) and the tubular BC showed comparable value demonstrating its potential to be employed as blood vessel to replace part of the carotid artery (Klemm et al. 2001). A clinical product named BActerial SYnthesized Cellulose (BASYC®) was used in microsurgery and presented favorable mechanical properties, including shape retention and tear resistance and a better mechanical strength than organic sheets, like polypropylene, polyethylene terephtalate or cellophane, and polyester (Dacron). The BASYC® tubes resisted to the blood pressure of the test animal (white rat) of 0.02 MPa (150 mmHg). After 4 weeks treated blood vessels showed that BASYC®-prosthesis was wrapped up with connective tissue, pervaded with small vessels like vasa vasorum. The BASYC®-interposition was completely incorporated in the body without any rejection. The regeneration nerve was improved after 10 weeks, compared to an uncovered anastomosed nerve (Belgacem and Gandini 2008). Recently, various properties and biology evaluation of BC tubes as blood vessel replacement have been investigated (Fink et al. 2010; Andrade et al. 2010; Esguerra et al. 2010).

At the beginning of this era there were two main specifications for tissue engineering scaffolds, the first was that the material had to be degradable, while

the second was that this degradable material shall to have prior approval by the FDA for use in medical devices. For this reason the first scaffold biomaterials were the bioabsorbable materials approved to be used to produce surgical sutures, plates, and drug delivery systems (Williams 2019). The second specification is highly questionable, in fact the regulatory approval for medical devices is predicated on the ability to show that the material does no harm, which means the material needs to be "biologically safe." Thus, depending on the precise application, the materials are subjected to the biological safety tests of ISO 10993 (International Standards Organization 2018) to show that they pass the tests that demonstrate a lack of cytotoxicity, acute systemic toxicity, reproductive toxicity, thrombogenicity, complement activation, and so on. Scheme 2 demonstrates some biological, mechanical, and morphological requirements for effectiveness of scaffolds. This scheme do not have relationship with ISO 10993.

The first requirement for tissue engineering development is the choice of "best" material to be changed and adaptable to implanted in the body. Of course, this material needs to be noncytotoxic, non immunogenic, and minimally pro-inflammatory. As already mentioned, the body needs to recognize the material and interact with it, which means the biomaterial should be capable of orchestrating molecular signaling to the target cells, either by directing endogenous molecules or delivering exogenous molecules. Scheme 3 demonstrates some modifications that can be made to improve the bioactivity of the biomaterials and cellular factors that affect cellular behavior.

6 Cellulose for Skin Tissue Engineering and Wound Healing

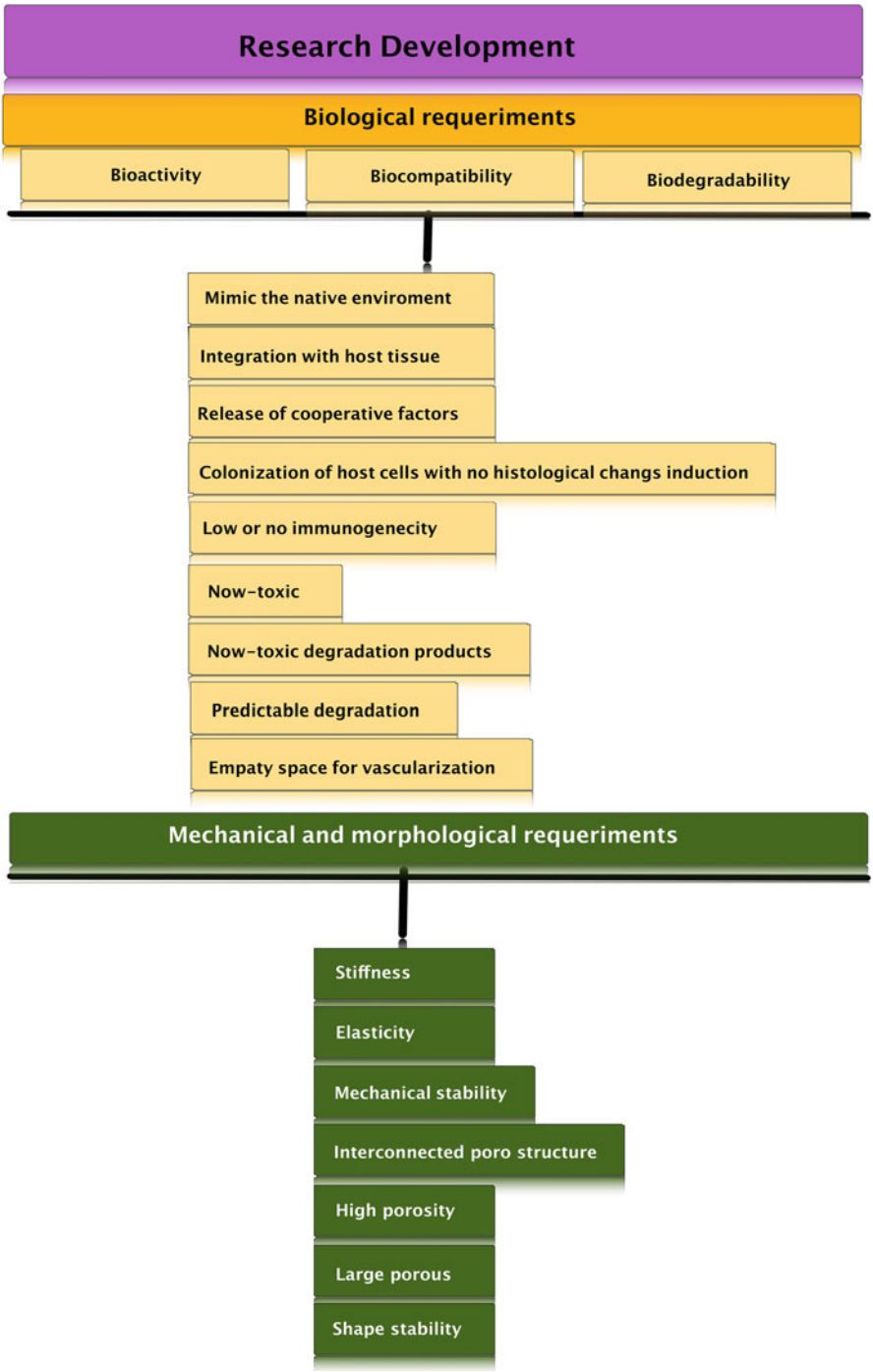
The largest organ in the human body is the skin with several vital functions mainly as barrier against adverse effects (chemical damage, radiation, e.g., by ultraviolet light, and microbial infection). Three layers epidermis, dermis, and the fat layer, hypodermis, compose the skin (Kanitakis 2002).

Bacterial cellulose is the most widely cellulose applied for reconstruction of skin layers due to its resemblance to natural soft tissues. The use of bacterial cellulose in skin wound therapy proposed as "temporary skin substitutes" for treating burns, ulcers, abrasions, and other skin injuries was first reported in 1990 (Fontana et al. 1990).

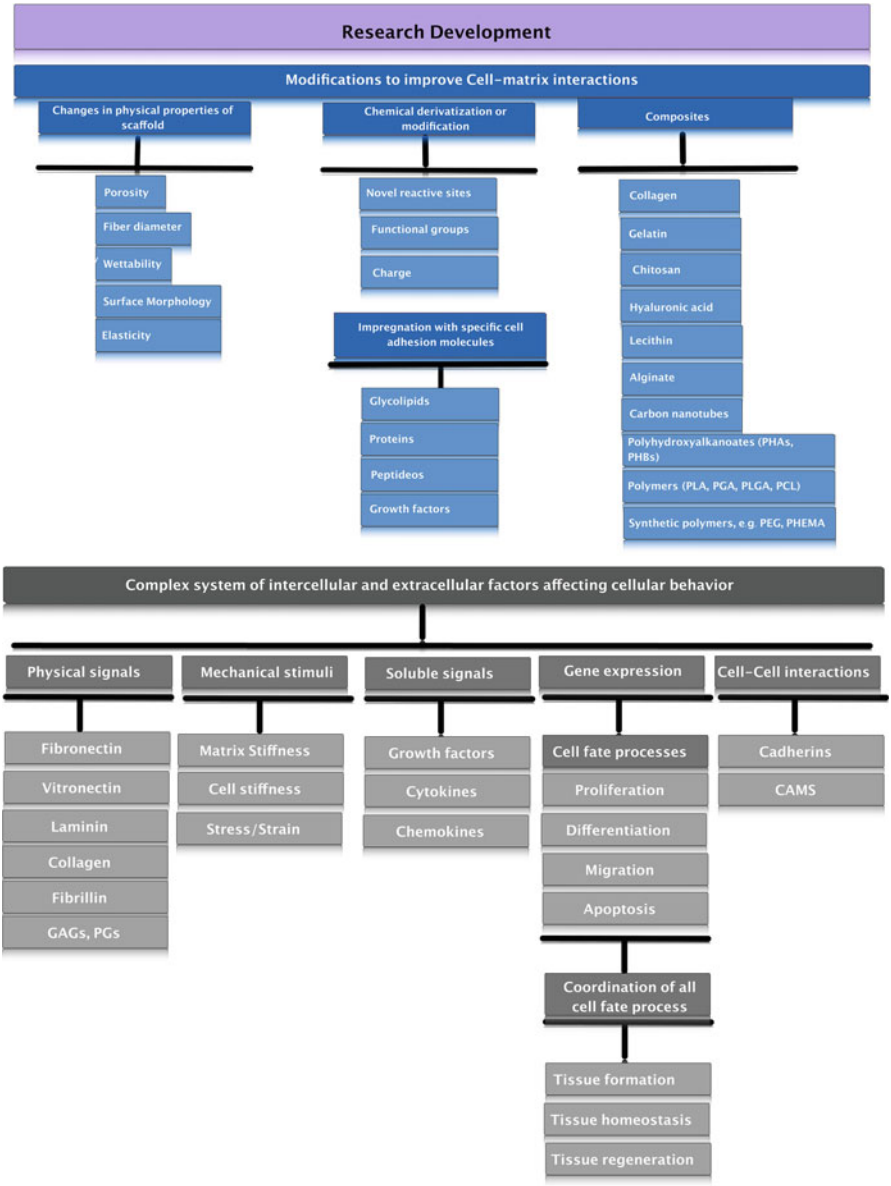
Joining mechanical and morphological characteristics to biological properties, nontoxic, biocompatible, and biodegradable, different biocomposites were produced and different properties were improved as reported by Portela et al. (2019).

Like bacterial nanocellulose, plant-derived nanocellulose has repeatedly been shown to be promising for skin tissue engineering, especially after its physical and chemical properties have been modified (e.g., cellulose nanofibrils (CNFs) modified by TEMPO-mediated oxidation) (or introduction of electrical charge functionalized with biomolecules, e.g., cell adhesion peptides (Trovatti et al. 2018) and silk fibroin (Shefa et al. 2017)).These modifications improved the capacity of nanocellulose for wound healing.

There are many reviews focusing the cellulose application in skin tissue and wound dressing (Sulaeva et al. 2015; Portela et al. 2019; Ullah et al. 2016; Czaja



Scheme 2 Biological requirements for development of scaffolds



Scheme 3 Modifications to improve cell-scaffold interactions and intracellular and extracellular factors that affect the response of cells

et al. 2007; Wu et al. 2017; Naseri-Nosar and Ziora 2018; Pinho and Soares 2018; Rasouli et al. 2019) between others. Then, the commercial products available for wound dressing and hemostatic agents based on cellulose will be highlighted.

Table 3 Some commercially available cellulose-based products as skin dressing and hemostatic agent

Manufacturer	Commercial name	Application	Source of cellulose
Bowil Biotech, Poland	Celmat®	Wound care	*Bacterial cellulose*
Lohmann & Rauscher, Germany	Suprasorb X® Suprasorb X® + PHMB Polyhexamethylene biguanide	Tissue repair and wound care and antimicrobial properties	*Bacterial cellulose*
Xylos Corporation, Langhorne, USA	xCell®	Wound dressing	Bacterial cellulose
Xylos Corporation, Langhorne, USA	BASYC®	*Artificial blood vessel*	*Bacterial cellulose*
Vuelo Pharma, Brazil	Membracel®	*Wound treatment*	*Bacterial cellulose*
Bionext Produtos Biotecnológicos Ltda, Brazil	Bionext®	*Wound treatment*	*Bacterial cellulose*
Bionext Produtos Biotecnológicos Ltda, Brazil	Gengiflex®	*Burns, ulcers, and treatment of periodontal diseases*	*Bacterial cellulose*
Seven Indústria de Produtos Biotecnológicos Ltda, Brazil	Nexfill®	*Wound treatment*	*Bacterial cellulose*
Johnson & Johnson, USA	Surgicel®	Control of capillary, venous, and small arterial hemorrhage	*Oxidized regenerate cellulose (gauze, fleece, fibrils, and power) cotton*
Becton Dickinson, USA	Oxycel®	Hemostatic agent Absorbable	*Oxidized cellulose (gauze) cotton*
Johnson & Johnson, USA	Evarrest®	Adjunct to hemostasis	*Oxidized regenerate cellulose (patch) cotton*
Coreva Health Science, LLC, USA	ActCel®	Hemostatic agent	*Oxidized regenerate cellulose cotton*
Gelita Medical, Germany	Gelita-Cel®	*Procoagulant dressing*	*Oxidized cellulose (gauze, fibrils, and powder) cotton*
Bioster, Czech Republic	Traumacel®	Hemostatic agent	*Oxidized regenerate cellulose with calcium (woven gauze or powder) cotton*
Synthesia, Czech Republic	Okcel®	Hemostatic agent	*Oxidized cellulose (gauze, felt pad, and powder) cotton*
CuraMedical BV, Dutch	CuraCel®	Hemostatic agent	*Oxidized regenerate cellulose (gauze and fibrils) cotton*

(continued)

Table 3 (continued)

Manufacturer	Commercial name	Application	Source of cellulose
Mascia Brunelli, Milano, Italy	Emosist®	Hemostatic agent	*Oxidized regenerate cellulose (gauze) cotton*
Medical Products Co, Ltd., China	Taikeling®	Hemostatic agent	*Oxidized regenerate cellulose (gauze, fibrils, and powders) cotton*

Oxidized cellulose is an excellent biodegradable and biocompatible derivate of cellulose, which has become one of the most important hemostatic agents used in surgical procedures. Oxidized cellulose-based hemostatic materials have proven local hemostatic efficacy and antibacterial activity and appear to be a longstanding, frequently used, safe, and effective hemostat for hemorrhage in the surgical setting (WO2016171633A1 2016).

Recently a biodegradable antibacterial nanocomposite based on oxidized bacterial nanocellulose was prepared and exhibited greater procoagulant properties and blood-clotting capability and higher adhesion of erythrocytes and platelets with concomitant lower blood loss, in addition to ultrafast cessation of bleeding, superior to the commercial hemostatic ORC product Surgicel™ gauze (Yuan et al. 2020).

Surgical products are procoagulant materials composed of a scaffold of cellulose polymers that have been in clinical use for more than 60 years and are manufactured in fibrillar consistency as well as woven sheets. The major benefit of these agents is that they are totally absorbable, which means that can be left on or within the wound; the wound can even be sutured closed over them.

Several strategies to adsorb, covalently bind, or physically entrap antimicrobial compounds in BC, including antibiotics, silver nanoparticles, chitosan, and cationic antiseptics, have been developed (Sulaeva et al. 2015). The efficacy of BC membranes and improved properties after inclusion of antimicrobial agents has been evidenced in the literature (Hosseini et al. 2020; Liu et al. 2018). A group of antimicrobial compounds that has been less extensively investigated in this context are antimicrobial peptides (AMPs) (Lei et al. 2019). Recently BC was functionalized with ε-poly-L-Lysine (ε-PLL) using carbodiimide chemistry and the growth of *S. epidermidis* on the membranes was inhibited without significant effects on the morphological structure and mechanical properties of BC. The cytocompatibility of BC functionalized to cultured human fibroblasts was the same of native BC (Fürsatz et al. 2018). At present, nanocellulose is produced on an industrial scale for commercial application. The manufacturers are mainly located in the USA, Brazil, and Poland. A summary of commercial products from bacterial cellulose and oxidized regenerate cellulose is shown in Table 3.

7 Conclusions, Future Directions, and Challenges

The aim of this chapter is to demonstrate the main aspects of cellulose and its applications in bone as well as cartilage, and skin tissue engineering focusing products already on the market. The remarkable versatility of cellulose permits

scientists to explore its unique mechanical and morphological properties associated with biocompatibility, nontoxicity, and biodegradability. The levels of structural organization and extraordinary supramolecular nanofiber 3D network permits the potential application in tissue engineering, an emerging and challenged research field. This chapter brings an overview of researches focusing in distinct steps of research development initially in the choice of material, chemical, and/or physical modification, physicochemical characterization, in vitro biological characterization, clinical evaluation, and the bioactive potential affecting the cellular response. Tissue engineering is a radically different approach to reconstruction of the body demanding interdisciplinary research, regulatory requirements for products and processes, and high cost of productive investments. Despite these challenges, the success of biopolymers application, variety of methods, and the results obtained ensure the terrific opportunity for the development of responsive structures focusing on the production of fully functional tissue.

References

Abouzeid RE, Khiari R, Beneventi D, Dufresne A. Biomimetic mineralization of three-dimensional printed alginate/TEMPO-oxidized cellulose nanofibril scaffolds for bone tissue engineering. Biomacromolecules. 2018;19(11):4442–52. https://doi.org/10.1021/acs.biomac.8b01325.

Ahlfeld T, et al. Methylcellulose – a versatile printing material that enables biofabrication of tissue equivalents with high shape fidelity. R Soc Chem. 2020:8(8)2102–2110.

Al-Sabah A, et al. Structural and mechanical characterization of crosslinked and sterilised nanocellulose-based hydrogels for cartilage tissue engineering. Carbohydr Polym. 2019;212 (February):242–51. https://doi.org/10.1016/j.carbpol.2019.02.057.

Andrade FK, Moreira SMG, Domingues L, Gama FMP. Improving the affinity of fibroblasts for bacterial cellulose using carbohydrate-binding modules fused to RGD. J Biomed Mater Res A. 2010;92(1):9–17. https://doi.org/10.1002/jbm.a.32284.

Anglès MN, Dufresne A. Plasticized starch/tunicin whiskers nanocomposite materials. 2: mechanical behavior. Macromolecules. 2001;34(9):2921–31. https://doi.org/10.1021/ma001555h.

Atalla RH, VanderHart DL. Native cellulose: a composite of two distinct crystalline forms. Science. 1984;223:283–5. https://doi.org/10.2307/1693060. American Association for the Advancement of Science.

Belgacem MN, Gandini A. Monomers, polymers and composites from renewable resources. Elsevier; 2008.

Bhatia S, Goli D. Introduction to pharmaceutical biotechnology, vol. 1. IOP Publishing; 2018.

Bose S, Vahabzadeh S, Bandyopadhyay A. Bone tissue engineering using 3D printing. Mater Today. 2013;16(12):496–504. https://doi.org/10.1016/j.mattod.2013.11.017.

Brien FJO. Biomaterials & scaffolds for tissue engineering. Mater Today. 2011;14(3):88–95. https://doi.org/10.1016/S1369-7021(11)70058-X.

Brown RM, Saxena IM. Cellulose biosynthesis: a model for understanding the assembly of biopolymers. Plant Physiol Biochem. 2000;38(1–2):57–67. https://doi.org/10.1016/S0981-9428(00)00168-6. ESME – Gauthier-Villars.

Chami Khazraji A, Robert S. Interaction effects between cellulose and water in nanocrystalline and amorphous regions: a novel approach using molecular modeling. J Nanomater. 2013. https://doi.org/10.1155/2013/409676.

Coelho F, et al. Bacterial cellulose membrane functionalized with hydroxiapatite and anti-bone morphogenetic protein 2: a promising material for bone regeneration. PLoS One. 2019;14(8):1–23. https://doi.org/10.1371/journal.pone.0221286.

Czaja WK, Young DJ, Kawecki M, Brown RM. The future prospects of microbial cellulose in biomedical applications. Biomacromolecules. 2007;8(1):1–12. https://doi.org/10.1021/bm060620d.

De Souza Lima MM, Borsali R. Rodlike cellulose microcrystals: structure, properties, and applications. Macromol Rapid Commun. 2004;25(7):771–87. https://doi.org/10.1002/marc.200300268.

de Windt TS, et al. Concise review: unraveling stem cell cocultures in regenerative medicine: which cell interactions steer cartilage regeneration and how? Stem Cells Transl Med. 2014;3(6):723–33. https://doi.org/10.5966/sctm.2013-0207.

De Witte TM, Fratila-Apachitei LE, Zadpoor AA, Peppas NA. Bone tissue engineering via growth factor delivery: from scaffolds to complex matrices. Regen Biomater. 2018;5(4):197–211. https://doi.org/10.1093/rb/rby013.

Delmer DP, Amor Y. Cellulose biosynthesis. Plant Cell. 1995;7(7):987–1000. https://doi.org/10.1105/tpc.7.7.987.

Dufresne A. Nanocellulose Processing Properties and Potential Applications 2019;(5):76–89.

Dutta SD, Patel DK, Lim K-T. Functional cellulose-based hydrogels as extracellular matrices for tissue engineering. J Biol Eng. 2019;13(1):55. https://doi.org/10.1186/s13036-019-0177-0.

Dzobo K, et al. Advances in regenerative medicine and tissue engineering: innovation and transformation of medicine. Stem Cells Int. 2018;2018:2495848. https://doi.org/10.1155/2018/2495848.

Engineering B, Wood W. Alginate sulfate – nanocellulose bioinks for cartilage bioprinting applications. Ann Biomed Eng. 2017;45(1):210–23. https://doi.org/10.1007/s10439-016-1704-5.

Esguerra M, et al. Intravital fluorescent microscopic evaluation of bacterial cellulose as scaffold for vascular grafts. J Biomed Mater Res A. 2010;93(1):140–9. https://doi.org/10.1002/jbm.a.32516.

Ferreira FV, et al. Nanocellulose/bioactive glass cryogels as scaffolds for bone regeneration. Nanoscale. 2019;11(42):19842–9. https://doi.org/10.1039/c9nr05383b.

Fink H, et al. Real-time measurements of coagulation on bacterial cellulose and conventional vascular graft materials. Acta Biomater. 2010;6(3):1125–30. https://doi.org/10.1016/j.actbio.2009.09.019.

Fontana JD, et al. Acetobacter cellulose pellicle as a temporary skin substitute. Appl Biochem Biotechnol. 1990;24–25(1):253–64. https://doi.org/10.1007/BF02920250.

Fu N, Dong T, Meng A, Meng Z, Zhu B, Lin Y. Research progress of the types and preparation techniques of scaffold materials in cartilage tissue engineering. Curr Stem Cell Res Ther. 2017;13(7):583–90. https://doi.org/10.2174/1574888x12666170718152611.

Fürsatz M, et al. Functionalization of bacterial cellulose wound dressings with the antimicrobial peptide ϵ-poly-L-Lysine. Biomed Mater. 2018;13(2):025014. https://doi.org/10.1088/1748-605X/aa9486.

Gadim TDO, et al. Nanostructured bacterial cellulose − poly(4-styrene sulfonic acid) composite membranes with high storage modulus and protonic conductivity. ACS Appl Mater Interfaces. 2014;6(10):7864–75.

Gong J, Mo L, Li J. A comparative study on the preparation and characterization of cellulose nanocrystals with various polymorphs. Carbohydr Polym. 2018;195:18–28. https://doi.org/10.1016/j.carbpol.2018.04.039.

Habibi Y, Lucia LA, Rojas OJ. Cellulose nanocrystals: chemistry, self-assembly, and applications. Chem Rev. 2010;110(6):3479–500. https://doi.org/10.1021/cr900339w.

Hasan A, Waibhaw G, Saxena V, Pandey LM. Nano-biocomposite scaffolds of chitosan, carboxymethyl cellulose and silver nanoparticle modified cellulose nanowhiskers for bone tissue engineering applications. Int J Biol Macromol. 2018;111:923–34.

Henriksson M, Henriksson G, Berglund LA, Lindström T. An environmentally friendly method for enzyme-assisted preparation of microfibrillated cellulose (MFC) nanofibers. Eur Polym J. 2007;43(8):3434–41. https://doi.org/10.1016/j.eurpolymj.2007.05.038.

Hickey RJ, Modulevsky DJ, Cuerrier CM, Pelling AE. Customizing the shape and microenvironment biochemistry of biocompatible macroscopic plant-derived cellulose scaffolds. ACS Biomater Sci Eng. 2018;4(11):3726–36. https://doi.org/10.1021/acsbiomaterials.8b00178.

Hosseini H, Zirakjou A, Goodarzi V, Mousavi SM, Khonakdar HA, Zamanlui S. Lightweight aerogels based on bacterial cellulose/silver nanoparticles/polyaniline with tuning morphology of polyaniline and application in soft tissue engineering. Int J Biol Macromol. 2020;152:57–67. https://doi.org/10.1016/j.ijbiomac.2020.02.095.

Huang L, et al. Bacterial cellulose nanofibers promote stress and fidelity of 3D-printed silk based hydrogel scaffold with hierarchical pores. Carbohydr Polym. 2019;221(December 2018):146–56. https://doi.org/10.1016/j.carbpol.2019.05.080.

Huh JB, et al. Effects of PMMA and cross-linked dextran filler for soft tissue augmentation in rats. Int J Mol Sci. 2015;16(12):28523–33. https://doi.org/10.3390/ijms161226112.

Hutchens SA, Benson RS, Evans BR, O'Neill HM, Rawn CJ. Biomimetic synthesis of calcium-deficient hydroxyapatite in a natural hydrogel. Biomaterials. 2006;27(26):4661–70. https://doi.org/10.1016/j.biomaterials.2006.04.032.

Ingole VH, Vuherer T, Maver U, Vinchurkar A, Ghule AV. Mechanical properties and cytotoxicity of differently structured nanocellulose-hydroxyapatite based composites for bone regeneration application. Nanomaterials (Basel). 2000;10(1):25.

Ingole VH, Vuherer T, Maver U, Kokol V, Vinchurkar A, Ghule AV. Mechanical properties and cytotoxicity of differently structured nanocellulose-hydroxyapatite based composites for bone regeneration application. Nano. 2020;10(1):25. https://doi.org/10.3390/nano10010025.

Ishikawa M, Oaki Y, Tanaka Y, Kakisawa H, Salazar-Alvarez G, Imai H. Fabrication of nanocellulose-hydroxyapatite composites and their application as water-resistant transparent coatings. J Mater Chem B. 2015;3(28):5858–63. https://doi.org/10.1039/c5tb00927h.

Kang HW, Lee SJ, Ko IK, Kengla C, Yoo JJ, Atala A. A 3D bioprinting system to produce human-scale tissue constructs with structural integrity. Nat Biotechnol. 2016;34(3):312–9. https://doi.org/10.1038/nbt.3413.

Kanitakis J. Anatomy, histology and immunohistochemistry of normal human skin. Eur J Dermatol. 2002;12(4):390–401.

Karageorgiou V, Kaplan D. Porosity of 3D biomaterial scaffolds and osteogenesis. Biomaterials. 2005;26(27):5474–91. https://doi.org/10.1016/j.biomaterials.2005.02.002. Elsevier BV.

Kim MH, Kim BS, Park H, Lee J, Park WH. Injectable methylcellulose hydrogel containing calcium phosphate nanoparticles for bone regeneration. Int J Biol Macromol. 2018;109:57–64. https://doi.org/10.1016/j.ijbiomac.2017.12.068.

Kirdponpattara S, Khamkeaw A, Sanchavanakit N, Pavasant P, Phisalaphong M. Structural modification and characterization of bacterial cellulose-alginate composite scaffolds for tissue engineering. Carbohydr Polym. 2015;132:146–55. https://doi.org/10.1016/j.carbpol.2015.06.059.

Klemm D, Schumann D, Udhardt U, Marsch S. Bacterial synthesized cellulose – artificial blood vessels for microsurgery. Prog Polym Sci (Oxford). 2001;26(9):1561–603. https://doi.org/10.1016/S0079-6700(01)00021-1. Elsevier Ltd.

Kummala R, et al. Human dermal fibroblast viability and adhesion on cellulose nanomaterial coatings: influence of surface characteristics. Biomacromolecules. 2020;21(4):1560–7. https://doi.org/10.1021/acs.biomac.0c00107.

Lam MT, Longaker MT. Comparison of several attachment methods for human iPS, embryonic and adipose-derived stem cells for tissue engineering. J Tissue Eng Regen Med. 2012;6(Suppl 3). https://doi.org/10.1002/term.1499.

Law N, et al. Characterisation of hyaluronic acid methylcellulose hydrogels for 3D bioprinting. J Mech Behav Biomed Mater. 2018;77:389–99. https://doi.org/10.1016/j.jmbbm.2017.09.031.

Leblanc Latour M, Tarar M, Hickey RJ, Cuerrier CM, Catelas I, Pelling AE. Plant-derived cellulose scaffolds for bone tissue engineering. bioRxiv. 2020;8:1–14. https://doi.org/10.1101/2020.01.15.906677.

Leguy J, Diallo A, Putaux JL, Nishiyama Y, Heux L, Jean B. Periodate oxidation followed by NaBH4 reduction converts microfibrillated cellulose into sterically stabilized neutral cellulose nanocrystal suspensions. Langmuir. 2018;34(37):11066–75. https://doi.org/10.1021/acs.langmuir.8b02202.

Lei J, et al. The antimicrobial peptides and their potential clinical applications. Am J Transl Res. 2019;11(7):3919–31.
Liu R, Dai L, Si C, Zeng Z. Antibacterial and hemostatic hydrogel via nanocomposite from cellulose nanofibers. Carbohydr Polym. 2018;195:63–70. https://doi.org/10.1016/j.carbpol.2018.04.085.
Long Y, et al. Collagen−hydroxypropyl methylcellulose membranes for corneal regeneration. ACS Omega. 2018;3(1):1269–75. https://doi.org/10.1021/acsomega.7b01511.
Lopes TD, Riegel-Vidotti IC, Grein A, Tischer CA, de S. Faria-Tischer PC. Bacterial cellulose and hyaluronic acid hybrid membranes: production and characterization. Int J Biol Macromol. 2014;67:401–8. https://doi.org/10.1016/j.ijbiomac.2014.03.047.
Ma PX. Scaffolds for tissue fabrication. Mater Today. 2004;7(5):30–40. https://doi.org/10.1016/S1369-7021(04)00233-0.
Man Z, Muhammad N, Sarwono A, Bustam MA, Kumar MV, Rafiq S. Preparation of cellulose nanocrystals using an ionic liquid. J Polym Environ. 2011;19(3):726–31. https://doi.org/10.1007/s10924-011-0323-3.
Mandrycky C, Wang Z, Kim K, Kim D. 3D bioprinting for engineering complex tissues. Biotechnol Adv. 2016;34:422–34.
Markstedt K, Mantas A, Tournier I, Martínez Ávila H, Hägg D, Gatenholm P. 3D bioprinting human chondrocytes with nanocellulose-alginate bioink for cartilage tissue engineering applications. Biomacromolecules. 2015;16(5):1489–96. https://doi.org/10.1021/acs.biomac.5b00188.
Martínez Ávila H, et al. Novel bilayer bacterial nanocellulose scaffold supports neocartilage formation invitro and invivo. Biomaterials. 2015;44:122–33. https://doi.org/10.1016/j.biomaterials.2014.12.025.
Martínez Ávila H, Schwarz S, Rotter N, Gatenholm P. 3D bioprinting of human chondrocyte-laden nanocellulose hydrogels for patient-specific auricular cartilage regeneration. Bioprinting. 2016;1–2:22–35. https://doi.org/10.1016/j.bprint.2016.08.003.
Matinfar M, Mesgar AS, Mohammadi Z. Evaluation of physicochemical, mechanical and biological properties of chitosan/carboxymethyl cellulose reinforced with multiphasic calcium phosphate whisker-like fibers for bone tissue engineering. Mater Sci Eng C. 2019;100:341–53. https://doi.org/10.1016/j.msec.2019.03.015.
Modulevsky DJ, Lefebvre C, Haase K, Al-Rekabi Z, Pelling AE. Apple derived cellulose scaffolds for 3D mammalian cell culture. PLoS One. 2014;9(5):e97835. https://doi.org/10.1371/journal.pone.0097835.
Modulevsky DJ, Cuerrier CM, Pelling AE. Biocompatibility of subcutaneously implanted plant-derived cellulose biomaterials. PLoS One. 2016;11(6):e0157894. https://doi.org/10.1371/journal.pone.0157894.
Möller T, et al. In vivo chondrogenesis in 3D bioprinted human. Plast Reconstr Surg Glob Open. 2017:1–7. https://doi.org/10.1097/GOX.0000000000001227.
Mondal S. Preparation, properties and applications of nanocellulosic materials. Carbohydr Polym. 2017;163:301–16. https://doi.org/10.1016/j.carbpol.2016.12.050. Elsevier Ltd.
Naseri-Nosar M, Ziora ZM. Wound dressings from naturally-occurring polymers: a review on homopolysaccharide-based composites. Carbohydr Polym. 2018;189:379–98. https://doi.org/10.1016/j.carbpol.2018.02.003. Elsevier Ltd.
Nelson KIM. Innovative nanocellulose process breaks the cost barrier. TAPPI J. 2014;13(5):19–23.
Nguyen D, et al. Cartilage tissue engineering by the 3D bioprinting of iPS cells in a nanocellulose/alginate bioink. Sci Rep. 2017;7(1):1–10. https://doi.org/10.1038/s41598-017-00690-y.
Niemczyk-Soczynska B, Gradys A, Kolbuk D, Krzton-Maziopa A, Sajkiewicz P. Crosslinking kinetics of methylcellulose aqueous solution and its potential as a scaffold for tissue engineering. Polymers (Basel). 2019;11(11):1772. https://doi.org/10.3390/polym11111772.
Nishiguchi A, Taguchi T. Osteoclast-responsive, injectable bone of bisphosphonated- nanocellulose that regulates osteoclast/osteoblast activity for bone regeneration. Biomacromolecules. 2019;20(3):1385–93. https://doi.org/10.1021/acs.biomac.8b01767.

Nishiyama Y. Structure and properties of the cellulose microfibril. J Wood Sci. 2009;55(4):241–9. https://doi.org/10.1007/s10086-009-1029-1.

Ogueri KS, Jafari T, Escobar Ivirico JL, Laurencin CT. Polymeric biomaterials for scaffold-based bone regenerative engineering. In: Regenerative engineering and translational medicine, 5, 2. Springer International Publishing; 2019. p. 128–54. https://doi.org/10.1007/s40883-018-0072-0.

Osorio DA, et al. Cross-linked cellulose nanocrystal aerogels as viable bone tissue scaffolds. Acta Biomater. 2019;87:152–65. https://doi.org/10.1016/j.actbio.2019.01.049.

Pinho E, Soares G. Functionalization of cotton cellulose for improved wound healing. J Mater Chem B. 2018;6(13):1887–98. https://doi.org/10.1039/c8tb00052b. Royal Society of Chemistry.

Portela R, Leal CR, Almeida PL, Sobral RG. Bacterial cellulose: a versatile biopolymer for wound dressing applications. Microb Biotechnol. 2019;12(4):586–610. https://doi.org/10.1111/1751-7915.13392. Wiley.

Puelacher WC, Kim SW, Vacanti JP, Schloo B, Mooney D, Vacanti CA. Tissue-engineered growth of cartilage: the effect of varying the concentration of chondrocytes seeded onto synthetic polymer matrices. Int J Oral Maxillofac Surg. 1994;23(1):49–53. https://doi.org/10.1016/S0901-5027(05)80328-5.

Qi Y, Cheng Z, Ye Z, Zhu H, Aparicio C. Bioinspired mineralization with hydroxyapatite and hierarchical naturally aligned nanofibrillar cellulose. ACS Appl Mater Interfaces. 2019;11(31):27598–604. https://doi.org/10.1021/acsami.9b09443.

Rasouli R, Barhoum A, Bechelany M, Dufresne A. Nanofibers for biomedical and healthcare applications. Macromol Biosci. 2019;19(2):1–27. https://doi.org/10.1002/mabi.201800256.

Rederstorff E, et al. Enriching a cellulose hydrogel with a biologically active marine exopolysaccharide for cell-based cartilage engineering. J Tissue Eng Regen Med. 2017;11(4):1152–64. https://doi.org/10.1002/term.2018.

Reid MS, Villalobos M, Cranston ED. Benchmarking cellulose nanocrystals: from the laboratory to industrial production. Langmuir. 2017;33(7):1583–98. https://doi.org/10.1021/acs.langmuir.6b03765.

Revol JF. Change of the d spacing in cellulose crystals during lattice imaging. J Mater Sci Lett. 1985;4(11):1347–9. https://doi.org/10.1007/BF00720097.

Ribeiro-Viana RM, Faria-Tischer PCS, Tischer CA. Preparation of succinylated cellulose membranes for functionalization purposes. Carbohydr Polym. 2016;148:21–8. https://doi.org/10.1016/j.carbpol.2016.04.033.

Roushangar Zineh B, Shabgard MR, Roshangar L. Mechanical and biological performance of printed alginate/methylcellulose/halloysite nanotube/polyvinylidene fluoride bio-scaffolds. Mater Sci Eng C. 2018;92(December 2017):779–89. https://doi.org/10.1016/j.msec.2018.07.035.

Ruoslahti E. RGD and other recognition sequences for integrins. Annu Rev Cell Dev Biol. 1996;12:697–715.

Saito T, Kimura S, Nishiyama Y, Isogai A. Cellulose nanofibers prepared by TEMPO-mediated oxidation of native cellulose. Biomacromolecules. 2007;8(8):2485–91. https://doi.org/10.1021/bm0703970.

Shefa AA, et al. In vitro and in vivo evaluation of effectiveness of a novel TEMPO-oxidized cellulose nanofiber-silk fibroin scaffold in wound healing. Carbohydr Polym. 2017;177:284–96. https://doi.org/10.1016/j.carbpol.2017.08.130.

Sigma-Aldrich. Attachment and matrix factors. BioFiles. 2008;3(8):1–28, [Online]. Available: https://www.sigmaaldrich.com/CA/en/technical-documents/technical-article/cell-culture-and-cell-culture-analysis/3dcell-culture/attachment-factors-for-cell-culture

Singh BN, Pramanik K. Generation of bioactive nano-composite scaffold of nanobioglass/silk fibroin/carboxymethyl cellulose for bone tissue engineering. J Biomater Sci Polym Ed. 2018;29(16):2011–34. https://doi.org/10.1080/09205063.2018.1523525.

Siqueira G, Tapin-Lingua S, Bras J, da Silva Perez D, Dufresne A. Morphological investigation of nanoparticles obtained from combined mechanical shearing, and enzymatic and acid hydrolysis of sisal fibers. Cellulose. 2010;17(6):1147–58. https://doi.org/10.1007/s10570-010-9449-z.

Šturcova A, His I, Apperley DC, Sugiyama J, Jarvis MC. Structural details of crystalline cellulose from higher plants. Biomacromolecules. 2004;5(4):1333–9. https://doi.org/10.1021/bm034517p.

Sugiyama J. High resolution observations of cellulose microfibrils. Mokuzai Gakkaishi. 1984;30:98–99, [Online]. Available: https://ci.nii.ac.jp/naid/80001913897. Accessed 10 July 2020.

Sukul M, Nguyen TBL, Min YK, Lee SY, Lee BT. Effect of local sustainable release of BMP2-VEGF from nano-cellulose loaded in sponge biphasic calcium phosphate on bone regeneration. Tissue Eng Part A. 2015;21(11–12):1822–36. https://doi.org/10.1089/ten.tea.2014.0497.

Sulaeva I, Henniges U, Rosenau T, Potthast A. Bacterial cellulose as a material for wound treatment: properties and modifications. A review. Biotechnol Adv. 2015;33(8):1547–71. https://doi.org/10.1016/j.biotechadv.2015.07.009.

Trache D, Hussin MH, Haafiz MKM, Thakur VK. Recent progress in cellulose nanocrystals: sources and production. Nanoscale. 2017;9(5):1763–86. https://doi.org/10.1039/c6nr09494e.

Trovatti E, et al. Enhancing strength and toughness of cellulose nanofibril network structures with an adhesive peptide. Carbohydr Polym. 2018;181:256–63. https://doi.org/10.1016/j.carbpol.2017.10.073.

Ullah H, Wahid F, Santos HA, Khan T. Advances in biomedical and pharmaceutical applications of functional bacterial cellulose-based nanocomposites. Carbohydr Polym. 2016;150:330–52. https://doi.org/10.1016/j.carbpol.2016.05.029. Elsevier Ltd.

Valentim RMB, et al. Composite based on biphasic calcium phosphate (HA/β-TCP) and nano-cellulose from the açaí tegument. Materials (Basel). 2018;11(11):2213. https://doi.org/10.3390/ma11112213.

VanderHart DL, Atalla RH. Studies of microstructure in native celluloses using solid-state 13C NMR. Macromolecules. 1984;17(8):1465–72. https://doi.org/10.1021/ma00138a009.

Williams DF. Challenges with the development of biomaterials for sustainable tissue engineering. Front Bioeng Biotechnol. 2019;7(May):1–10. https://doi.org/10.3389/fbioe.2019.00127.

WO2016171633A1 – Regenerated oxidized celulose based hemostatic materialcontaining anti-fibrolytic agents – Google Patents. 2016. https://patents.google.com/patent/WO2016171633A1/nl. Accessed 11 July 2020.

Wu S, et al. Oxidized regenerated cellulose/collagen dressings: review of evidence and recommendations. Adv Ski Wound Care. 2017;30(11):S1–S18. https://doi.org/10.1097/01.ASW.0000525951.20270.6c.

Yuan H, Chen L, Hong FF. A biodegradable antibacterial nanocomposite based on oxidized bacterial nanocellulose for rapid hemostasis and wound healing. ACS Appl Mater Interfaces. 2020;12(3):3382–92. https://doi.org/10.1021/acsami.9b17732.

Zhang L, Hu J, Athanasiou KA. The role of tissue engineering in articular cartilage repair and regeneration. Crit Rev Biomed Eng. 2009;37(1–2):1–57. https://doi.org/10.1615/CritRevBiomedEng.v37.i1-2.10.

Zhang X, et al. Biocompatiable silk fibroin/carboxymethyl chitosan/strontium substituted hydroxyapatite/cellulose nanocrystal composite scaffolds for bone tissue engineering. Int J Biol Macromol. 2019a;136:1247–57.

Zhang X, et al. Aligned electrospun cellulose scaffolds coated with rhBMP-2 for both in vitro and in vivo bone tissue engineering. Carbohydr Polym. 2019b;213(February):27–38. https://doi.org/10.1016/j.carbpol.2019.02.038.

Glycoconjugate for Tissue Engineering

49

Hevi Wihadmadyatami and Dwi Liliek Kusindarta

Contents

Abstract

Currently, tissue engineering is progressing rapidly, one of which is the application of one of the tissue engineering products, namely biomaterials in biomedical and regenerative medicine. One of the main factors that are very important in the biomaterial design is the extracellular matrix (ECM), where ECM plays a crucial role as the microenvironment that surrounds and supports the cells of each tissue and organ. One of ECM's main constituents is carbohydrate or glycan, which is in the form of glycoconjugate and its derivatives. In its development, there are various kinds of polymers formed from glycan in the form of nature or synthetics known as glycopolymers. Glycopolymers have recently used as a biomaterial for therapeutic methods, medical adhesives, drug delivery systems, and scaffolds.

H. Wihadmadyatami · D. L. Kusindarta (✉)
Department of Anatomy, Universitas Gadjah Mada, Yogyakarta, Indonesia
e-mail: heviwihadmadyatami@ugm.ac.id; indarta@ugm.ac.id

J. M. Oliveira et al. (eds.), *Polysaccharides of Microbial Origin*,
https://doi.org/10.1007/978-3-030-42215-8_60

Thus, a comprehensive understanding of glycoconjugate's role in tissue engineering is essential for knowledge and research in the field of biomaterial studies. This review article highlights commonly used glycopolymer on the biomedical applications.

Keywords

Tissue engineering · ECM · Glycoconjugate · Glycopolymer

1 Introduction

Tissue engineering is a research area that focuses on the engineering of functional tissue. Functional tissue that develops by tissue engineering can be used to replace organs that have been damaged by disease or injury (Mandrycky et al. 2017). Tissue engineering and regenerative medicine are two inseparable fields. The technology related to regenerative medicine has developed rapidly and includes two main concepts: (1) cell therapy without the use of a scaffold (cell therapy) and (2) tissue engineering that needs a scaffold to support tissue regeneration. Recent advances in technology related to these two concepts are already widely applied in the field of tissue engineering and include (1) biomaterial system, which will be implanted to the body and undergo to the process of tissue unification (e.g., 3D biomaterial scaffold, decellularized natural material); (2) cell system (e.g., reprogramming adult stem cell into multipotent stem cells, stem cell transplantation); (3) cell and biomaterial complex system, on this system the cells are seeded on the biomaterials then implanter into the body; thus, it will repair and regenerate the tissue or organ (Xu et al. 2019).

Technology advanced in the field of tissue engineering opens the possibility to be able to make organs or tissues following the design needed by each patient. However, this technology also currently has limitations, especially in limiting to the in vivo repair and regeneration using uniform or single tissue scaffold, mechanic failure, and the possibility of infection. Besides, the main problems currently in organ transplants including the shortage of donated organs due to immune rejection, although immunosuppressive therapy and HLA matching recently applied and much advanced (Ikada 2006; O'Brien 2011). There are three significant approaches in advancing tissue engineering including

1. Design of biomaterials that can serve as implantable scaffolds which can be remodeled by the body's cells in vivo
2. Design of scaffolds that able to implanted after passed the in vitro remodeling
3. Involvement of multipotent stem cells, which can promote to expand the scaffold (Polo-Corrales et al. 2014; Chen et al. 2016)

Scaffold design is one of the most crucial aspects of tissue engineering. The construction of scaffold should fulfill the ability so that both of mechanical and

chemical properties could create an environment which can supports cellular growth, able to induce the growth factors, promote remodeling, and promote neovascularization (Barthes et al. 2014). Currently, the regenerative 3D scaffold considered the best type of scaffold, since the scaffold can deliver cells in vivo and has different mechanical properties or porosity and emerging applications in soft tissue engineering. In general, criteria for ideal 3D scaffold are determined by the following characteristics, such as type of biomaterial, biocompatibility, co-culture of cells, biodegradability, porosity, incorporation of variants of extracellular matrix (ECM), pore size, mechanical properties (tensile strength and elastic modulus), shape and size, inter-connectivity, orientation, incorporation of physical signals and abilities to entrap soluble signals (Alaribe et al. 2016).

One of the main factors that are very important in the design of the scaffold is the extracellular matrix (ECM). In the body, ECM plays a crucial role as the microenvironment that surrounds and supports the cells of each tissue and organ. The properties of the ECM vary significantly from tissue to tissue and are in a state of balance with the tissue cells. Designs of tissue engineering scaffolds mainly based on the properties of the ECM from the organ or tissue that will be replaced (Fernandez 2009; Barthes et al. 2014). Constructing of a scaffold to mimic the mechanical and chemical properties of the ECM can be achieved either by incorporating the same chemical compounds and macromolecules or by including synthetic constructs with the same chemical, structural, and mechanical properties (Weyers and Linhardt 2013; Eltom et al. 2019).

ECM in the body consists of fibrillar proteins (collagen family, elastin fiber) and glycoconjugate including glycoproteins (vitronectin, fibronectin, laminin, integrin), proteoglycans, and glycosaminoglycans (Stegemann et al. 2007; Fernandes et al. 2009). At present, there have been developed synthetic molecules or natural compounds that already chemically manipulated to mimic of species found in the ECM based on the protein content. Some commercialized products in ECM tissue engineering based on nature compounds have already been introduced, for example, Biocart ™ II (autologous chondrocytes within freeze-dried fibrin and hyaluronan), Hyalograft®-C, a nonwoven mesh of hyaluronic acid-based microfibers, NeoCart® a bovine type I collagen, and Novocart® product, a 3D autologous chondrocyte implant system composed on a bioresorbable biphasic collagen scaffold (Williams 2019). Nevertheless, with the development of science that continues to progress, the manipulation of ECM in tissue engineering continues to experience improvements which involve glycoconjugate in making 3D scaffolds. Glycoconjugate plays important structural and regulatory roles in the ECM and is involved in many crucial cellular signaling processes governing tissue growth and development. Both ECM and glycoconjugate are involved in cell signaling, proliferation, adhesion, and migration (Sasaki and Toyoda 2013; Djerbal et al. 2017) Thus, their mechanical, biological, and chemical properties make glycoconjugate critical components of tissue engineering scaffolds. Recently the presentation of glycoconjugate engineering may be involved in the therapeutic medication mainly in regenerative medicine. In this part will review the definition of glycoconjugate including structure, type, and the participation of glycoconjugate on tissue engineering. Besides, the involvement of glycoconjugate as part of the soluble material in tissue engineering will be discussed.

2 Glycoconjugate: Definition, Structure, and Type

Glycoconjugates are molecules of one or more carbohydrate (glycans) linked covalently to oligosaccharides or other compounds. Frequently, the glycoconjugate found to attach to the specific protein or lipid. If the glycoconjugate bound to the protein, they will form a glycoprotein and proteoglycan. Meanwhile, if they linked to the lipid, it will form glycoglycerolipids and glycosphingolipids. In the extraordinary chase, glycoconjugate bound to the both and form glycosylphosphatidylinositol-anchored proteins (Fig. 1). Due to attachment of glycoconjugate, three main types of protein modification are found in mammals: N-linked glycosylation, O-linked glycosylation, and the attachment of glycosylphosphatidylinositol anchors as C-mannosylation (Varki et al. 2009; Reily et al. 2019). The biosynthesis of glycoconjugates occurs in two specialized areas as the part of the membranous biosynthetic compartments within the cell: the endoplasmic reticulum and the Golgi apparatus. Meanwhile, the

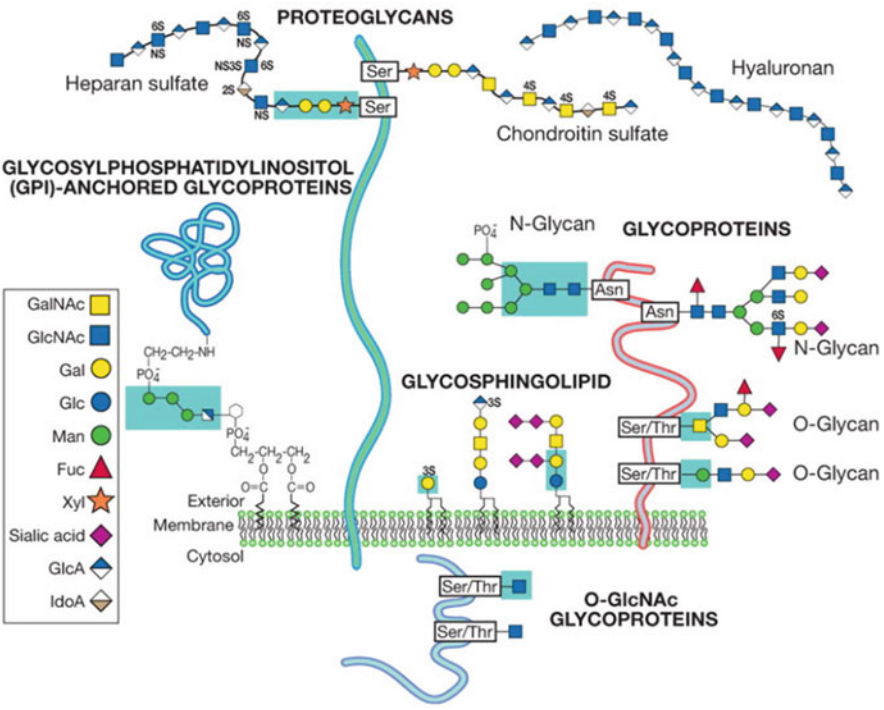


Fig. 1 Common glycan linkage regions on animal cell glycoconjugates. The most common types of glycans found in animal glycoconjugates are shown, with an emphasis upon the linkage region between the oligosaccharide and the protein or lipid. Other rarer types of linkage regions and free oligosaccharides that can exist naturally are not shown. (Reproduced with permission from the John Willey and Sons, originally Figure 12.1.1 in Varki A, Cummings RD, Esko JD, Freeze HH, Stanley P, Bertozzi CR, Hart GW, Etzler ME, Overview of Glycoconjugate Analysis, Curr Protoc Protein Sci. 2009)

degradation of glycoconjugates occurs in the lysosome, another specialized membranous digestive compartment. The two main enzymes that mediate the biosynthesis and degradation of glycoconjugates are glycosyltransferases and glycoside hydrolases. Besides, the N-glycosylation occurs on the endoplasmic reticulum, on the other hand, the O-glycosylation happens on the Golgi apparatus.

2.1 Glycoprotein

Glycoproteins are oligosaccharides chains (glycans) or carbohydrate molecules which are connected to protein with the covalent junction and attached in the position of polypeptide side-chains in different quality and order. The carbohydrate will be attached to the protein during co-translational modification and post-translational modification. All the process is known as glycosylation. The binding between protein core to the acidic components such as uronic sugar (iduronic or glucuronic acid) and aminoglycan, will described as proteoglycans or glycosaminoglycan (Kamiya et al. 2014; Jakab 2016). Besides, the difference between glycoprotein and proteoglycan lies in the number of conjugated carbohydrate monomers. In the proteoglycan, the amount of carbohydrates is more than the protein, and this situation is inversely proportional to glycoprotein where protein is more than carbohydrate monomers.

According to the binding site of the glycan to the protein, there are three major groups of glycoproteins: N-linked glycosylation, O-linked glycosylation, and glycosyl phosphatidylinositol proteins. In O-linked glycoproteins, the glycan model is short and simple and is attached to the OH group of serine or regional side chain via acyl linkage. In N-linked glycoproteins, the glycan moiety attached to asparagine via N-acetylglucosamine linkage. The glycan component in N-linked glycoproteins is complex but ubiquitous. There are two types of N-linked glycans: the high mannose type and the acidic type (Saraswathy and Ramalingam 2011). The last type of glycation in the glycoprotein is glycosyl phosphatidylinositol proteins. This glycation occurs when the carboxyl terminal amino acid of a protein via a phosphoryl-ethanolamine moiety joined to an oligosaccharide (glycan), linked via glucosamine to phosphatidylinositol (PI) (Welsh et al. 2016). Glycoproteins take part in critical cellular functions such as protein folding, embryonic development, cell-cell recognition, cell adhesion, immune functions, and pathogen identification.

2.2 Glycolipids

Glycolipids, depending on the structure and the lipid moiety, are divided into two categories: glycosphingolipids (GSLs) and glycoglycerolipids. The term of glycoglycerolipid is used to define the glycolipids containing mono-, di-, or trisaccharides linked to glycosidically to the hydroxyl group of diglycerides. Meanwhile, glycosphingolipid describes as the lipids which contain at least one residue of monosaccharide linked to ceramide. Ceramides are amides of fatty acids completed with a

long chain of di- or trihydroxy bases. The glycosphingolipids could be divided into four types: (1) neutral glycosphingolipids, (2) galactosylceramides, (3) glucosylceramide, and (4) oligoglycosylceramides. Glycolipids are vital components of cellular membranes of most eukaryotes. Glycolipid also takes an essential role due to the mechanism of signal transducer and cell proliferation.

3 Glycan-Based Polymer

Carbohydrates and polysaccharides are the most abundant biopolymers ubiquitous in living systems. In the cell surface, glycans or carbohydrates, in the form of glycoproteins, glycolipids, glycosaminoglycans (GAGs), proteoglycans, and other glycoconjugates, are known to play crucial biological roles in correlation to the metabolism processes, protein and lipid modification, cellular structure, signal transduction, as well as mediators of cell-cell interactions and host-pathogen interactions. The protein-glycan interaction mediates all of this interaction; these interactions are usually weak, but the multivalent effect of clustered glycan supports the interaction; for example, the glycolipids on the cell surfaces form the densely packed saccharide domain such as raft and caveolae and interact with protein effectively. Some research already reported synthetic interaction between glycan and protein to have a similar signal and ability to the natural glycan ligands (e.g., glycopeptides, glycoparticle, and glycocalixarene). Since glycan takes a crucial biological significance of this class of biomolecules, there is very important to securing access to these biopolymers for a broad range of biomedical, regenerative medicine, and tissue engineering as well as materials science applications. Recently, biopolymers used in biomedical engineering can be categorized into three main groups: natural, synthetic, natural, and synthetic blend polymers.

3.1 Nature Glycopolymers

Polysaccharides are nature polymers containing between 30-103-4 monosaccharides derived from a variety of typical glycan monomers such as glucose, maltose, galactose, N-acetylglucosamine, N-acetylgalactosamine, and fucose. Meanwhile, the nature of glycopolymers could be defined as a large family of polysaccharides derived from animals, plants, algae, and microorganisms (Table 1). Because of the diversity of their chemical structures as well as physical and biological properties, nature glycopolymers have found many varieties of applications in biomedicine, pharmaceutical research, and another industrial field (Miura 2007; Pramudya and Chung 2019).

3.1.1 Chitin/Chitosan

Chitin is the second most abundant natural polysaccharide, after cellulose, and is a linear polymer composed of repeating β(1,4)-N-acetylglucosamine units. Chitin presents in the shells of arthropods, for example, crabs, shrimps, and insects.

Table 1 The examples of the most common type polysaccharide found in nature

Source	Polysaccharides
Animal	Chitin/chitosan, glucosamynoglycans (GAGs), glycogen
Algae	Laminaran, insulin, carrageenan, agar, alginate, agarosa
Plant	Cellulose, pectin, galactomannas, glucomannans
Microbial	Dextran, cyclodextrin, xanthan, gellan, curdian

Chitosan is the partial portion of the N-deacetylated analog of chitin, which is a heteropolysaccharide consisting of D-glucosamine and N-acetyl-D-glucosamine. The existence of free amino groups makes chitosan a natural cationic polymer and presents chemical functionality for facile derivatization of the chitosan polymer. Both chitin and chitosan are rigid and crystalline polymers, contributing to their strength and insolubility in the water at neutral pH. In acidic conditions, chitosan can be dissolved due to free amino groups' protonation, while chitin is insoluble. The molecular weight of chitin and chitosan can be as high as 106 Dalton (Komi and Hamblin 2016; Zarrintaj et al. 2020).

In biomedicine, chitin and chitosan have multiple applications, including drug delivery vehicles, wound dressing materials, and potential tissue engineering matrices. In drug delivery, chitosan has been of interest due to its ability to interact with cell membranes, increase cellular permeability, and increase the residence time of drugs in the gastrointestinal tract. Positively charged chitosan are used in gel formation with acidic drugs to protect bioactive molecules from the gastric environment and enhance dissolution. Chitosan possesses a new characteristic in vivo: due to the lack of specific hydrolytic enzymes for chitosan in the small intestine, orally delivered chitosan survives passage into the colon. Therefore, chitosan-based drug carriers are explored for the treatment of colon diseases. In wound healing, hydrogels made from chitosan can protect the wound from bacterial infection, maintain a hydrated local environment, and reduce scarring. Chitosan can be used together with growth factors for local delivery and to speed up the wound healing process. Recently, chitosan and its derivatives have been used extensively as porous scaffolds for the regeneration of various tissues or organs, including skin, bone, cartilage, liver, nerve, and blood vessel.

3.1.2 Glycosaminoglycans

GAGs are distinct class of linear polysaccharides that consist of repeating disaccharide units containing at least one deoxyamino sugar. GAGs reflect a large number of polymers with significant chemical and structural differences that arise from the patterns of disaccharide building blocks (e.g., N-acetylglucosamine (GlcNAc)–glucuronic acid (GlcA) and uronic acid–glucosamine). GAGs play a vital role in many cell surfaces and connective tissues and extracellular matrix (ECM). They can regulate many biological processes through their interactions with numerous effector proteins. In biomedicine, the GAGs take a role as scaffold matrix on the stabilization of connective tissue, ECM organization, hydration and water homeostasis,

receptor-mediated signaling, morphogenesis, and tissue homeostasis, regulation of the inflammatory response, tissue remodeling, and also cellular migration.

3.1.3 Agarosa

Agarosa or agarose is a linear polysaccharide extracted from agarophyte red algae. Agarose contains two fractions: neutral agarose and anionic agaropectin. Agarose is made up of alternating (1–4)-linked 3,6anhydrous-α-L-galactopyranose and (1–3)-linked β-D-galactose units. Agarose functions as solid growth media in the research area; thus, it is fabricated in different forms (e.g., microspheres and films). In the fabricated form, the agarose take a role to encapsulate molecules for sustained-drug delivery or immobilize proteins for tissue engineering. Due to the gelation property of agar, it is most often used as a hydrogel. To realize sustained-drug delivery, agar hydrogels have been modified by integrating other biopolymers to form a penetrated network. This network structure of agar hydrogels can improve the mechanical property of the drug delivery systems and extend the drug release profile. In tissue engineering, agar hydrogels with high porosity have shown promising results to promote cell adhesion as well as cell proliferation.

3.1.4 Gelatin

Gelatin is a protein-based material achieved from collagen hydrolysis. Gelatin is known as a biocompatible, biodegradable, and affordable material. Collagen can be found in cellular ECM and due to such similarity with ECM gelatin has attracted significant attention in biomedical applications.

3.2 Synthetic Polymer

Natural or native polymers, as mention above, such as chitosan, GAGs, agarose, are well-known sources for preparing scaffold in tissue engineering. Although nature glycopolymers usually have good bioactivity, their mechanical properties are often weak; they found difficulties in handling the structure of heterogeneity and impurity, a high risk of contamination, and challenging to handle quality from batch to batch (Liu et al. 2017). Compared with other materials, the properties of the synthetic polymer could be quickly designed and made by changing the molecular structure and processing parameters to adapt and develop milieu as same as extracellular matrix. These synthetic polymers are highly popular as scaffold material, as have defined chemistry, secure processing, and tailoring ability, and can be modified to achieve desired properties for specific applications. Other advantages include cost-efficacy, ability to be produced in large quantity uniformly and longer shelf time, also the physicochemical and mechanical properties such as tensile strength, elastic modulus, and degradation rate (Dwivedi et al. 2019). However, these polymers are not bioactive; hence, they can elicit inflammatory responses inside the host. Several synthetic polymers already familiar are used in the field of regenerative medicine mainly in the field of tissue regenerative, such as polyethylene glycol (PEG), lactide- and glycolide-derived polyesters (Sakiyama-Elbert et al. 2012), polycaprolactones (Silva et al. 2010), poly

(2-hydroxyethyl Methacrylate), NeuroGel ™ (N- (2-hydroxypropyl) methacrylamide or HPMA), poly lactic-co-Glycolic Acid (PLGA), poly (ε-caprolactone (PCL), poly L-lactic acid (PLLA) and conducting polymers (polypyrrole, polyaniline) (Subramanian et al. 2009), poly(N-methacryloylglucosamine) (pMAG) and poly (N-methacryloylmannosamine) (pMAM), poly(azobenzene methacrylate) (PMAzo), and poly(3-O-4-vinylbenzoyl-D-glucopyranose) (PBG).

3.2.1 Polyethylene Glycol (PEG)

Polyethylene glycol (PEG) or polyethylene oxide (PEO) is ethylene oxide macromolecules. Those having molecular weights less than 20,000 g/mol are named PEG whereas those having values above 20,000 g/mol are called PEO. PEG structure is composed of PEG diol with two hydroxyl end groups, which can be converted into other functional groups, such as methyloxyl, carboxyl, amine, thiol, azide, vinyl sulfone, azide, acetylene, and acrylate. The two functional end groups can be the same (symmetric) or different (asymmetric). These two functional end groups are essential for hydrogel formation or for conjugating with covalently linking biomolecules. PEG is soluble in water, ethanol, acetonitrile, benzene, and dichloromethane, while it is insoluble in diethyl ether and hexane. PEG is available in many different structures, such as comb-like branched and star macromolecules. PEGylation is a process in which PEG is bonded to another molecule, which is promising in therapeutic methods. PEG is a nonbiodegradable, hydrophilic polymer widely used in biomedical applications. It has excellent biocompatibility and non-immunogenic and is resistant to protein adsorption. Hydrogels based of PEG are compromising scaffolding materials for regenerating and repairing a variety of tissues because they can give a highly swollen 3D environment similar to soft tissues (Zarrintaj et al. 2020).

3.2.2 Polycaprolactone (PCL)

PCL is a biodegradable polyester, having a melting point ranging between 59 and 64 °C and glass transition temperature of −60 °C. PCL has been widely used in the synthesis of polyurethane because of its appropriate properties like resistance to water, oil, and solvent. Appropriate degradability along with proper biocompatibility introduces PCL as an excellent candidate for biomedical applications mainly as the replacement of hard tissue. PCL has been utilized in drug release and long-term implants, but because of its low mechanical properties and low cell adhesion, it should be blended with other polymers to exhibit the appropriate properties. PCL has been widely used to enhance natural polymers mechanical properties. PCL is a hydrophobic polymer, while natural polymers are hydrophilic; therefore, blending PCL and natural polymers results in phase separation, a defect in polymer blending. Hence, compatibilizer is a crucial factor in PCL usage. PCL exhibits a longer degradation time (2–3 years) and is degraded by microorganisms or by hydrolysis of its aliphatic ester linkage under physiological conditions. Due to the presence of five hydrophobic moieties in its repeating units, PCL degrades slowest among all the biodegradable polyesters (poly-glycolic acid-PGA and polylactic acid -PLA).

3.2.3 Polylactic Acid (PLA)

PLA has been known as a bioplastic in various fields such as packaging and biomedical applications. As a thermoplastic aliphatic polyester, PLA can be derived from different bio- and renewable sources such as starch, corn, and cassava. PLA is widely synthesized through the ring opening polymerization of lactic acid in the presence of the various metal catalysts like octoate. PLA is a semicrystalline polymer, and the chemistry of PLA involves the processing and polymerization of lactic acid monomer. Since lactic acid is a chiral molecule, PLA has stereoisomers, such as poly(L-lactide) (PLLA), poly(D-lactide) (PDLA), and poly(DL-lactide) (PDLLA) (Lopes et al. 2012). PLA is known as a thermal plasticity material which have suitable mechanical properties as biodegradable and biocompatible polymer, thus made the PLA widely used in biomedical engineering. PLA should be blended with other polymers as a consequence of its brittleness to exhibit acceptable performance in biomedical uses. Its main application includes surgical sutures, implants for bone fixation, and drug delivery devices and materials for tissue engineering. In tissue engineering, cells can be grown in a PLA scaffold that is inserted at the site of organ defect. When inserted in vivo, it is able to degrade simply by hydrolysis without any use of enzymes or catalysts; thus, a second surgical removal of implant is deemed unnecessary. PLA is obtained from lactic acid and converted back to the latter one when hydrolytically degraded. Lactic acid is a naturally occurring organic acid that can be produced by fermentation of sugars obtained from renewable resources such as sugarcane. Although there are multiple ways to fabricate PLA, none of them is simple or easy to execute. PLA synthesis requires rigorous control of conditions (temperature, pressure, and pH), the use of catalysts, and long polymerization times, which implies high energy consumption. Nanofibrous PLA materials find wide applicability not only as scaffolds for tissue regeneration but also as drug delivery vehicles, especially when fabricated via electrospinning and also musculoskeletal tissue engineering (Santoro et al. 2017).

3.2.4 Poly Lactic-Co-Glycolic Acid (PLGA)

PLGA is a linear aliphatic copolymer obtained at different proportions between its constituent monomers, lactic acid (LA) and glycolic acid (GA). It can be synthesized with any ratio of LA and GA and molecular weights (Mw) with a wide range from below 10,000 up to 200,000 g/mol. In addition, PLGA can be made in completely amorphous or highly crystalline forms. It has been reported that the polymer with less than 70% LA is amorphous in nature (Jain 2000). The amorphous form shows low mechanical strength and is found to be suitable for drug release; PLGA is categorized to its application forms: scaffolds, fibers, hydrogels, or microspheres; and composite constructs based on PLGA and hydroxyapatite.

3.3 Natural, Synthetic, Natural-Synthetic Polymer Blends

Blends polymer, both natural and synthetic, were made to overcome the drawback of the single polymer (Table 2). Natural polymers cannot solely satisfy biomedical

Table 2 The example of natural, synthetic, natural-synthetic polymer blends

Type of polymer	Blends polymer	Application	References
Natural	Chitosan/agarose/gelatin (CAG)	Cartilage regenerations	Bhat et al. (2011)
Natural	Chitosan/agarose/gelatin (CAG)	Cardiac and skin tissue engineering	Bhat and Kumar (2012)
Synthetic	Poly(azobenzene methacrylate) (PMAzo) and poly(3-O-4-vinylbenzoyl-D-glucopyranose) (PBG)	Drug delivery system	Muñoz-Bonilla and Fernández-García (2015)
Natural and synthetic	PLGA/pluronic F127	Nerve regeneration	Oh et al. (2008)
Natural and synthetic	Gelatin/PVA	Hard tissue regeneration	Kim et.al. (2018)
Natural and synthetic	Gelatin/PLLA	Bone tissue engineering	Liu et.al. (2017)
Natural and synthetic	Poly(6-methacryloyl-α-D-galactopyranose) (polyMG), has been introduced into collagen	Corneal substitution	Deng et.al. (2010)

applications because of mechanical defects; therefore, their blends have been utilized to overcome insufficient mechanical properties. Conveniently, natural polymers need a cross-linking agent to form a hydrogel, which makes them prone to toxicity. The synthetic polymer also cannot individually provide appropriate properties for biomedical applications as well as the natural polymer. In this essence, blending synthetic polymers have been utilized to achieve synergic properties. Moreover, synthetic and natural biocompatible polymer blends can exhibit synergic properties in biomedical applications. The biomimicking scaffold can appropriately regenerate the damaged tissue. A combination of the synthetic compatibilization of polymer blends and natural polymers can emulate the tissue behavior where synthetic polymer enhances the mechanical properties. In contrast, natural polymer provides ECM-like properties.

Finally, currently, a variety of natural and synthetic glycans-based polymer are becoming available in sufficient quantity and purity and therefore are used in a broad range of studies into the regenerative medicine associated with infectious diseases and cancer, developing vaccines, drug delivery, and tissue engineering.

4 Glycoconjugate and Scaffolding Material in Tissue Engineering

Recently, polymers based on glycan scaffolds have gained interest not only for their mechanic properties combined with the biodegradability but also as biomaterials for biomedical applications. Glycan-based polymer scaffolds have been extensively used in the field of tissue engineering. Every year millions of patients suffer the

loss or failure of an organ or tissue due to accident and or disease. Thus, it is crucially needed to find new strategies for treatment, which may give solutions to these patients by using a tissue engineering approach. Polymer scaffolds are one of these kinds of solutions, which is widely used to repair and regenerate tissue as they serve to support, reinforce, and organize regenerating tissue. In some cases, polymers scaffolds also serve to release bioactive substances. To build successfully application of the scaffold in the transplantation, these polymer scaffolds should fulfill specific characteristics, including functional biocompatibility/avoid immune incompatibility, low toxicity, precise three-dimensional microstructure, appropriate mechanical and physical properties, also biodegradability (Li et al. 2018). Recently, some collagen-based 3D scaffold constructs are successfully being used in regenerative medicine. In most cases, their mechanical strength is several orders of magnitude lower than native tissue strength and needs to be improved before they can be used in vivo. Widely recognized problem for TE constructs is their lack of ability to produce and retain PGs and GAGs, essential components of the ECM.

Proteoglycans (PGs) part of glycoconjugate is a significant component of the ECM. Proteoglycans (PGs) and their glycosaminoglycans (GAG) are essential for building the milieu cell environments as they are responsible for many essential functions in development and tissue homeostasis biophysical properties and roles in cell signaling and developing of the extracellular matrix. In the effort to capture these biological functions, a range of biomaterials are designed to incorporate PGs and GAGs, which are typically isolated from animal sources, for tissue engineering, drug delivery, and regenerative medicine applications. Proteoglycan-based biomaterials could imitate the unique, tissue-specific GAG profiles and native GAG presentation in human tissues. PGs' protein core offers biological functionality, including growth factor and extracellular matrix binding domains, as well as sites for protein immobilization. Finally, PGs can be produced as a recombinant protein, which expressed in mammalian cells and thus offers genetic manipulation and metabolic engineering opportunities for control over the protein and GAG structures and functions (Rnjak-Kovacina et al. 2018).

PGs consist of one or more glycosaminoglycan (GAG) chains attached via a link tetrasaccharide to serine residues within a core protein. GAGs are long chains of repetition of disaccharide units that are variably sulfated. There are four main classes of GAGs, including chondroitin sulfate (CS) and dermatan sulfate (DS), hyaluronan (HA), heparan sulfate (HS), and keratan sulfate (KS), of which the only HA does not covalently attach to a PGs core protein via a link tetrasaccharide (Fig. 2). PGs as a group exhibit great structural diversity because each type of PGs may contain several types of GAGs, different numbers and lengths of GAG chains, modifications in the repetitive patterns of the disaccharides by a complex of sulfate groups, and a different core protein structure. PGs can be visualized in monomeric form or can form aggregates by complexing with HA. Both the PGs core protein and the GAG chains play an important role in tissue remodeling, intracellular signaling, uptake of proteins, cell migration, and many other crucial functions in native tissues. PGs are generally categorized according to their location, whether they are located intracellular, cell surface, pericellular, and in the extracellular space (Table 3).

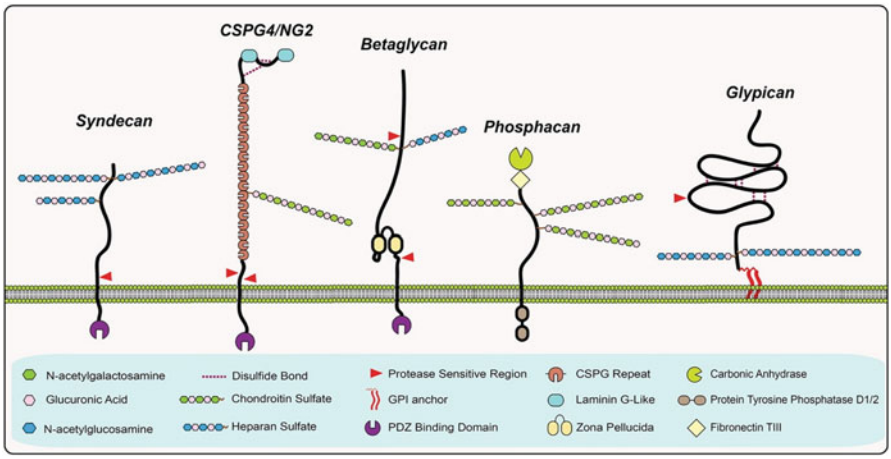


Fig. 2 Schematic representation of the cell surface proteoglycans, which comprise transmembrane type I (the N-terminus is outside of the plasma membrane) proteoglycans (four syndecans, CSPG4/NG2, betaglycan, and phosphacan) and six GPI-anchored proteoglycans, glypicans 1–6. The type of GAG chain and the major protease-sensitive sites are indicated. The key for the various modules is provided in the bottom panel. (Reproduced with permission from the Elsevier, originally Figure 2 in Lozzo, VR, Schaefer L, Proteoglycan form and function: A comprehensive nomenclature of proteoglycans, Matrix Biology. 2009)

PGs production can be stimulated in tissue engineering constructs using growth factors and mechanical stimulation. To promote tissue organization, regeneration, and growth factor binding, PGs have already been incorporated into collagen, GAG, and matrigel scaffolds and have achieved ECM composition that is closer to native tissues than those produced by scaffolds without PGs. PGs also show great promise in the development of engineered tissues to model disease states, such as investigating how the small leucine-rich proteoglycans roles in collagen fibrillogenesis may be altered in connective tissue diseases. Overall, PGs have immense possibilities in the structural development and healing of engineered tissues and should be modulated to achieve native ECM-like composition or to study disease mechanisms.

PGs participate in intracellular signaling by acting as receptors on the cell surface to bind to signaling molecules, such as growth factors, and other proteins. This PG binding can immobilize, block, or delay the release of proteins, such as proteolytic enzymes and proteases, involved in cell migration and tissue remodeling. The GAG chains of the PG bind to proteins present on the cell surface, as well as to many soluble and ECM proteins. There are numerous reports relevant to both native and engineered tissues, which give the prespective of the use of PGs in the production of other ECMs. For example, PGs and their GAG chains have been reported to influence elastic fiber assembly.

Furthermore, many GAG-rich PGs, particularly the hyalectins, will aggregate with HA; this local accumulation of negatively charged GAGs creates an osmotic imbalance that draws in large amounts of water, thus creating stiff supramolecular assemblies that can influence the mechanical characteristics of connective tissues.

Table 3 The type of proteoglycan and glycosaminoglycan based on the location of proteoglycan

Location of Proteoglycans	Proteglycans	Glycosaminoglycans
Intracellular	Serglycin	Chondroitin sulfate/dermatan sulfate
		Heparan sulfate/heparin
Cell surface	Betaglycan	
	Glypicans	
	Phosphacan	
	Syndecans	
Pericellular	Agrin	Heparan sulfate
	Collagen XV	Chondroitin sulfate/heparan sulfate
	Collagen XVIII	Heparan sulfate
	Perlecan	Chondroitin sulfate/heparan sulfate
		Keratan sulfate
Extracellular	Aggrecan	Chondroitin sulfate/keratan sulfate
	Aspirin	
	Biglycan	Chondroitin sulfate
	Brevican	Chondroitin sulfate
	Chondroadherin	
	Decorin	Dermatan sulfate
	ECM2	
	ECMX	
	Epiphycan	Chondroitin sulfate/dermatan sulfate
	Fibromodulin	Keratan sulfate
	Keratocan	Keratan sulfate
	Lubricin	Chondroitin sulfate/keratan sulfate
	Lumican	Keratan sulfate
	Neurocan	Chondroitin sulfate
	Nyctalopin	
	Opticin	
	Osteoadherin	Keratan sulfate
	Osteoglycin	
	Podocan	
	Podocan-like 1	
	PRELP	
	Testicans	Heparan sulfate
	Tsukushi	
	Versican	Chondroitin sulfate
	Proteglycans	Glycosaminoglycans
Intracellular	Serglycin	Chondroitin sulfate/dermatan sulfate
		Heparan sulfate/heparin
Cell surface	Betaglycan	
	CSPG4 (NG2)	
	Glypicans	
	Phosphacan	
	Syndecans	

This advantage of PGs thus concludes that PGs are able to have substantial contributions in tissue-engineered (TE) scaffolds, and they can be exogenously added, or their synthesis stimulated to impart natural tissue-like properties. Many researches have already been conducted with the application of PG and or GAG together with the bio- scaffold in tissue engineering (Table 4).

5 Scaffolds Based on Extracellular Matrix Promote Neural Tissue Regeneration

In recent years, along with increasing life expectancy, brain disorders caused by neurotrauma, stroke ischemia, and/or neurodegenerative diseases are the most challenging medical conditions. Financial losses from this condition throughout the world are very high every year (Gooch et al. 2017; Kusindarta et al. 2018; Shimba et al. 2019). It is estimated that these costs will continue to increase, mainly due to the limitations of effective treatment options. Currently the therapy used is still in the limits to reduce clinical symptoms and not to limit or restore cell loss. In some conditions, such as Parkinson's disease, Alzheimer's disease, treatment is targeted at the performance of certain neurotransmitters such as dopamine, acetylcholine, neuropeptide Y. However, continuous cell loss due to nerve degeneration cannot be reversed as a reversible process. This continuous loss of cells results in tissue atrophy which gradually shrinks brain tissue. In contrast to tissue atrophy, acute brain injury, such as stroke and penetration of traumatic brain injury, results in volumetric tissue loss characterized by cavitation due to cell and matrix loss. Pharmacological therapies, such as neuroprotective agents, are mainly focused on saving acute dying neurons, whereas anti-inflammatory agents target the immune system's response to damage caused, with the aim of reducing secondary tissue damage (Neuhaus et al. 2017).

Conventionally, autologous grafts are the gold standard and have been used to treat nerve defects (Amilo et al. 1995; Nikkhah et al. 1997). However, autografts have limitations that include lack of nerve material because they are taken from patients. In addition, there is a mismatch of donor-site nerve size with the recipient site, apart from that the possibility of human leucocyte antigen (HLA) mismatch is also a consideration. Autograft can also cause neuroma formation and lack of functional recovery from nerves. Allogeneic grafts, which are isolated from corpses, are not limited by supplies but immunity factors remain a problem that causes rejection of allograft (Subramanian et al. 2009). To overcome immune rejection, several studies have been conducted to examine the potential of acellular nerve graft. However, this method is constrained by delayed nerve regeneration and extracellular matrix remodeling.

Because of the generally significant increase in the population with aging and the consequences of an increased incidence of neurodegenerative diseases, the development of therapies, which can help and replace cells damaged by neurodegeneration, is the highest priority (Kusindarta et al. 2018). The brain's extracellular matrix (ECM) is a macromolecular composed of polysaccharides and proteins that occupy the space

Table 4 Several research applications in the use of combination glycan biomaterial and the synthetic polymer in tissue engineering

Biomaterial	Application	References
Collagen in corporation to glycosaminoglycan/ chondroitin sulfate	Nerve and bone regeneration	Stang et al. (2005)
Matrigel together with perlecan	Cardiac and vascular tissue	Abilez et al. (2006)
Lumican peptide covalently attached to a hexadecyl lipid to produce a self-assembling peptide	Epithelial	Hamley et al. (2015)
Decorin peptide, KLER, and RGD copolymerized with PEG	Cartilage	Salinas et al. (2013)
A dermatan sulfate chain attached to a collagen binding peptide inspired by decorin incorporated into collagen type I scaffolds through collagen fibrillogenesis	Vascular	Paderi et al. (2011)
A chondroitin sulfate chain modified with hyaluronic acid(HA)-binding peptides inspired by aggrecan bound to HA incorporated into collagen type I scaffolds through collagen fibrillogenesis	Cartilage	Bernhard and panitch (2012), Sharma et al. (2013)
A dermatan sulfate chain modified with HA-binding peptides inspired by decorin in an HA vehicle	Dermal	Stuart et al. (2011)
Bovine cartilage-derived aggrecan decorated with chondroitin sulfate incorporated into agarose-poly (ethylene glycol) diacrylate interpenetrating network hydrogels	Cartilage	Ingavle et al. (2013)
Titanium coated with collagen type II fibrils with bound bovine decorin or biglycan each decorated with chondroitin sulfate/dermatan sulfate	Bone	Douglas et al. (2007, 2008)
Perlecan isolated from human endothelial cells decorated with heparan sulfate physisorbed onto ePTFE	Vascular graft	Lord et al. (2009)
Collagen and gelatin electrospun scaffolds crosslinked with glutaraldehyde surface functionalized with perlecan domain I		Casper et al. (2007)
Perlecan domain I covalently immobilized on HA-based, microscopic hydrogel particles via a PEG linker		Srinivasan et al. (2012)
Perlecan domain V decorated with chondroitin sulfate and heparan sulfate physisorbed or covalently bound to silk	Vascular	Rnjak-Kovacina et al. (2016)
Perlecan domain I DNA delivered in a lyophilized single-phase tricalcium phosphate scaffold for local expression of perlecan domain I decorated with heparan sulfate and chondroitin sulfate	Bone	DeCarlo et al. (2012)
Perlecan domain I and VEGF189 transgene delivered in a chitosan scaffold for local expression of perlecan domain I decorated with heparan sulfate and chondroitin sulfate and VEGF189	Angiogenesis and wound healing	Lord et al. (2017)

between neurons and glia and accounts for about 20% of the total volume in the adult brain. ECM is synthesized by neurons and glial cells, meanwhile, brain ECM is mainly composed of glycoconjugate such as glycosaminoglycans (e.g., hyaluronan), proteoglycans (e.g., neurocan, brevican, versican, and aggrecan), glycoprotein (e.g., tenascin-R). In addition, there are also fibrous proteins in ECM such as collagen, fibronectin, and vitronectin. Structurally, ECM acts as a physical barrier to reduce the diffusion of soluble and membrane-related molecules and cell migration. ECM also functions to regulate a number of primary neural processes during brain development and can play a role in physiological and pathological conditions in the adult brain, including neuronal growth, synaptogenesis, synaptic stabilization, and injury-related plasticity. ECM, therefore, is an important target for the development of therapies for central nervous system disorders, which involve progressive neurodegeneration.

A tissue engineering approach using a transplant mechanism for replacing neurons with scaffolds composed of extracellular matrix has begun to be developed by targeting the stimuli for the formation of the new neurons. The use of biomaterials and polymers has proven to be an important strategy for maximizing the success of cell transplants. The success of cell transplantation that ultimately leads to neurogenesis is a very complex process that depends on: (a) sending or migrating stem sets into areas where cells can interact, (b) induction of signaling molecules, and (c) angiogenesis and vascularization. Ideal scaffold cells are required to be physiochemically similar to the surrounding tissue, nontoxic, do not stimulate immunogenic reactions, biodegradable, suitable for various structures, promote cell adhesion and proliferation, and better contact for neuronal growth, able to stimulate endogenous stem cells and highly porous to allow increased density when cell seeding processes occurs (Fig. 3) (Subramanian et al. 2009). Scaffold cells occur in various forms, such as hydrogels, sponges, membranes, and tubules that contain channels. These scaffold cells are made based on natural or synthetic matrix components.

Scaffolds based on extracellular matrix made from natural components of protein and glycoconjugate are generally very biocompatible and easily available. Some examples of natural components that are often used include collagen, hyaluronic acid, alginate, agarose, fibronectin, chitosan, and fibrin. Several studies have reported the use of collagen as a base for scaffold cells, combined with neurotropic factors such as Brain derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3); these scaffold cells are able to stimulate axonal regeneration and partial functional recovery after transection of the spinal cord (Han et al. 2010). The combination of collagen with the addition of neuronal stem cells (NSCs) also shows improvement in remyelination and recovery in mouse models (Hatami et al. 2009). Fibrin, a natural component of blood clotting, can also be used as a base for injected or biodegradable scaffold cells. Fibrin has the ability to increase the migration of nerve support cells and stimulate the expression of NSCs (Johnson et al. 2010). Scaffold cells using hyaluronic acid show the ability to inhibit lymphocytes and astrocytes. Hyaluronic acid-based scaffold has been shown to reduce glial scarring (Wei et al. 2010). Whereas the application of alginate, agarose, and chitosan in scaffold cells proved to be able to stimulate the expression of growth factors (Barminko et al. 2011).

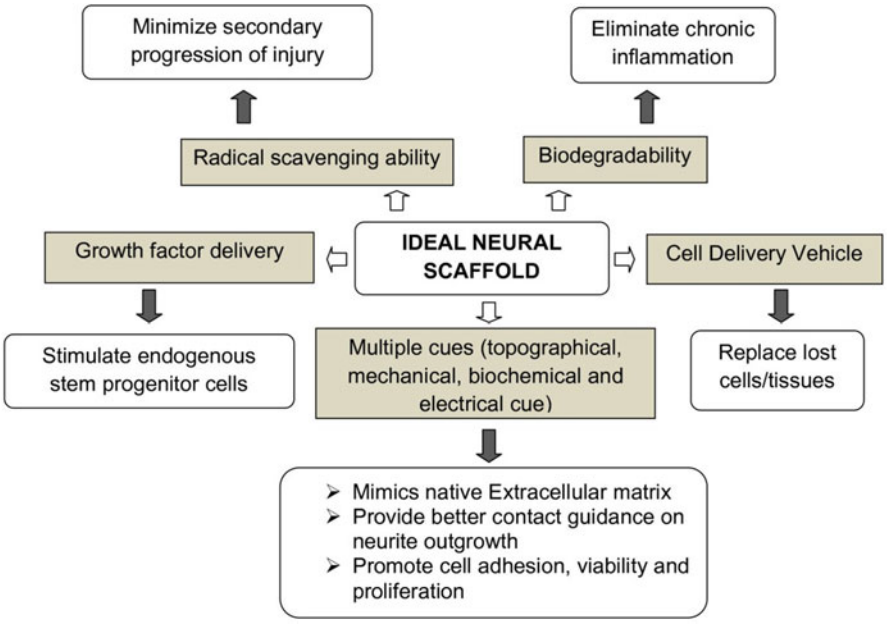


Fig. 3 The properties for ideal neural scaffold. (Reproduced with permission from the Springer Nature, originally Figure 1 in Subramanian A, Krishnan UM, Sethuraman S, Development of biomaterial scaffold for nerve tissue engineering: Biomaterial mediated neural regeneration, Journal of Biomedical Science. 2009)

Synthetic scaffold materials are generally developed with the intention of making matrices with structural characteristics that resemble those of the original ECM. Although two-dimensional fibrillar substrates can cause distorted cellular forms and behavior, they have proven quite successful in neural network engineering approaches where they have been able to facilitate CNS regeneration (Lutolf and Hubbell 2005). Making synthetic ECM offers several advantages including: (1) this technology allows the manufacture of fibrillar biomaterials from the nanometer scale (nanoparticles) through polymer processing or supramolecular assembly; (2) the characteristic of natural hydrogel ECM can be copied physico-chemically by synthetic hydrogel. Synthetic hydrogels can be formed in situ, which makes the application of hydrogels very attractive for cell-containing hydrogels, which require special experimental protocols (Lutolf and Hubbell 2005). (3) The possibility of combining bioactive signals in fibrillary and hydrogel-based nerve ECM makes it a highly prospective scaffold material for tissue regeneration; (4) synthetic matrices allow the creation of two types of matrices that can be degraded or not degraded. It is now known that several materials are used as basic of synthetic scaffold in the neuroengineering including: polyethylene glycol (PEG), lactide- and glycolide-derived polyesters (Sakiyama-Elbert et al. 2012), polycaprolactones (Silva et al. 2010), Poly (2-hydroxyethyl Methacrylate), NeuroGel™ (N- (2-hydroxypropyl) methacrylamide or HPMA), poly lactic-co-glycolic acid (PLGA), poly ε-caprolactone (PCL), poly L-lactic acid (PLLA),

and conducting polymers (polypyrrole, polyaniline) (Subramanian et al. 2009). In addition, interesting research also proves fluorescent loaded polymeric nanoparticle and lipid-anchored GAG-mimicking glycopolymers (lipo-pSGF) showed an activity in promoting neural differentiation on the mouse model (Liu et al. 2017) (Fig. 4).

In addition to manipulating intrinsic extracellular matrices to produce an environment that supports nerve regeneration, alternative strategies involve the use of nano-size scaffolds that are made artificially to overcome the body's natural barriers to repair. The use of tissue-engineered scaffolds offers a way to solve the unavailability of CNS regeneration capacity to reconstruct cavities that form due to of brain injury because of neurodegeneration, thus may reconnect neuronal processes. The function

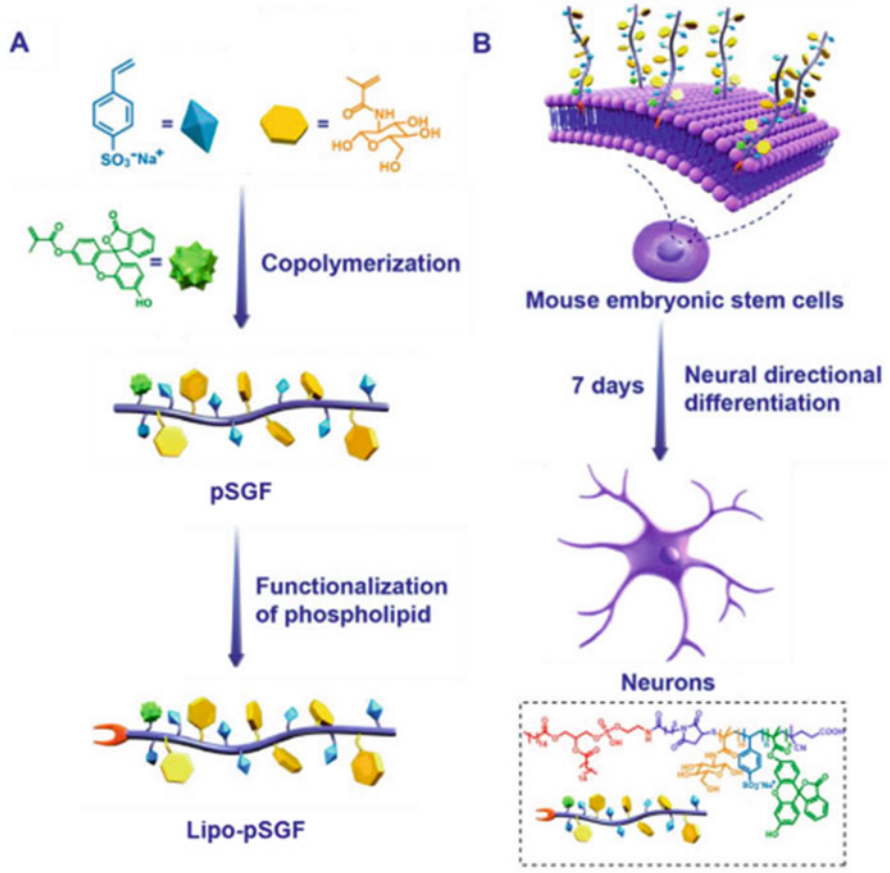


Fig. 4 Synthesis of the lipid-anchored biomimetic GAGs (Lipo-pSGF) (**a**) and their cooperation integration into cell membranes in the central nervous system and promoting mouse embryonic stem cells differentiation into neurons (**b**). (Reproduced with permission from the American Chemical Society, originally Figure 1 in Liu Q, Lyu Z, Yu Y, Zhao Z, Hu S, Yuan L, Chen G, Chen H, Synthetic Glycopolymers for Highly Efficient Differentiation of Embryonic Stem Cells into Neurons: Lipo- or not?, Applied Materials and Interface. 2017)

of an artificial scaffold to improve communication between cells allows for increased proliferation, migration, and differentiation. Studies have shown the efficacy of scaffold in CNS (Huang et al. 2012), but regeneration and functional recovery in mouse models that have lesions have been enhanced by the addition of other substrates such as vascular endothelial growth factor (Zhang et al. 2007) or hyaluronic acid with laminin. Transplantation can help neuro-regeneration not only by differentiating to replace damaged or degenerative cell types (Zhu et al. 2019), but also by removing various neurotropic factors such as nerve growth factors and brain-derived neurotropic factors (BDNF) (Lu and Tuszynski 2008) and by inhibiting T cell activation, thereby preventing further injury (Bacigaluppi et al. 2009). Schwann cells stimulate regeneration of the central nervous system by naturally expressing various surface adhesion molecules and growth factors and by producing components of ECM, laminin and fibronectin. Recently, ECM scaffolds formed from nature protein (type 1 collagen) and glycan have already been successfully used widely in peripheral nerve (Prest et al. 2018), peripheral nervous system (Prest et al. 2018), spinal cord (Tukmachev et al. 2016), as well as the brain (Faust et al. 2017).

6 Glycopolimer on Cancer Immunotherapy

In a recent study, glycopolymers reported playing an essential role in immunology. In particular, cancer-based immunotherapy uses the immune system to eliminate cancer cells. This process is programming to allow immune cells to trap cancer cells as the target accurately. The development of artificial glycocalyx based on self-assembled glyco-nanoparticles called glyco-NPs was given an ability to reverse the immunosuppressive phenotype, which impairs the antitumor immune response (Su et al. 2015). Then, the enhanced immune-function of the macrophage may promote tumor immunotherapy. The reversal of the immunosuppressive phenotype is controlled by macrophage polarization caused by the synthesized glycopolymer. As an additional example of anticancer immunotherapy, two synthetic glycopolymers, poly(N-methacryloylglucosamine) (pMAG) and poly(N-methacryloylmannosamine) (pMAM), have been used to create engineered tumor cell membranes. In this example, tumor cell membranes were engineered with pMAG and pMAM. pMAG and pMAM function as binding sites allowing selective recognition to macrophage lectins, such as a mannose receptor (MR), and complement receptor three (CR3). The binding, between pMAG-MR and pMAM-CR3, triggers immune responses, which lead to the elimination of the tumor cells. These examples of glycopolymer application in immunology would offer strong potential in the development of immunotherapy for cancer treatment.

7 Conclusion and Outlook

Taken together, all this current knowledge of methodologies on the synthesis of glycoconjugate-based polymers has a great potential for future studies to enhance their specific recognition properties and to develop the best therapeutic and

biological agents in tissue engineering and regenerative medicine. It is believed that in the next decades, interdisciplinary approaches on the designed and synthesis glycopolymer based on nature, synthetic, or mix of nature and synthetic on the medical or pharmaceutical applications for therapeutic purposes will be greatly revolutionizing due to the medical treatments needed.

References

Abilez O, Benharash P, Mehrotra M, Miyamoto E, Gale A, Picquet J, Xu C, Zarins C. A novel culture system shows that stem cells can be grown in 3D and under physiologic pulsatile conditions for tissue engineering of vascular grafts. J Surg Res. 2006;132(2):170–8. https://doi.org/10.1016/j.jss.2006.02.017.

Alaribe FN, Manoto SL, Motaung SCKM. Scaffolds from biomaterials: advantages and limitations in bone and tissue engineering. Biologia. 2016;71(4):353–66. https://doi.org/10.1515/biolog-2016-0056.

Amilo S, Yanez R, Barrios RH. Nerve regeneration in different types of grafts: experimental study in rabbits. Microsurg 1995;16:621–630.

Bacigaluppi M, Pluchino S, Peruzzotti-Jametti L, Kilic E, Kilic U, Salani G, Brambilla E, West MJ, Comi G, Martino G, Hermann DM. Delayed post-ischaemic neuroprotection following systemic neural stem cell transplantation involves multiple mechanisms. Brain. 2009;132(Pt 8):2239–51. https://doi.org/10.1093/brain/awp174.

Barminko J, Kim JH, Otsuka S, Gray A, Schloss R, Grumet M, Yarmush ML. Encapsulated mesenchymal stromal cells for in vivo transplantation. Biotechnol. Bioeng. 2011;108:2747–2758.

Barthes J, Özçelik H, Hindié M, Ndreu-halili A, Vrana NE. Cell microenvironment engineering and monitoring for tissue engineering and regenerative medicine: the citation accessed citable link cell microenvironment engineering and monitoring for tissue engineering and regenerative medicine: the recent advances. Biomed Res Int. 2014. https://doi.org/10.1155/2014/921905.

Bernhard JC, Panitch A. Synthesis and characterization of an aggrecan mimic. Acta Biomater. 2012;8(4):1543–50. https://doi.org/10.1016/j.actbio.2011.12.029. Epub 2011 Dec 29. PMID: 22248525.

Bhat S, Kumar A. Cell proliferation on three-dimensional chitosaneagaroseegelatin cryogel scaffolds for tissue engineering applications. J Biosci Bioeng. 2012;114(6):663–70. https://doi.org/10.1016/j.jbiosc.2012.07.005.

Bhat S, Tripathi A, Kumar A. Supermacroprous chitosan-agarose-gelatin cryogels: in vitro characterization and in vivo assessment for cartilage tissue engineering. J R Soc Interface. 2011;8(57):540–54. https://doi.org/10.1098/rsif.2010.0455. Epub 2010 Oct 13. PMID: 20943683; PMCID: PMC3061127.

Casper CL, Yang W, Farach-Carson MC, Rabolt JF. Coating Electrospun Collagen and Gelatin Fibers with Perlecan Domain I for Increased Growth Factor Binding. Biomacromolecules. 2007;8(4):1116–1123. https://doi.org/10.1021/bm061003s.

Chen F-M, Liu X, Polym P, Author S. Advancing biomaterials of human origin for tissue engineering HHS Public Access Author manuscript. Prog Polym Sci. 2016;53:86–168. https://doi.org/10.1016/j.progpolymsci.2015.02.004.

DeCarlo A, Belousova M, Ellis AL, Petersen D, Grenett H, Hardigan P, O'Grady R, Lord M, Whitelock JM. Perlecan domain 1 recombinant proteoglycan augments BMP-2 activity and osteogenesis. BMC Biotechnol. 2012;12(60). https://doi.org/10.1186/1472-6750-12-60.

Deng C, Li F, Hackett JM, Chaudhry SH, Toll FN, Toye B, Hodge W, Griffith M. Collagen and glycopolymer based hydrogel for potential corneal application. Acta Biomater. 2010;6(1):187–94. https://doi.org/10.1016/j.actbio.2009.07.027.

Djerbal L, Lortat-Jacob H, Kwok J. Chondroitin sulfates and their binding molecules in the central nervous system. Glycoconj J. 2017;34(3):363–76. https://doi.org/10.1007/s10719-017-9761-z.

Douglas T, Heinemann S, Hempel U, Mietrach C, Knieb C, Bierbaum S, Worch H. Characterization of collagen II fibrils containing biglycan and their effect as a coating on osteoblast adhesion and proliferation. J Mater Sci-Mater M. 2007;19(4):1653–1660. https://doi.org/10.1007/s10856-007-3250-z.

Douglas T, Hempel U, Mietrach C, Viola M, Vigetti D, Heinemann S, Worch H. Influence of collagen-fibril-based coatings containing decorin and biglycan on osteoblast behavior. J Biomed Mater Res Part A. 2008;84A(3):805–816. https://doi.org/10.1002/jbm.a.31501.

Dwivedi R, Kumar S, Pandey R, et al. Polycaprolactone as biomaterial for bone scaffolds: Review of literature. J Oral Biol Craniofac Res. 2020;10(1):381–388. https://doi.org/10.1016/j.jobcr.2019.10.003.

Eltom A, Zhong G, Muhammad A. Scaffold techniques and designs in tissue engineering functions and purposes: a review. Adv Mater Sci Eng. 2019. https://doi.org/10.1155/2019/3429527.

Faust A, Kandakatla A, Van Der Merwe Y, Ren T, Huleihel L, Hussey G, et al. Urinary bladder extracellular matrix hydrogels and matrixbound vesicles differentially regulate central nervous system neuron viability and axon growth and branching. J Biomater Appl. 2017;31:1277–95. https://doi.org/10.1177/0885328217698062.

Fernandes H, Moroni L, van Blitterswijk C, de Boer J. Extracellular matrix and tissue engineering applications. J Mater Chem. 2009;19(31):5474. https://doi.org/10.1039/b822177d

Gooch CL, Pracht E, Borenstein AR. The burden of neurological disease in the United States: a summary report and call to action. Ann Neurol. 2017;81:479–84. https://doi.org/10.1002/ana.24897.

Hamley IW, Dehsorkhi A, Castelletto V, Walter MN, Connon CJ, Reza M, Ruokolainen J. Self-Assembly and Collagen-Stimulating Activity of a Peptide Amphiphile Incorporating a Peptide Sequence from Lumican. Langmuir. 2015;31(15):4490–5. https://doi.org/10.1021/acs.langmuir.5b00057. Epub 2015 Apr 13. PMID: 25835126.

Han Q, Jin W, Xiao Z, Ni H, Wang J, Kong J, Wu J, Liang W, Chen L, Zhao Y, Chen B, Dai J. The promotion of neural regeneration in an extreme rat spinal cord injury model using a collagen scaffold containing a collagen binding neuroprotective protein and an EGFR neutralizing antibody. Biomaterials. 2010;31(35):9212–20. https://doi.org/10.1016/j.biomaterials.2010.08.04.

Hatami M, Mehrjardi NZ, Kiani S, Hemmesi K, Azizi H, Shahverdi A, Baharvand H. Human embryonic stem cell-derived neural precursor transplants in collagen scaffolds promote recovery in injured rat spinal cord. Cytotherapy. 2009;11(5):618–30.

Huang KF, Hsu WC, Chiu WT, Wang JY. Functional improvement and neurogenesis after collagen-GAG matrix implantation into surgical brain trauma. Biomaterials. 2012;33(7):2067–75. https://doi.org/10.1016/j.biomaterials.2011.11.040.

Ikada Y. Challenges in tissue engineering. J R Soc Interface. 2006;3(10):589–601. https://doi.org/10.1098/rsif.2006.0124.

Ingavle GC, Frei AW, Gehrke SH, Detamore MS. Incorporation of aggrecan in interpenetrating network hydrogels to improve cellular performance for cartilage tissue engineering. Tissue Eng Part A. 2013;19(11–12):1349–1359. https://doi.org/10.1089/ten.TEA.2012.0160.

Jain RA. The manufacturing techniques of various drug loaded biodegradable poly(lactide-*co*-glycolide) (PLGA) devices. Biomaterials. 2000;21(23):2475–90. https://doi.org/10.1016/S0142-9612(00)00115-0.

Jakab L. A heterogén glikoproteinszerkezetek biológiai szerepe. Orv Hetil. 2016;157(30):1185–92. https://doi.org/10.1556/650.2016.30436.

Johnson PJ, Tatara A, McCreedy DA, Shiu A, Sakiyama-Elbert SE. Tissue-engineered fibrin scaffolds containing neural progenitors enhance functional recovery in a subacute model of SCI. Soft Matter. 2010;6(20):5127–37. https://doi.org/10.1039/c0sm00173b.

Kamiya Y, Satoh T, Kato K. Recent advances in glycoprotein production for structural biology: toward tailored design of glycoforms. Curr Opin Struct Biol. 2014;26:44–53. https://doi.org/10.1016/j.sbi.2014.03.008. Epub 2014 May 20. PMID: 24841384.

Kim H, Yang GH, Choi CH, Cho YS, Kim G. Gelatin/PVA scaffolds fabricated using a 3D-printing process employed with a low-temperature plate for hard tissue regeneration: fabrication and characterizations. Int J Biol Macromol. 2018;120:119–27. https://doi.org/10.1016/j.ijbiomac.2018.07.159.

Komi DEA, Hamblin MR. Chitin and chitosan: production and application of versatile biomedical nanomaterials. Int J Adv Res (Indore). 2016;4(3):411–27.

Kusindarta DL, Wihadmadyatami H, Jadi AR, Karnati S, Lochnit G, Hening P, Haryanto A, Auriva MB, Purwaningrum M. Ethanolic extract Ocimum sanctum. Enhances cognitive ability from young adulthood to middle aged mediated by increasing choline acetyl transferase activity in rat model. Res Vet Sci. 2018;118:431–8. https://doi.org/10.1016/j.rvsc.2018.04.005.

Li Q, Niu Y, Xing P, Wang C. Bioactive polysaccharides from natural resources including Chinese medicinal herbs on tissue repair. Chin Med. 2018;13(1):1–11. https://doi.org/10.1186/s13020-018-0166-0.

Liu M, Zeng X, Ma C. et al. Injectable hydrogels for cartilage and bone tissue engineering. Bone Res. 2017;5:17014. https://doi.org/10.1038/boneres.2017.14.

Liu Q, Lyu Z, Yu Y, Zhao Z, Hu S, Yuan L, Chen G, Chen H. Synthetic glycopolymers for highly efficient differentiation of embryonic stem cells into neurons: Lipo- or not? Appl Mater Interface. 2017;9(13):11518–27. https://doi.org/10.1021/acsami.7b01397.

Lopes M, Jardini A, Filho R. Poly (Lactic Acid) production for tissue engineering applications. Procedia Engineering. 2012;42:1402–1413. https://doi.org/10.1016/j.proeng.2012.07.534.

Lord MS, Yu W, Cheng B, Simmons A, Poole-Warren L, Whitelock JM. The modulation of platelet and endothelial cell adhesion to vascular graft materials by perlecan. Biomater. 2009;30(28):4898–906. https://doi.org/10.1016/j.biomaterials.2009.05.063. Epub 2009 Jun 21. PMID: 19540587.

Lord MS, Ellis AL, Farrugia BL, Whitelock JM, Grenett H, Li C, O'Grady RL, DeCarlo AA. Perlecan and vascular endothelial growth factor-encoding DNA-loaded chitosan scaffolds promote angiogenesis and wound healing. J Control Release. 2017;250:48–61. https://doi.org/10.1016/j.jconrel.2017.02.009. Epub 2017 Feb 9. PMID: 28189628.

Lu P, Tuszynski MH. Growth factors and combinatorial therapies for CNS regeneration. Exp Neurol. 2008;209(2):313–320. https://doi.org/10.1016/j.expneurol.2007.08.004.

Lutolf MP, Hubbell JA. Synthetic biomaterials as instructive extracellular microenvironments for morphogenesis in tissue engineering. Nat Biotechnol. 2005;23(1):47–55. https://doi.org/10.1038/nbt1055.

Mandrycky C, Phong K, Zheng Y. Tissue engineering toward organ-specific regeneration and disease modeling. MRS Commun. 2017;7(3):332–47. https://doi.org/10.1557/mrc.2017.58.

Miura Y. Synthesis and biological application of glycopolymers. J Polym Sci A Polym Chem. 2007;45:5031–6. https://doi.org/10.1002/pola.22369.

Muñoz-Bonilla A, Fernández-García M. Glycopolymeric materials for advanced applications. Materials. 2015;8(5):2276–96. https://doi.org/10.3390/ma8052276.

Neuhaus AA, Couch Y, Hadley G, Buchan AM. Neuroprotection in stroke: the importance of collaboration and reproducibility. Brain. 2017;140(8):2079–92. https://doi.org/10.1093/brain/awx126.

Nikkhah G, Carvalho GA, Samii M. Nerve transplantation and neurolysis of the brachial plexus after post-traumatic lesions. Orthopade 1997;26:612–620.

O'Brien FJ. Biomaterials & scaffolds for tissue engineering. Materials Today. 2011;14(3):88–95. https://doi.org/10.1016/S1369-7021(11)70058-X.

Oh SH, Kim JH, Song KS, Jeon BH, Yoon JH, Seo TB, Namgung U, Lee IW, Lee JH. Peripheral nerve regeneration within an asymmetrically porous PLGA/Pluronic F127 nerve guide conduit. Biomaterials. 2008;29(11):1601–9. https://doi.org/10.1016/j.biomaterials.2007.11.036.

Paderi J, Stuart K, Snyder PW, Freeman L, Panitch A. Collagen-binding peptidoglycans inhibit MMP mediated collagen degradation and reduce dermal scarring. PLoS One. 2011;2011(6):e22139.

Polo-Corrales L, Latorre-Esteves M, Ramirez-Vick JE. Scaffold design for bone regeneration. J Nanosci Nanotechnol. 2014;14(1):15–56. https://doi.org/10.1166/jnn.2014.9127.

Pramudya I, Chung H. Recent Progress of Glycopolymer Synthesis for Biomedical Applications. Biomaterials Science. 2019. https://doi.org/10.1039/c9bm01385g.

Prest TA, Yeager E, Lopresti ST, Zygelyte E, Martin MJ, Dong L, et al. Nerve-specific, xenogeneic extracellular matrix hydrogel promotes recovery following peripheral nerve injury. J Biomed Mater Res A. 2018;106:450–9. https://doi.org/10.1002/jbm.a.36235.

Reily C, Stewart TJ, Renfrow MB, Novak J. Glycosylation in health and disease. Nature Reviews Nephrology. 2019. https://doi.org/10.1038/s41581-019-0129-4.

Rnjak-Kovacina J, Tang F, Whitelock JM, Lord MS. Silk biomaterials functionalized with recombinant domain V of human perlecan modulate endothelial cell and platelet interactions for vascular applications. Colloids and Surfaces B: Biointerfaces. 2016;148:130–138. https://doi.org/10.1016/j.colsurfb.2016.08.

Rnjak-Kovacina J, Tang F, Whitelock JM, Lord MS. Glycosaminoglycan and Proteoglycan-Based Biomaterials: Current Trends and Future Perspectives. Adv Healthc Mater. 2018;7(6): e1701042. https://doi.org/10.1002/adhm.201701042. Epub 2017 Dec 6. PMID: 29210510.

Sakiyama-Elbert S, Johnson PJ, Hodgetts SI, Plant GW, Harvey AR. Scaffolds to promote spinal cord regeneration. Handb Clin Neurol. 2012;109:575–94. https://doi.org/10.1016/B978-0-444-52137-8.00036-X.

Salinas A, Esbrit P, Vallet-Regí M. A tissue engineering approach based on the use of bioceramics for bone repair. Biomaterials Science. 2013;1:40. https://doi.org/10.1039/c2bm00071g.

Santoro M, Menegaz BA, Lamhamedi-Cherradi SE, et al. Modeling Stroma-Induced Drug Resistance in a Tissue-Engineered Tumor Model of Ewing Sarcoma. Tissue Eng Part A. 2017;23(1–2):80–89. https://doi.org/10.1089/ten.TEA.2016.0369.

Saraswathy N, Ramalingam P. Glycoproteomics. In: Concepts and techniques in genomics and proteomics; 2011. p. 213–8. https://doi.org/10.1533/9781908818058.213.

Sasaki, N, Toyoda, M. Glycoconjugates and related molecules in human vascular endothelial cells. J Vasc Med. 2013. https://doi.org/10.1155/2013/963596.

Sharma S, Lee A, Choi K, Kim K, Youn I, Trippel SB, Panitch A. Biomimetic aggrecan reduces cartilage extracellular matrix from degradation and lowers catabolic activity in ex vivo and in vivo models. Macromol Biosci. 2013;13(9):1228–37. https://doi.org/10.1002/mabi.201300112. Epub 2013 Jul 8. PMID: 23836445.

Shimba K, Chang CH, Asahina T, Moriya F, Kotani K, Jimbo Y, Gladkov A, Antipova O, Pigareva Y, Kolpakov V, Mukhina I, Kazantsev V, Pimashkin A. Functional scaffolding for brain implants: engineered neuronal network by microfabrication and iPSC technology. Front Neurosci. 2019;13:1–6. https://doi.org/10.3389/fnins.2019.00890.

Silva MLAD, Crawford A, Mundy JM, Correlo VM, Sol P, Bhattacharya M, Hatton PV, Reis RL, Neves NM. Chitosan/polyester-based scaffolds for cartilage tissue engineering: assessment of extracellular matrix formation. Acta Biomater. 2010;6(3):1149–57. https://doi.org/10.1016/j.actbio.2009.09.006. Epub 2009 Sep 27. PMID: 19788942.

Srinivasan PP, McCoy SY, Jha AK, Yang WD, Jia XQ, Farach-Carson MC, Kirn-Safran CB. Injectable perlecan domain 1-hyaluronan microgels potentiate the cartilage repair effect of BMP2 in a murine model of early osteoarthritis. Biomed Mater. 2012;7(2):024109. https://doi.org/10.1088/1748-6041/7/2/024109.

Stang F, Fansa H, Wolf G, Reppin M, Keilhoff G. Structural parameters of collagen nerve grafts influence peripheral nerve regeneration. Biomaterials. 2005;26(16):3083–91. https://doi.org/10.1016/j.biomaterials.2004.07.060.

Stegemann JP, Kaszuba SN, Rowe SL. Review: advances in vascular tissue engineering using protein-based biomaterials. Tissue Eng. 2007;13(11):2601–13. https://doi.org/10.1089/ten.2007.0196. PMID: 17961004; PMCID: PMC2257983.

Stuart K, Paderi J, Snyder PW, Freeman L, Panitch A. Collagen-binding peptidoglycans inhibit MMP mediated collagen degradation and reduce dermal scarring. PLoS One. 2011;6(7):e22139. https://doi.org/10.1371/journal.pone.0022139.

Su L, Zhang W, Wu X, Zhang Y, Chen X, Liu G, Chen G, Jiang M. Glycocalyx-mimicking nanoparticles for stimulation and polarization of macrophages via specific interactions. Small. 2015;11(33):4191–200. https://doi.org/10.1002/smll.201403838.

Subramanian A, Krishnan UM, Sethuraman S. Development of biomaterial scaffold for nerve tissue engineering: biomaterial mediated neural regeneration. J Biomed Sci. 2009;16(1):108. https://doi.org/10.1186/1423-0127-16-108.

Tukmachev D, Forostyak S, Koci Z, Zaviskova K, Vackova I, Vyborny K, Sandvig I, Sandvig A, Medberry CJ, Badylak SF, Sykova E, Kubinova S. Injectable Extracellular Matrix Hydrogels as Scaffolds for Spinal Cord Injury Repair. Tissue Eng Part A. 2016;22(3–4):306–17. https://doi.org/10.1089/ten.TEA.2015.0422. PMID: 26729284; PMCID: PMC4799710.

Varki A, Cummings RD, Esko JD, Freeze HH, Stanley P, Bertozzi CR, Hart GW, Etzler ME. Overview of glycoconjugate analysis. Curr Protoc Protein Sci. 2009;12. https://doi.org/10.1002/0471140864.ps1201s57.

Wei Y-T, He Y, Xu C-L, Wang Y, Liu B-F, Wang X-M, Xu Q-Y. Hyaluronic acid hydrogel modified with nogo-66 receptor antibody and poly-L-lysine to promote axon regrowth after spinal cord injury. Journal of Biomedical Materials Research Part B: Applied Biomaterials. 2010;95B (1):110–117. https://doi.org/10.1002/jbm.b.31689.

Welsh KJ, Kirkman MS, Sacks DB. Role of glycated proteins in the diagnosis and management of diabetes: research gaps and future directions. Diabetes Care. 2016;39(8):1299–306. https://doi.org/10.2337/dc15-2727.

Weyers A, Linhardt RJ. Neoproteoglycans in tissue engineering. FEBS J. 2013;280(10):2511–22. https://doi.org/10.1111/febs.12187.

Williams DF. Challenges with the development of biomaterials for sustainable tissue engineering. Front Bioeng Biotechnol. 2019;7(1–10). https://doi.org/10.3389/fbioe.2019.00127.

Xu Y, Chen C, Hellwarth PB, Bao X. Biomaterials for stem cell engineering and biomanufacturing. Bioact Mater. 2019;4:366–79. https://doi.org/10.1016/j.bioactmat.2019.11.002.

Zarrintaj P, Mahmodi G, Manouchehri S, Mashhadzadeh AH, Khodadadi M, Servatan M, Mozafari M. Zeolite in tissue engineering: Opportunities and challenges. MedComm. 2020. https://doi.org/10.1002/mco2.5.

Zhang Y, Song J, Shi B, Wang Y, Chen X, Huang C, Yang X, Xu D, Cheng X, Chen X. Combination of scaffold and adenovirus vectors expressing bone morphogenetic protein-7 for alveolar bone regeneration at dental implant defects. Biomaterials. 2007;28(31):4635–42. https://doi.org/10.1016/j.biomaterials.2007.07.009. Epub 2007 Jul 30. PMID: 17664001.

Zhu B, Eom J, Hunt RF. Transplanted interneurons improve memory precision after traumatic brain injury. Nature Communications. 2019;10(1). https://doi.org/10.1038/s41467-019-13170-w.

3D Printing of Microbial Polysaccharides

50

V. Nalbantova, P. Lukova, G. Pierre, N. Benbasat, P. Katsarov, P. J. P. Espitia, C. A. Fuenmayor, A. Nesic, M. S. Carranza, P. Michaud, and C. Delattre

Contents

V. Nalbantova · P. Lukova
Department of Pharmacognosy and Pharmaceutical Chemistry, Medical University-Plovdiv, Plovdiv, Bulgaria
e-mail: vanya.nalbantova@mu-plovdiv.bg; paolina.lukova@mu-plovdiv.bg

G. Pierre · P. Michaud
Université Clermont Auvergne, Clermont Auvergne INP, CNRS, Institut Pascal, Clermont-Ferrand, France
e-mail: guillaume.pierre@uca.fr; philippe.michaud@uca.fr; cedric.delattre@uca.fr; philippe.michaud@uca.fr

N. Benbasat
Department of Pharmacognosy and Pharmaceutical Chemistry, Medical University-Plovdiv, Plovdiv, Bulgaria

Department of Pharmacognosy and Pharmaceutical Botany, Medical University Sofia, Sofia, Bulgaria
e-mail: niko.benbasat@mu-plovdiv.bg

P. Katsarov
Department of Pharmaceutical Sciences, Medical University, Plovdiv, Bulgaria

Research Institute at Medical University-Plovdiv, Medical University – Plovdiv, Plovdiv, Bulgaria
e-mail: plamen.katsarov@mu-plovdiv.bg

J. M. Oliveira et al. (eds.), *Polysaccharides of Microbial Origin*,
https://doi.org/10.1007/978-3-030-42215-8_61

Abstract

From the last three decades, innovative 3D printing processes have been progressively more investigated for food and regenerative medicine topics due to modern technological advances of 3D printers. In tissue engineering, 3D bioprinting technologies are increasingly improved by the continuous development of efficient bioinks. In this area, biodegradable, cell-biocompatible and nontoxic biopolymers such as microbial polysaccharides have been successfully used as hydrogel biomaterial for bone, skin, etc., tissue regeneration.

This chapter, specially dedicated to 3D bioprinting of biopolymers, aims to give a recent overview on the main chemical characterization (monosaccharide compositions, glycosyl linkage...) and physicochemical properties (gelling properties...) of bacterial polysaccharides used as promising bioinks bioprintable materials for tissue engineering fields.

P. J. P. Espitia
Nutrition and Dietetics School, Universidad del Atlántico, Puerto Colombia, Atlántico, Colombia

C. A. Fuenmayor
Institute of Food Science and Technology (ICTA), Universidad Nacional de Colombia, Bogotá, DC, Colombia
e-mail: cafuenmayorb@unal.edu.co

A. Nesic
Department of Chemical Dynamics and Permanent Education, Vinca Institute for Nuclear Sciences, University of Belgrade, Belgrade, Serbia
e-mail: anesic@vin.bg.ac.rs

M. S. Carranza
Graduate School of Engineering Science, Osaka University, Osaka, Japan

College of Science, De La Salle University, Manila, Philippines
e-mail: mscarranza@cheng.es.osaka-u.ac.jp; mary_stephanie_carranza@dlsu.edu.ph

C. Delattre (✉)
Université Clermont Auvergne, Clermont Auvergne INP, CNRS, Institut Pascal, Clermont-Ferrand, France

Institut Universitaire de France (IUF), Paris, France
e-mail: cedric.delattre@uca.fr

Keywords

Microbial Polysaccharide · 3D-bioprinting · Regenerative medicine · Hydrogel · Nanocellulose

1 Introduction

Three-dimensional printing, also called additive manufacturing, is a technology including numerous processes in which material is amalgamated or solidified under computer control to generate a three-dimensional entity from digital models. It is an emerging field that is being integrated into numerous applications such as prototyping, automotive, aerospace engineering, art, construction, computers, robots, oceanography, food fabrication, wound dressing, tissue engineering, and regenerative medicines (Mohammed 2016; Stanton et al. 2015; Sun et al. 2015; Zhang et al. 2019). For example, 3D printing is a promising technology to make 3D tissues based on copies of patient's organ model obtained with tomography (Yanagawa et al. 2016). This process was firstly described and used in 1981 by H. Kodama of the Nagoya Municipal Industrial Research Institute to manufacture layer by layer three-dimensional plastic materials using a photosensitive polymer (Kodama 1981). 3D printing referred to the polymer technologies contrary to additive manufacturing used in metal working. The main 3D printing processes are fused deposition modeling (based on continuous deposition of a thermoplastic by a print head), stereolithography integrating digital light processing (photochemical cross-linking of monomers, oligomers, or polymers layer by layer) and selective laser sintering, a photochemical technique that uses a laser power source to sinter a powder material at points in space defined by a 3D model (Liu et al. 2019). When microbial polysaccharides are used as polymers in 3D printing, specific technologies derived from those described above have to be involved such as light-assisted 3D printing, inkjet-based printing, extrusion-based printing, and particle fused-based 3D printing (Liu et al. 2019; Tai et al. 2019). Hydrogels have been published as the best promising bases for bioprinted inks and many of them are composed of microbial polysaccharides (McCarthy et al. 2019a,b). Polysaccharides are biopolymers of monosaccharides (mainly pentoses and hexoses with furanose or pyranose ring structures) linked by glycosidic linkages and exhibiting complex homo- or heteropolymeric structures sometimes covalently associated with no-sugar groups. They are linear or ramified and have different solubility depending on their structures. Even if these biopolymers are present in all organisms (animals, terrestrial plants, seaweeds bacteria, fungi, and microalgae), their diversity is probably higher when they derive from microorganisms (Cruz et al. 2020; Gaignard et al. 2019; Morris and Harding 2009; Sutherland 2007). Much of these polysaccharides are extracellularly produced by microorganisms (exopolysaccharides), which makes their extraction and purification easier after their production in controlled bioreactors or photobioreactors. These ecofriendly, renewable, and sustainable polymers are currently used in a large

area of applications such as materials, cosmetic, pharmaceutical, food, medicine, and others. The main microbial polysaccharides used in these applications are bacterial cellulose, alginate, hyaluronic acid, dextran, gellan, and xanthan (McCarthy et al. 2019a,b). Three-dimensional printing of polysaccharides-bio-based materials is still limited to biomaterial and food engineering despite the abundance of these biopolymers. The 3D printing polysaccharide-based biomaterials encountered large success not only with regard to their structural similarities to glycosaminoglycans of extracellular cell matrix but also to their physico-chemistry, biodegradability, nontoxicity, cell-biocompatibility, non-mutagenicity, and non-carcinogenicity. These properties and their printability have to be considered before using them as an ink constituent for 3D printing. The low printing performances of some polysaccharides can be overcome after their chemical modification or their combination with other components such as low-costs biodegradable polyesters. Addition of some antimicrobial or antibiofilm agents to polysaccharides before the 3D printing process is also explored to increase their biocompatibility and limit the risk of infections (McCarthy et al. 2019a,b). Three-dimensional printing of polysaccharide-based materials for food engineering is currently more prospective and at this time only some proofs of this concept have been done such as snacks or chocolates. It is based on the manufacturing of food products, which can be customized in texture, color, shape, nutrition, or flavor. The main advantage of this food processing is to adapt the printing material to the individual needs of the consumers and also to make a prototype for food developments. As explained in the review of Sun et al. (2015), it is fundamental to distinguish the differences between food printing and robotic-based food manufacturing. In the first one, a fabrication process integrates digital gastronomy and 3D printing manufactured foods with defined and adapted parameters such as nutrient contents or color. On the contrary, the robotic-based technologies design the automatization and the robotization of food production. Polysaccharides and mainly microbial polysaccharides are good candidates for food 3D printing as their behavior in water allows to form hydrogels with various textures and abilities to encapsulated numerous compounds. The aim of this review is to explore the potential of microbial polysaccharides to be used as bioink in 3D printing applications, especially in food engineering, regenerative medicine and tissue engineering.

2 3D Printing Technologies

2.1 Concept and Definition

Additive manufacturing (AM), also known as 3D printing, is a set of technologies aimed at producing three-dimensional objects with a layer-by-layer approach. A project created via a modeling software is used, which is interfaced with instruments that are able to effectively lay different layers of materials, according to a predefined geometric layout. The International Organization for Standardization (ISO) and the

American Society for Testing and Materials (ASTM) define 3D printing as a «process of joining materials to make parts from 3D model data, usually layer upon layer, as opposed to subtractive manufacturing methodologies and manufacturing training» (ISO/ASTM 2015).

The 3D printing processes have been developed according to the following conceptual steps (Fig. 1): (i) an idea of the object is developed in concept, including the selection of the desired composition and features; (ii) a 3D model of the object is designed in a CAD software; (iii) a file (e.g., .stl, .amf) is developed in a software to enable the interpretation of the geometrical information by the AM equipment; (iv) the object is manufactured by an AM technique, selected according to the desired material and features; (v) cleaning and post-processing of the object are often needed to improve the final product and mechanical features (Jiménez et al. 2019).

There are several AM techniques available, and they are frequently classified in the following categories (ISO/ASTM 2015): binder jetting, directed energy deposition, material extrusion, material jetting, powder bed fusion, sheet lamination, and vat photopolymerization. However, other classifications have been proposed based on the type of base material or ink, the energy source, the machinery, tools, and the technology or know-how (Jiménez et al. 2019).

In **binder jetting technology** (Gibson et al. 2015a), the print head releases a binding fluid on the base material in powder form. A solid layer is thus formed by the interaction between the material or ink and the binder, and the process is repeated until the desired object is made. A variety of metals, ceramics, and polymers have been used (Ziaee and Crane 2019), and several other materials can be used in the same process. Indeed, this technique has been explored for the manufacturing of food and feed by using food-grade biopolymers and other substances, both as ink

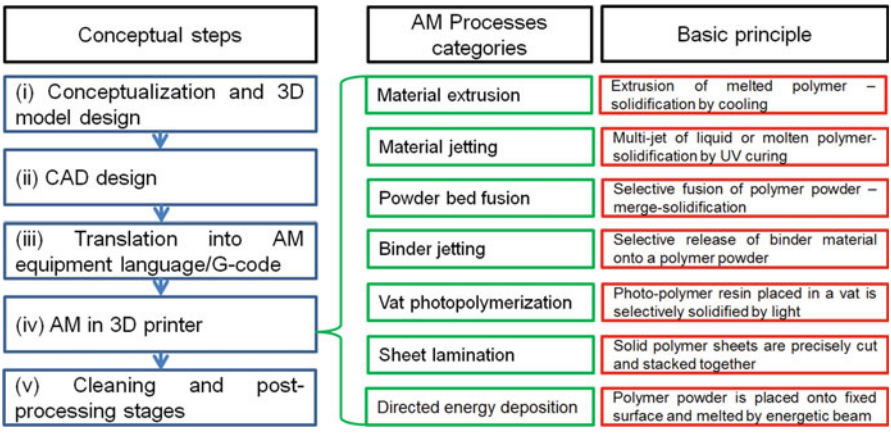


Fig. 1 A scheme of the phases and categories of AM processing of polymers for 3D-printed objects. (Source: Elaborated based on Dizon et al. (2018), Jiménez et al. (2019), González-Henríquez et al. (2019))

materials and binders (Holland et al. 2019). In this technique, post-processing is required. For instance, to increase the mechanical resistance, the object is often hardened in a special heated chamber, the excess powder must be removed, usually using forced air convection, and the surface can be treated downstream to make it more compact. The surface definition of the objects manufactured with binder jetting technology is relatively low, but, on the other hand, this technique has the advantage of not requiring the use of supports.

In **material jetting** technology, multi-jet print heads, moving horizontally, release a liquid or a molten material onto the surface of the print layer. At each release, the layer is cured or solidified using UV light rays. In this way, it is possible to create objects with very well-defined surfaces (Yap et al. 2017). Although a more narrow variety of polymers have been used as inks in material jetting AM, this technique has allowed the fabrication of freestanding composite objects with increased functionality (MacDonald and Wicker 2016).

In **material extrusion** (or melting extrusion) processes, the most common of which is **fused deposition modeling** (FDM), a filament of a solid thermoplastic polymeric material, used as the ink, is pushed through a heated nozzle, melts, and is deposited on a construction platform, where the filament cools, forming the solid 3D printed object. It is generally not fast or particularly accurate, but it can be cost-effective and used with different materials, and therefore, its use in industrial, research, and domestic 3D printing has become widespread. A variety of commercially available polymers can be used as FDM inks, which includes biodegradable or biocompatible polymers (Liu et al. 2019; Baran and Erbil 2019). An interesting mode of material extrusion-based 3D printing is the gel-forming extrusion. Hot solutions of hydrogel-forming polymers, which are often edible or biocompatible, such as methylcellulose, agarose, collagen, gelatin are 3D printed onto cooled stages by sol-gel transitions (Kirchmajer et al. 2015; Godoi et al. 2019; Tai et al. 2019).

Another type of AM deposition process is the **directed energy deposition** (DED) (Gibson et al. 2015b), in which materials, originally in the form of powder or wire, are deposited onto a fixed surface or object, and then melted by an energy source, for example, a laser or an electron beam. The 3D printed object is created as each layer of material solidifies. However, the applicability of DED for polymer materials is limited (González-Henríquez et al. 2019), and usually metal powders are used as ink materials.

With **powder bed fusion** (PBF) a layer of the ink material in powder form is released on a platform; then a heat source, a laser (also known as selective laser sintering or SLS), or an electron beam is used to melt the powder particles. With a roller, the subsequent layers are deposited and merged one by one (King et al. 2015). It can be used with polymers, ceramics, and metals as ink materials, offering a resolution and accuracy that highly depend on the size of the powder particles. However, it is a relatively slow technique. The great variety of polymers and polymer blends that can be used as inks is an advantageous feature of PBF and SLS (González-Henríquez et al. 2019).

In **sheet lamination systems** (Gibson et al. 2010), thin sheets of paper or other materials are cut and stacked together onto each other, layer by layer, until the final

object is obtained. The technique is often used to print colored objects with a high degree of detail, with high production speed. The most common methods are laminated object manufacturing (LOM) and ultrasonic additive manufacturing (UAM): In the first case, the sheets are laser cut, in the second they are consolidated via ultrasound. The most common ink materials for this process are paper and thermoplastic polymers (González-Henríquez et al. 2019).

Vat photopolymerization (Gibson et al. 2015c) is a category of 3D printing in which a liquid photopolymer (resin) is first placed in a vat and then selectively solidified layer by layer, with a light-activated polymerization process. Stereolithography (STL), digital light processing (DLP), continuous liquid interface production (CLIP), and multiphoton polymerization (MPP) belong to this category of AM. Post-processing is mainly aimed at removing excess resin. This technique allows high accuracy and precise finishes (Lee et al. 2017). The use of synthetic and natural biocompatible polymers for STL applications has been highlighted for the manufacturing of high-resolution customizable tissue scaffolds (Mondschein et al. 2017).

Material extrusion, powder bed fusion, and vat photopolymerization are AM techniques that require more specific polymeric materials (González-Henríquez et al. 2019). However, polymer materials, including biopolymers, have been explored as ink materials in all the different 3D printing technologies. Figure 1 shows the basic principles of each one of these techniques when applied to polymer materials (Dizon et al. 2018; González-Henríquez et al. 2019; Tai et al. 2019).

2.2 Main Applications in Food and Regenerative Medicine

Three-dimensional printing is an innovative technology that allows creating objects in three-dimension by layer-by-layer deposition (Tai et al. 2019; McCarthy et al. 2019a,b), with a complex shape and at a considerable quick speed. These features make the 3D printing technology very interesting for the industrial field, especially the food industry and regenerative medicine. In this context, the 3D printing process is developed by a computer-controlled system that follows the design to shape the new 3D desired object. This results in the creation of new edible products with a new texture, flavor, taste, and structure from food printer (Fig. 2) and new active structure and specific shape from bioprinter using the four types of printing technology such as: laser-assisted bioprinting, micro-extrusion bioprinting, stereolithography bioprinting, and inkjet bioprinting (Fig. 3).

Also, the nutritional value of the newly developed food product can be customized according to specific nutritional requirements of consumers. There are different motivations for the development of a 3D printed food product, including the consumer's increasing interest for healthier food; specific nutritional requirements from target consumers group, such as athletes, children, and elderly; and the desire to meet consumer's criteria regarding sensory food properties by innovating their taste or texture (Severini et al. 2018).

However, other published research has focused on studying the effect of food materials as edible ink for the development of 3D printed food.

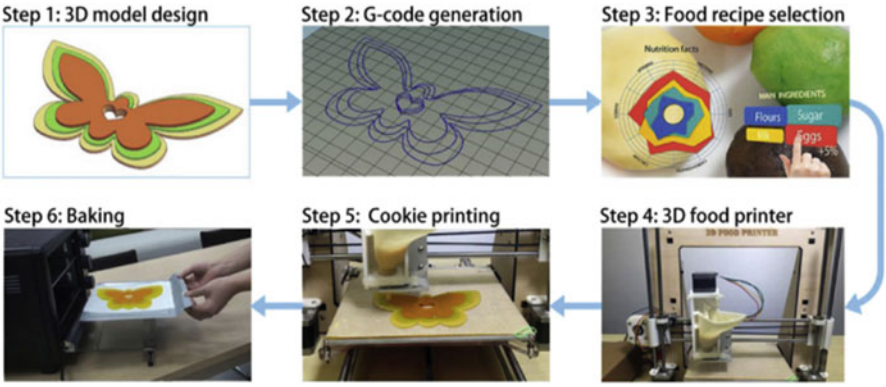


Fig. 2 Schematic diagram of Extrusion-based 3D Food Printing Process. (Source: Reproduced with permission from Sun et al. 2015)

Moreover, the technology of 3D printing has acquired increasing attention in the field of regenerative medicine due to the potential use of biomaterials and their interesting application advantages, which include nontoxicity and biodegradability (McCarthy et al. 2019a, b; Tai et al. 2019). The application of 3D printing has allowed the prototyping of medical devices and personalized therapeutic solutions (McCarthy et al. 2019b). One of the major applications of 3D technology is related to the production of complex tissues in vitro using living organisms and biomaterials as ink in the 3D printing process. The areas of application of this technology include bone, tendon, skin, cardiovascular, and other types of tissue engineering. In this concern, the bacterial polysaccharides have stood out as a potential ingredient in bioinks for 3D printing due to their printability, and other technological properties, such as thermal resistance, mechanical performance, biocompatibility, and biodegradability (Lee et al. 2017; McCarthy et al. 2019b; Tai et al. 2019). However, one of the main limitations of the 3D printing application in the medical field is the restricted use of bacterial polysaccharides as biomaterials, mostly due to their costly and challenging production, and their scalability (McCarthy et al. 2019b).

In the field of regenerative medicine, polymers such as acid hyaluronic, alginate, chondroitin sulfate, chitosan, agarose, gellan, pullulan, and cellulose have been widely used as bioink in 3D printing for developing scaffolds and hydrogels (Tai et al. 2019). Some of their main advantages include the mechanical resistance and rheological properties required for their use, their biocompatibility, biodegradability, and safety (Tai et al. 2019). Moreover, the use of bioinks based on the aforementioned biopolymers has been indicated as a potential alternative to diminish the risk of bacterial biofilm formation and development during an implantation process (McCarthy et al. 2019a).

Most of the bioinks tested in the field of regenerative medicine are obtained from bacterial source. In this regard, alginates, which can be obtained from algal genera (*Laminaria, Macrocystis, Ascophyllum, Ecklonia, Lessonia*, and *Durvillaea*), and also from bacteria genera *Azotobacter* and *Pseudomonas*, have

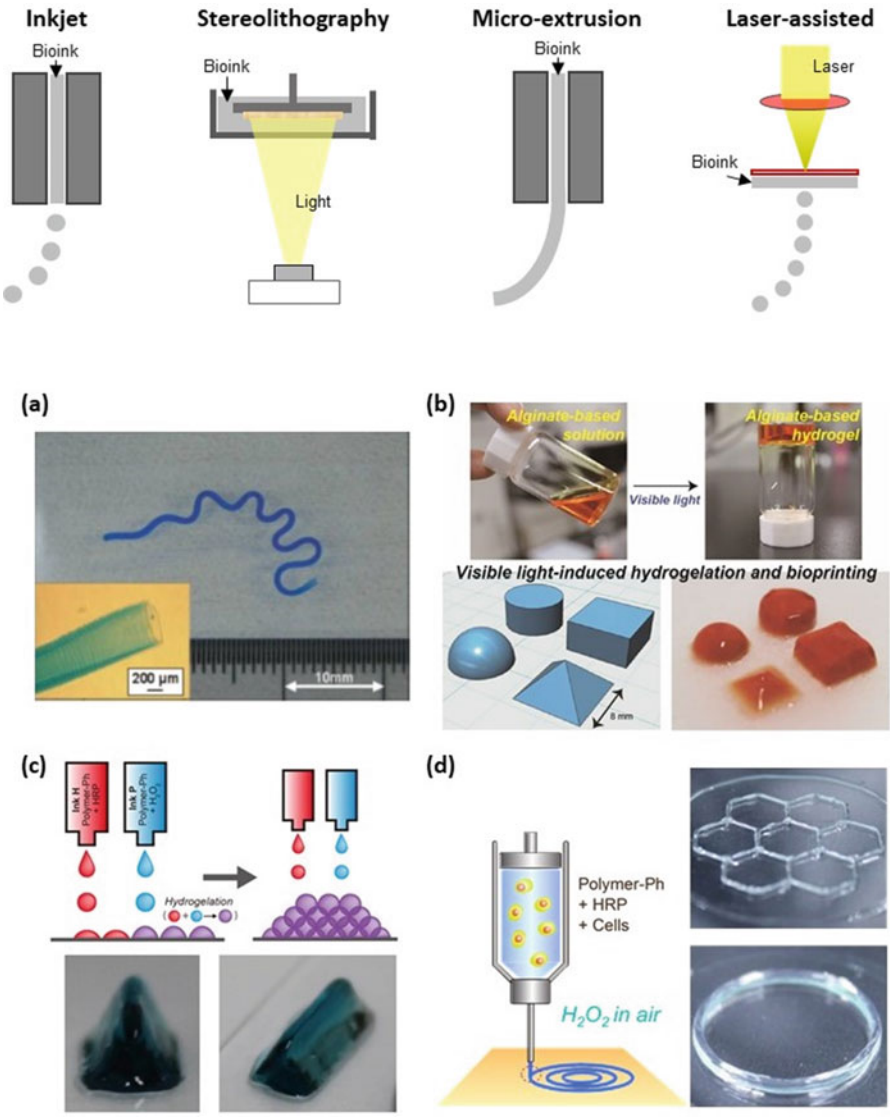


Fig. 3 (**a**) Ca-alginate tube prepared through inkjet bioprinting, and hydrogel constructs obtained from (**b**, **c**) alginate derivative and (**d**) hyaluronic acid (HA) derivative possessing phenolic hydroxyl moieties through (**b**) stereolithograpy bioprinting, (**c**) inkjet bioprinting, and micro-extrusion bioprinting. (Source: Tai et al. 2019)

shown potential as bioink due to its ability to preserve their 3D structure when added with cross-linkers, such as $CaCl_2$ and $CaSO_4$ (Fig. 3). Cellulose is another bacterial biopolymer (obtained from several bacterial genera, including *Acetobacter, Agrobacterium, Achromobacter, Aerobacter, Azotobacter, Sarcina*

ventriculi, Salmonella, Escherichia, and *Rhizobium*) widely used when testing the 3D printing technology in the field of regenerative medicine due to its ability to conform a nanofibrous network (**see paragraph 4.**). Hyaluronic acid (**see paragraph 4.**), obtained from bacteria including *Streptococci* spp., *Pasteurella multocida*, and *Cryptococcus neoformans*, has presented potential to be used as bioink for applications as wound healing, and surface coating. Gellan gum (**see paragraph 4.**), produced by *Sphingomonas elodea*, has been also attractive for applications as bioink and 3D printing due to its low immunogenicity, rheological properties, and gelling properties, allowing it to be applied for the development of bone scaffolds, fibroblast, and for neural cultures (McCarthy et al. 2019a).

3 Description of the Main Microbial Polysaccharides

3.1 Polysaccharides Definition

Polysaccharides, which are condensed and complex polymers, are the most abundant biological molecules in Nature. They are usually named Carbohydrates by Anglo-Saxons due to their general gross formula $(CH2O)_n$ but are finally "just" polyhydroxyketones or polyhydroxyaldehydes composed of three to seven carbon atoms. The basic constitutive unit is called monosaccharide. It can be linked to another one by O-glycosidic bonds forming glycans. According to the IUPAC-IUB (International Union of Pure and Applied Chemistry-International Union of Biochemistry) nomenclature, polysaccharides can be subdivided into two categories according to their Degree of Polymerization (DP): (i) oligosaccharides with a DP ranging from two to ten and (ii) polysaccharides whose DP is greater than ten. The line between polysaccharides and oligosaccharides is in practice more unclear and low molecular weight polysaccharides are often assimilated to oligosaccharides (Lehninger 2008). Polysaccharides generally have high molecular weights and can establish numerous inter- and intramolecular interactions due to their free hydroxyl groups. Thus, water-soluble polysaccharides have the capacity to considerably increase the viscosity of media. These rheological properties (gelling, stabilizing, thickening, etc.) are used in food, petroleum industry, cosmetics, paints, adhesives, and even bio-based/biosourced materials (also because of their biocompatibility and biodegradability).

Polysaccharides undoubtedly constitute one of the richest families of biopolymers in terms of structures and uses. This structural variability comes from the large number of available monosaccharide units and the possibility to glycosidically link the anomeric hydroxyl group of one monosaccharide to any of the hydroxyl groups of another residue. For comparison, 20 amino acids and 5 different bases can be used for constituting amino and nucleic acids, respectively, always associated in both cases with one type of bond. Here, the formation of a disaccharide (Glc-Glc) with two identical hexoses offers no less than five different possibilities since the first glucose unit can be linked by its anomeric carbon (α or β) to the five hydroxyl groups

of the second glucose, finally resulting in 11 different structural isomers (including O-pyranosyl or furanosyl forms). The presence of non-osidic units covalently associated to specific hydroxyl groups of the constitutive monosaccharides (sulfate/methyl/acetyl/succinate/pyruvate groups, organic acids, etc.) also contribute to this structural diversity (Lehninger 2008).

Polysaccharides are identified regarding the main constitutive monosaccharide followed by the "-ane" suffix. If the polysaccharide structure contains several types of monosaccharides, the second (and even the third one) will be used for naming, e.g., galactomannan or glucuronoarabinoxylan. Their complete hydrolysis (e.g., by using specific acids or enzymes such as polysaccharide hydrolases and lyases) allows the release of their constitutive monosaccharides. D-glucose (Glc) is the predominant monosaccharide found in Nature although D-fructose (Fru), D- and L-galactose (Gal), D-xylose (Xyl), and L-arabinose (Ara) residues are also often present. Other specific monosaccharides in natural polysaccharides can be commonly found such as D-glucosamine (GlcN), D-galactosamine (GalN), D-glucuronic and galacturonic acids (GlcA, GalA), N-acetyl-neuraminic acid (Neu5Ac) or N-acetyl-muramic acid (MurNAc). Depending on the origin (animal, plant, algal, or microbial), polysaccharides can be linear (e.g., amylose, cellulose, curdlan, glucuronan), substituted (e.g., galactomannan), or more or less branched (e.g., amylopectin, gum arabic, pectin, xanthan, etc.) (Lehninger 2008).

Overall, polysaccharide structure should always be defined in terms of: (i) flexibility of the macromolecular chain, a parameter influenced by the nature of the involved glycosidic bonds, (ii) nature of the main backbone (homo- or heteropolysaccharide) and repetition patterns, (iii) nature of the glycosidic units, which can introduce and/or enhance ionic interactions (uronic acids, GlcN/NAc-GlcN, GalN/NAc-GalN, etc.), (iv) molar mass and mass distribution, (v) type and ratio of functional groups carried by the macromolecular chain (substitution). The direct consequence of these intrinsic structural parameters is the wide diversity in the role of carbohydrates in the living organisms. They can provide (i) structural support as a constituent of the cell wall (e.g., cellulose, pectin, hemicellulose, or chitin), (ii) storage capacity in the cell (such as starch, galactomannan, or glycogen), (iii) adaptation to abiotic/biotic changes (bacterial exopolysaccharides) but may also be involved (iv) in cellular communication and control processes as reported for glycosaminoglycans (e.g., heparan, dermatan, hyaluronic acid, chondroitin) commonly called GAGs (Berteau and Mulloy 2003). Finally, polysaccharides are namely present in all biotopes (Fig. 4): (i) plants (e.g., cellulose, starch), (ii) algae (e.g., alginate, carrageenan, fucoidan), (iii) bacteria (e.g., curdlan, xanthan, dextran, succinoglycan) (Morris and Harding 2009), (iv) fungi (e.g., chitin, scleroglucan) (Park and Khan 2009), (v) lichens (e.g., lichenin, pustulan), and (vi) animals (e.g., chitin for insects and crustaceans, heparan).

The following sections give an emphasis on some polysaccharides from microorganisms. Note that bacterial polysaccharides are mainly described as exopolysaccharides (EPS), capsular polysaccharides (CPS), bound polysaccharides (BPS), teichoic acids (TA), lipopolysaccharides (LPS), and peptidoglycans (McCarthy et al. 2019a,b).

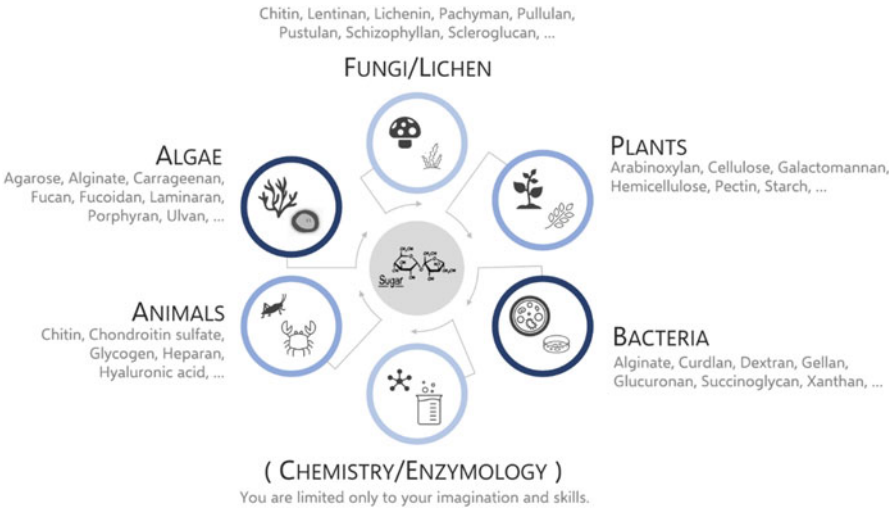


Fig. 4 Main polysaccharides from various sources currently described in the literature

3.2 Bacterial Cellulose

Bacterial cellulose (BC) is a highly pure form of nanocellulose synthesized by several genera of bacteria (i.e., *Acetobacter*, *Rhizobium*, *Agrobacterium*, *Aerobacter*, *Achromobacter*, *Azotobacter*, *Salmonella*, *Escherichia,* and *Sarcina*) as the primary metabolite in the oxidation of sugars and alcohols. *Acetobacter* negative-gram strains are known to be capable of polymerizing 200,000 glucose molecules per second into β-1,4-glucan chains to form a stretchable cellulose nanofiber pellicle. *Komagataeibacter* (previously known as *Gluconacetobacter*/*Acetobacter*) secretes ribbon-like nanofibers, reputable for its high purity, crystalline form, and durability (Florea et al. 2016; Moniri et al. 2017). In the presence of carbon and oxygen, the fibers are formed by the membrane-anchored protein, cellulose synthase, and thus, a row of 50–80 pore-like synthetic sites along the axis of the cell is evident. A cellulose matrix is formed and allows the obligate aerobe to gain access to higher tension oxygen at the surface of the medium. The cellulose matrices condense to form a floating thick pellicle of bacterial cellulose, which is used in a variety of applications.

Komagataeibacter is a bacteria genus that is naturally derived from fruits, wine, or vinegar, where acetic acid bacteria can thrive. Furthermore, the strain has been propagated in starter cultures for ease of use at industrial scales. Research has shown that spatial availability, source of carbon, nitrogen, oxygen, and culture conditions will affect the purity and amount of the BC produced. Various carbon sources have been explored from the perspective of both economical and research-practical applications. However, studies have repeatedly proven that d-glucose over sucrose, D-fructose, D-xylose, and other sugars is superior in effect of the quality and quantity of the final output. As the carbon source is fed into the metabolism,

D-glucose undergoes a conversion into substrate units of UDP-glucose, which in turn is converted to cellulose through the membrane-anchored protein, cellulose synthase. This leads to the fabrication of cellulose chains in the extracellular space of the microbe. Oxygen supply is key to the growth of the BC and, accordingly, the metabolism of the bacteria, or in the case of *Komagataeibacter*, the consumption of D-glucose. Oxygen acts as the final electron acceptor in the electron transfer chain. When absent, nicotinamide adenine dinucleotide (NADH) is unable to carry electrons and ultimately produce ATP since it is in its reduced form. This would lead to a plateau in the growth rate of the bacteria as well as the production of the nanofibers. Levels of gaseous oxygen in the atmosphere and dissolved oxygen would also have altering effects on the quality of the BC sheet. Another key factor worth discussing is the pH of the culture medium and its effect on the metabolism of the bacteria. *Acetobacter* microbes are sensitive to changes in these factors, as they would influence the metabolism and the morphology of the cell. In addition to the glacial acetic acid needed in the medium, the bacteria produce secondary metabolites that contribute to further lowering the pH of the medium. These metabolites include: gluconic, acetic, or lactic acids produced by the consumption of sugars and nitrogen sources.

Several studies have contributed to the optimization of the growth of BC as it has become a central focus for scientists to find ways to mass-produce BC at an industrial scale without losing the purity and durability of the hydrogel. The production cost of BC has also proven to be high due to the expensive culture media and the bioprocessing costs of the raw product (Azeredo et al. 2019). Alternative media have been tested for more practical industrial applications which has led to a wider range of resulting BC that can exhibit favorable characteristics for specific applications. Factors such as biocompatibility, durability, moldability, and elasticity are a few of the many features that can be controlled by the regulation of the culture parameters. More importantly, this demonstrated that BC is a viable biomaterial for a range of industries from medical to agricultural uses. BC sheets embedded with bioactive components have been used as wound dressings, providing a steady release of anti-inflammatory medicine as well as constant aeration (due to high porosity) for the wound to properly clot. Not only is BC a versatile material in pure form, but it has also proven to be a favorable material in its composite forms with other polymers such as the highly biocompatible silk fibroin, a protein derivative of raw silk.

As research continues to unfold the metabolism of the cellulose-producing bacteria (Fig. 5), new technology has allowed the microbes to take on more specialized roles that would meet the specific culture parameters in order to produce the unique and promising BC fibers.

3.3 Xanthan

Xanthan is a charged heteropolysaccharide composed of a main chain of β-(1→4)-D-glucose units with trisaccharide branches on the third position. The side chains

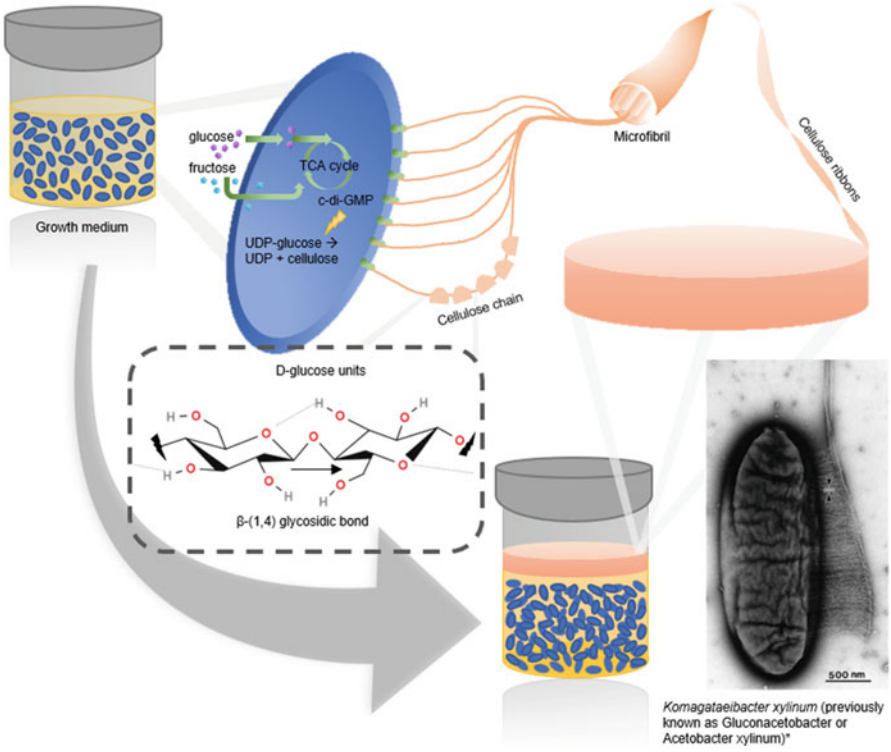


Fig. 5 Metabolism of the cellulose-producing microbe, Komagataeibacter xylinum. (Source: SEM bacterium image reprinted from Hirai et al. (2002) with permission from © SpringerNature)

consist of one D-glucuronic acid and two D-mannose residues, which could be linked with acetic and pyruvic acids (Fig. 6).

The organic acids are responsible for the anionic character of the polysaccharide (Cote and Ahlgren 2000; Garcia-Ochoa et al. 2000). Naturally, xanthan is produced by the bacterium *Xanthomonas campestris*, which occurs on the cabbage and bean plants. Commercially, xanthan is obtained by biotechnological fermentations in a growing medium with substrate glucose, additionally enriched with succinic, pyruvic, or other organic acids and controlled oxygen uptake (Garcia-Ochoa et al. 2000; Morris and Harding 2009).

The fermentation conditions during the production of xanthan, as well as the possibility of aggregation between some saccharide chains, influence its molecular weight, which can vary between 2×10^6 and 20×10^6 Da (Garcia-Ochoa et al. 2000). Xanthan is easily soluble in water. Its solutions are pseudoplastic or shear thinning, and highly viscous even at low concentrations (Garcia-Ochoa et al. 2000; Morris and Harding 2009). The viscosity of the solutions depends on the dissolution temperature, which can influence the confirmation structure of xanthan (helix or random coil), also on pH, the polymer concentration and the presence of salts.

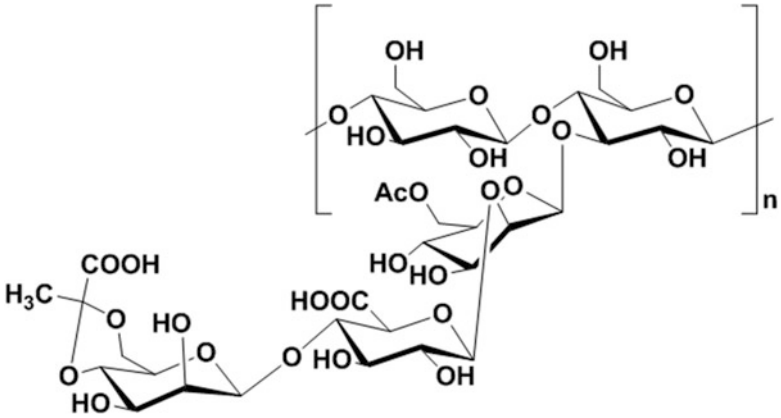


Fig. 6 Chemical structure of xanthan. (Source: Adapted from Cote and Ahlgren 2000)

A great advantage of xanthan in industrial application is its ability to form complexes with galactomannans and synergistically incrementing the solution viscosity (Garcia-Ochoa et al. 2000).

The good thickening, suspending, and stabilizing ability of xanthan, as well its water-solubility, freeze–thaw stability, compatibility, and nontoxicity make it one the most widely used polysaccharides in food, biomedical, pharmaceutical, cosmetic, and textile industries (Garcia-Ochoa et al. 2000; Morris and Harding 2009).

3.4 Gellan

Gellan is a linear extracellular heteropolysaccharide that is produced by the microorganism *Pseudomonas elodea*, but also can be found in the aquatic plant elodea (Paul et al. 1986). It is composed of the repeating tetrasaccharide sequence: [β-(1→3)-D-glucose, β-(1→4)-D-glucuronic acid, β-(1→3)-D-glucose, α-(1→4)-L-rhamnose] (Fig. 7).

The native gellan, as well as the biosynthesized ones, have additionally two acyl groups, acetate and glycerate, which are linked to the glucose unit. Commercially, glyceryl and acetyl groups are separated by alkaline extraction and the obtained polysaccharide is known as "gellan gum" (Morris et al. 2012).

Mixed with water, gellan has the ability to swell and expand its volume by forming a branched structure. Furthermore, the addition of monovalent (Na^+, K^+) and mostly divalent cations (Ca^{2+}, Mg^{2+}) in an aqueous gellan solution tends to the formation of gels with desired functional properties (versatile texture, optical activity, stability on temperature and pH variations, water dispersibility, and compatibility with other polymers) (Morris et al. 2012; Paul et al. 1986). Gellan gels can be either firm or elastic, which depends on the number of acetyl groups in the polysaccharide chain. The high content of acetyl groups leads to the formation of elastic and soft gels, while in the absence of acetyl groups in the structure, the formed gels are brittle

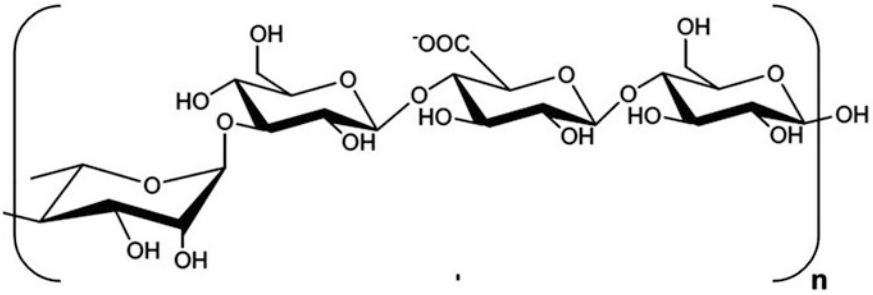


Fig. 7 Chemical structure of gellan. (Source: Adapted from Morris et al. 2012)

and fragile (Giavasis et al. 2000). Additionally, temperature also plays a role in the structure of gellan gels and more precisely in their viscosity. The high temperatures decrease the viscosity of the solution, while by cooling gellan has the ability to return to its original condition (Morris et al. 2012).

Gellan gels have a wide range of applications in both food and pharmaceutical industries. They are used as stabilizers, texturizers, suspending and film-forming agents, growth media for some bacteria and plant tissue cultivation, and also for antibody gel diffraction, enzyme matrices, and cell immobilization (Morris et al. 2012; Giavasis et al. 2000).

3.5 Alginate

Alginates are linear copolymers of (1→4)-linked residues of β-D-mannuronic acid (M) and α-L-guluronic acid (G), which form M-, G-, and MG-consecutive block sequences (Fig. 8) (Morris and Harding 2009; McCarthy et al. 2019a,b; Tai et al. 2019).

They are obtained generally from brown algae by an alkaline extraction; however some bacteria from *Pseudomonas* and *Azotobacter* genus could also produce alginates. Microbial alginates differ from those of algae in their chain length, the G/M ratio, and the presence of *O*-acetyl groups (Cote and Ahlgren 2000). Alginate derived from *P. aeruginosa* has a lower amount of guluronic acid and does not form polyguluronic blocks, while *A. vinelandii* alginate, similar to seaweed alginate, consists of M-, G-, and MG-consecutive block structures (Morris and Harding 2009). Generally, because of the low production cost, brown algae are still the main industrial source for obtaining alginate. Nevertheless, there are several advantages of bacterial alginate such as the ability to control the molecular weight, homogeneity between G/M residues, and the degree of acetylation. This could be obtained by controlling the synthesis of the enzyme epimerase, which is responsible for the conversion of D-mannuronic to L-guluronic acid. The production of the enzyme acetylase could be controlled, which as well can be utilized to change the amount of *O*-acetyl groups and therefore the viscosity of alginate solutions (Cote and Ahlgren 2000; Morris and Harding 2009).

However, the epimerase is not able to convert acetylated mannuronic acid residues to guluronic acid, which could have a high impact on the structure and properties of the bacterial alginates (McCarthy et al. 2019a,b).

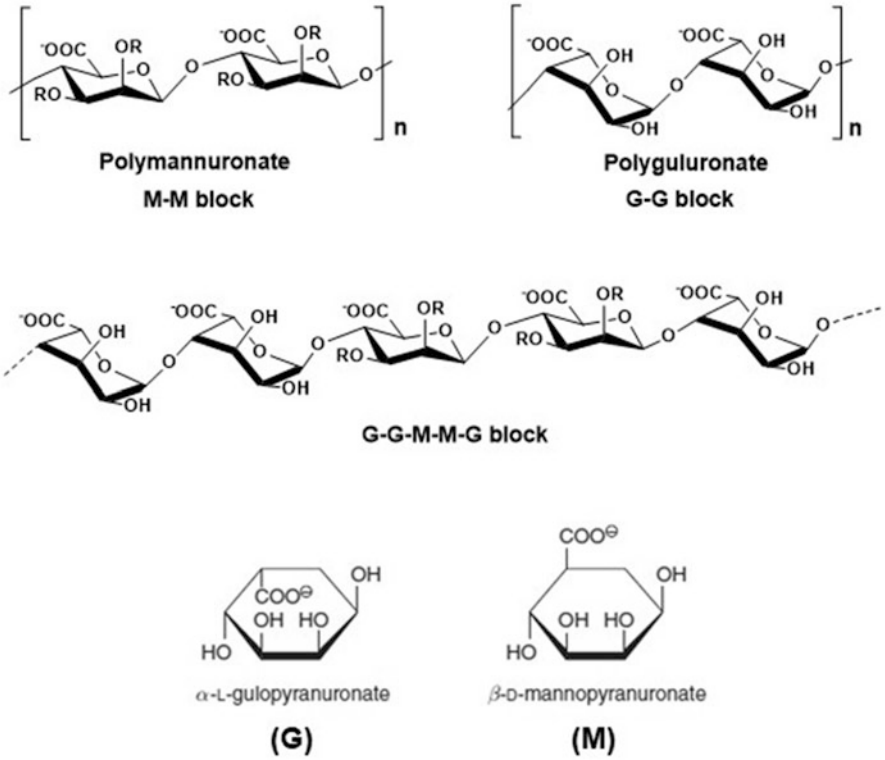


Fig. 8 Guluronic and mannuronic acid residues of alginates structures. (Source: Adapted from: Morris and Harding 2009; Tai et al. 2019)

Alginates have well-established gelling properties in the presence of cations such as Na^{+}, Ca^{2+}, Mg^{2+} and also at pH lower than 3.5. The gelling properties are associated with the high content of guluronic acid (Cote and Ahlgren 2000; McCarthy et al. 2019a,b). The applications of bacterial and algal alginates are generally the same and refer mainly to their ability of forming viscous solutions, gels, films, and suspensions. However, compared to seaweed alginates, bacterial alginates are preferred for biomedical applications due to their homogeneity in G/M residues, more stable molecular weight, and viscoelastic properties (Cote and Ahlgren 2000; McCarthy et al. 2019a,b).

3.6 Dextran

Dextrans are extracellular bacterial homopolysaccharides, defined as D-glucans and composed mainly from (1→6) linked α-D-glucopyranose residues, sometimes with ramified regions in α-(1→2), α-(1→3) or α-(1→4) positions (Fig. 9).

They are produced commonly by the lactic acid bacteria *Leuconostoc mesenteroides*; however some *Lactobacillus*, *Streptococcus*, *Weissella*, *Pediococcus*,

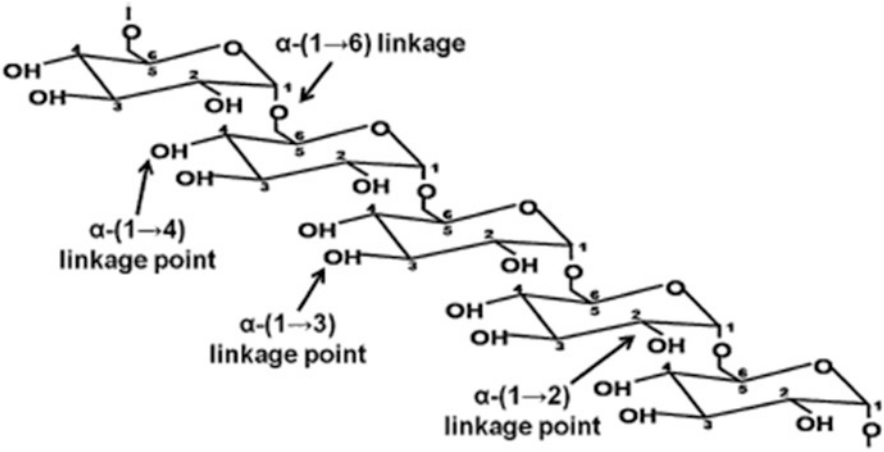


Fig. 9 Structure of dextran illustrating the main chain with α-(1→6) glycosidic bonds and possible side chains with α-(1→2), α-(1→3) and α-(1→4) linkages. (Source: Adapted from Kothari et al. 2014)

and *Acetobacter* strains are also a source of dextrans (Kothari et al. 2014). These bacteria synthesize the enzyme dextransucrase, which is responsible for the conversion of sucrose into dextran with the concomitant release of D-fructose. Dextrans are naturally found in refined sugar, maple syrup, honey, fermented foods, and beverages, also as a component of the dental plaque. Commercially dextrans are produced by biotechnological fermentations of *L. mesenteroides* strains in a medium enriched with sucrose and in the presence of purified dextransucrase (Cote and Ahlgren 2000; Kothari et al. 2014).

Depending on the microbial strain, the specific dextransucrase and the cultivation conditions, dextrans can vary in their molecular weight, the percentage of α-(1→6) glycosidic bonds in the main chain, and the type and length of the side branches (Kothari et al. 2014). Generally, dextrans are composed of short side chains and the branches percentage is between 5% and 33%. The α-(1→6) glycosidic bonds in the commercial dextrans are usually about 95% and the other 5% of the structure consist of α-(1→3) linked side chains (Morris and Harding 2009). The differences in the structure of dextrans have a significant effect on their properties and applications. The amount of dextran side chains determines their solubility in water. Dextrans with no branching are more soluble in water and behave as Newtonian fluids, while dextrans with >43% branching through α-(1→3) linkages are water insoluble exhibiting non-Newtonian pseudoplastic behavior (Kothari et al. 2014).

The molecular weight of dextrans can vary from 3 kDa to 50×10^5 kDa. Generally, enzymatic synthesis using purified dextransucrase is applied to obtain dextrans with specific molecular mass. Dextrans with Mw $> 2 \times 10^3$ kDa behave as an expandable coil and are utilized mostly in food industry as gelling, viscosifying, texturing, and emulsifying agent, also in photo film manufacturing, cosmetic, paper, petroleum, and textile industries. Commonly, dextrans with lower molecular weight

are more rod-like and used mainly in medicine (as blood plasma substituents, anticoagulants, prebiotics, in treating wounds) (Cote and Ahlgren 2000; Kothari et al. 2014).

3.7 Others Bacterial Polysaccharides

Curdlan is a non-charged extracellular β-(1→3)-D-glucan (Fig. 10), produced mainly from *Alcaligenes faecalis*, and also from some *Rhizobium*, *Cellulomonas,* and *Agrobacterium* strains (Morris and Harding 2009). It is a high molecular weight polysaccharide (Mw $> 2.0 \times 10^6$ Da) with a granule shape, forming a similar to starch structure. Curdlan produces gels at both high and low temperatures, as well as after treatment with alkaline solutions. The formed gels have a texture that combines the resistance of agar and the elasticity of gelatin. Curdlan finds application in various industries: in the food industry as a gelling agent, a texture modifier, a thickener, a stabilizer and fat replacer; in regenerative medicine as a scaffold for bone and tissue regeneration (Zhang et al. 2020).

Hyaluronic acid is a linear high molecular weight mucopolysaccharide consisting of alternating *N*-acetyl-β-(1→3)-D-glucosamine and β-(1→4)-D-glucuronic acid residues. It is a naturally occurring biopolymer, found in skin, joints, and vitreous fluid of animals and humans. However, the production of hyaluronic acid by animal organisms has some disadvantages such as the high cost, the long extraction time, and the high protein contamination. Some biotechnological methods for obtaining hyaluronic acid by *Staphylococcus*, *Pasteurella,* and *Cryptococcus* bacterial strains have been developed, which are preferred for industrial production. Hyaluronic acid is characterized by biodegradability, biocompatibility, viscoelasticity, and low immunogenicity, which makes it a suitable polymer for bioprinting, wound healing, tissue engineering, formulations with modified release, etc. (McCarthy et al. 2019a,b; Morris and Harding 2009).

Pullulan is an extracellular, non-charged, linear α-D-glucan found in fungus *Aureobasidium pullulans*. It is composed of maltotriose units with the following structure: [→6)α−D−Glcp(1→4)α−D−Glcp(1→4)α−D−Glcp(1→]n. Depending on the method of production, the molecular weight of pullulan can vary between 10 kDa and 25×10^3 kDa (Cote and Ahlgren 2000). Pullulan is a water-soluble polymer, which forms clear and viscous hydrogels. It is approved by FDA as a biocompatible, biodegradable, nontoxic, and non-immunogenic polymer and it is used widely in regenerative medicine, cosmetics, and food industry. Nanocomposites

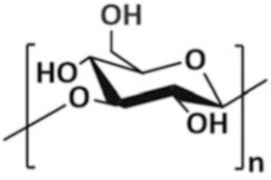


Fig. 10 Chemical structure of curdlan. (Source: Adapted from Zhang et al. 2020)

with pullulan and hydroxyapatite have been successfully applied for bone tissue regenerations (Della Giustina et al. 2019).

4 Use of Polysaccharides As Bioink

4.1 Cellulose Applications

Three-dimensional bioprinting is an emerging technology that has become an increasingly attractive solution to meet the demand in organ replacement and tissue engineering (Tai et al. 2019). It enables the precise control of composition, spatial distribution, and architecture of biomimetic, volumetric tissues. The additive manufacturing technique requires a selection of multicomponent bioink that exhibits both the rheological properties (i.e., viscosity and shear thinning) and biocompatibility to be able to mimic an extracellular matrix for the cells to grow and thrive (Jiménez et al. 2019; Tai et al. 2019; McCarthy et al. 2019a,b). Bioink composites would exhibit both liquid and solid-like attributes to be able to form hydrogel scaffolds, which could also promote the growth and survival of embedded cells (Tai et al. 2019; Jiménez et al. 2019). One of the most abundant, accessible, affordable, and practical polymers used in bioink fabrication is cellulose. The plant-sourced renewable biopolymer is further processed to form highly ordered cellulose nanomaterial. These nanocellulose-derived polymers have proven to be viable for bioprinting applications due to the high surface area to volume ratio, mechanical strength, durability, and modifiable chemical structure – allowing versatility in the ink application. These nanomaterials vary in origin and hierarchical organization leading to differences in the final form, such as cellulose nanofibers (CNF), cellulose nanocrystals (CNC), and bacterial nanocellulose (BNC) (Moniri et al. 2017; Azeredo et al. 2019).

Cellulose fibers treated with mechanical shearing processes such as grinding and high-pressure mixing lead to microfibrils (also termed, nanofibers). These fibers can also be obtained by chemical treatments such as acid hydrolysis, enzymatic reactions, and oxidation reactions on extracted raw pulp (Moniri et al. 2017; Azeredo et al. 2019). This would break the interfibrillated hydrogen bonds and form fibers in micrometer size length and nanometer size width. Cellulose nanocrystals are whisker-shaped or rod-like particles with dominant crystalline regions obtained by acid hydrolysis of all noncrystalline regions, leaving the remaining crystals in nanometer dimension. Finally, bacterial nanocellulose is considered the purest form of cellulose and is synthesized by several microbial genera including the well-known *Acetobacter* in culture medium (Börjesson and Westman 2014).

Cellulose nanocrystals (CNC) has been incorporated into bioink composites to improve the mechanical strength and shear thinning behavior of the ink (Jessop et al. 2019; Müller et al. 2017). In a study by Wu et al. (2018), a hybrid bioink of alginate and cellulose nanocrystals was used to construct a liver-mimicking construct. The polymer composite was mixed with trypsinized fibroblast and human hepatoma cells. The cell-laden ink was then extruded through a nozzle and molded into a

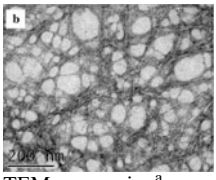
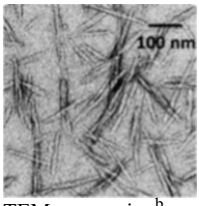
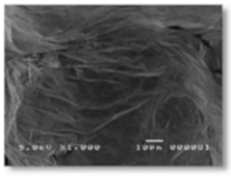
slab, which was further cross-linked in $CaCl_2$ bath in supplemented $CaCl_2$ medium. Results showed minimal cell death and demonstrated the composite would be a good potential bioink. Certain highly biocompatible and cell-growth enhancing polymers exhibit poor viscosity properties and structural integrity; with the addition of nanocellulose material the overall rheology of the ink would change (Table 1).

In another study by Shin et al. (2017), inclusion of CNFs in a gelatin methacrylamide bioink demonstrated an increase in mechanical stability allowing the printing of nose and ear constructs (Fig. 11). The structural integrity of the hydrogel network was maintained for over 7 days while absorbing culture medium. Fixed concentrations of gelatin methacrylamide and variable concentrations of CNF (ranging from 0 to 2.0% w/v) showed that an increase in the compressive modulus of the resulting hydrogels was directly correlated to the increase in the CNF concentration.

The incorporation of nanocellulose materials in bioink has not only improved mechanical integrity of the scaffolds but has improved biochemical and biophysical behavior of ECM to promote the interaction of cells and its environment.

Cellulose nanofibers have been used in combination with other polymers to form scaffolds that are physically and chemically tuned to be biocompatible, capable of vascularization, and scalable in production. Cytotoxicity tests done in an

Table 1 Description of the three types of nanocellulose

	Fabrication	Microscope images	Dimensions
Cellulose nanofibers (CNF)	Acidic or enzymatic treatment before or after High pressure homogenization Grinding microfluidization	 TEM suspension[a]	5–20 nm width, varying length in μm
Cellulose nanocrystals (CNC)	Acidic hydrolysis of wood pulp Enzymatic hydrolysis	 TEM suspension[b]	5–70 nm width, 100–250 nm in length (from plant cellulose), 100 nm to several μm from other sources
Bacterial nanocellulose (BNC)	Metabolic by-product of microbial strains in the presence of a low-molecular weight sugars, alcohols, oxygen, and low pH	 SEM surface fibers	20–100 nm width, varying length in μm

Source: Images adapted from [a]Wu et al. (2018) TEM image of CNF and [b]Chen et al. (2013) TEM image of CNC reprinted with permission of ©SpringerNature

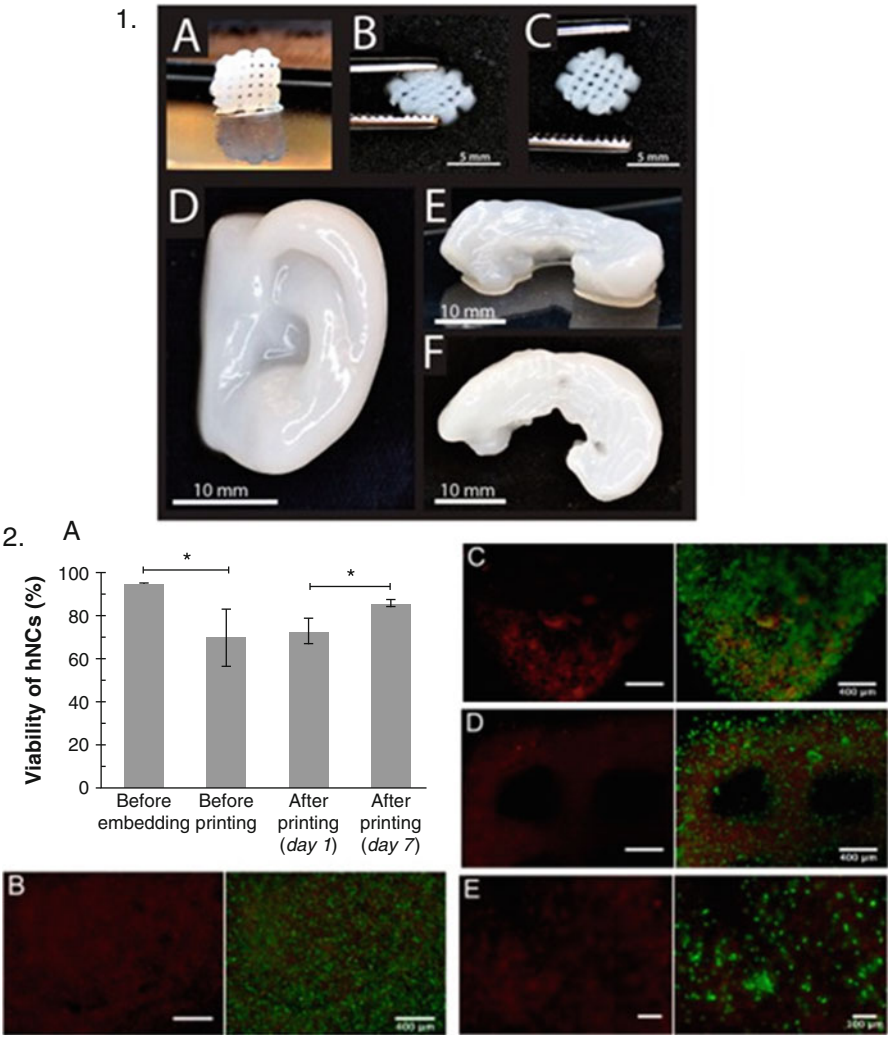


Fig. 11 (1) Cellulose composite bioink used to print ear constructs that were later cross-linked and B. (before) and C. (after) squeezing; (2) Cell biocompatibility on printed structures with dead (red) and live (green) cells B. (before) and C. (after) hNC (human nasoseptal chondrocyte) cells were cultured for 1 day while D. and E. images depict 4x and 10x magnifications after 7 days of culture. (Source: Adapted images borrowed from Markstedt et al. (2015) reprinted with the permission of © American Chemical Society)

investigation by Markstedt et al. (2015) showed that bioprinting with human nasoseptal cells was successful after the homogeneous distribution of the cells in both nonprinted and 3D bioprinted constructs. The bioink was composed of nanofibrillated cellulose and alginate mixtures, which were cross-linked by $CaCl_2$ after careful deposition. After printing and cross-linking, the printed structures

maintained their shape and resembled the 3D human printed ear and sheep meniscus. Multiple studies have shown that with the versatility of nanocellulose as a composite in bioink, the application must be considered for the successful proliferation and differentiation of the cells. Factors such as porosity and thickness would influence the diffusion of cells and nutrients throughout the scaffold. Furthermore, the anchoring ability of cells onto the surface of the material is also an important feature. Complex structures bio-inspired by nature allows the subsequent modification of the material for specific applications.

Bacterial nanocellulose is recognized for its unique origin and high purity compared to its CNF and CNC counterparts. The microbial nanofibers have demonstrated potential in more specialized applications for biomimetic and stimuli-responsive constructs, which can be used to model drug delivery systems or simulate bacterial resistance. Inclusion of bacterial cellulose in bioink composites is being continuously studied for innate features of the micro−/nanofibers that can provide the microporous environment required for cell proliferation and structural reinforcement. Huang et al. (2019) conducted a study testing the bioink potential of extracted silk derivatives together with bacterial cellulose nanofibers (BCNF) synthesized using *A. xylinum* strains (Fig. 12). Scaffolds were synthesized with predetermined amounts of silk, BCNF, glycerol, and genipin. This study also conducted subcutaneous implantation on mice under the dorsal skin for 4 weeks. Results showed that bioink with incorporated BCNF improved the degradability of the scaffolds and therefore the tissue response as the cells differentiated. This material also showed to

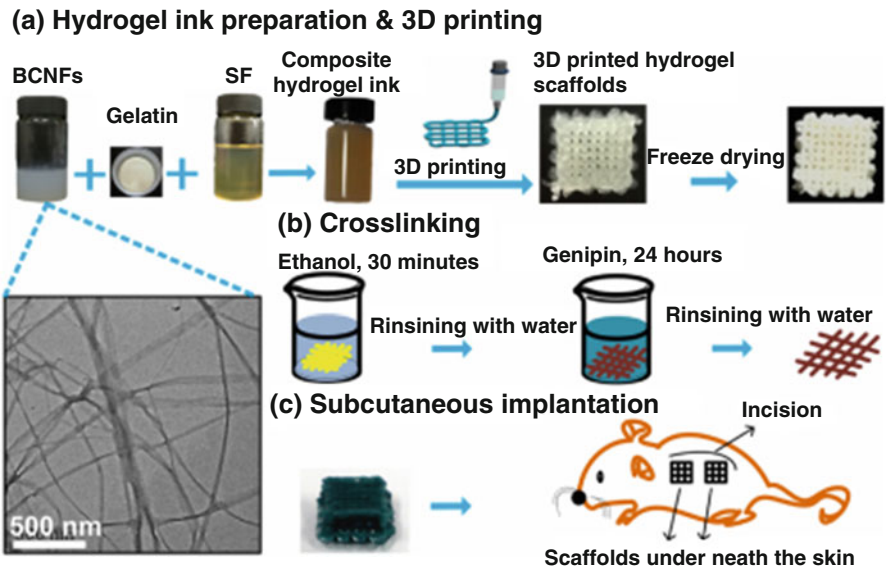


Fig. 12 Schematic diagram of the composite preparation of SF/gelatin-BCNF mixture to form hydrogel scaffolds followed by subcutaneous implantation of the 3D bioprinted construct. (Source: Huang et al. (2019) reprinted with the permission of © Elsevier)

trigger moderate acute inflammatory tissue response during the initial implantation mimicking the bio-responsive feature of this material. The resulting porous hydrogel also allowed cells to infiltrate and further accelerate the regeneration of new tissues within the scaffold. This proved that BCNF plays a unique role in providing the structural integrity and mechanical features for cells to successfully differentiate and form tissues that follow biochemical and biophysical cues.

Nanocellulose materials have demonstrated to be high quality nanocomposites for the improvement in printing ability, mechanical strength, and cell compatibility. Not only is this material sustainable, environment-friendly, affordable, and nontoxic, but the adaptability of the simple structure of cellulose holds much more potential in further functionality. A range of cross-linking methods can be applied to the glucan-modified units to not only meet the requirements of 3D bioprinting technology, but to cater to the environment-responsive cells. While nanocellulose has been well explored as a material, its potential as a bioink composite in more specialized applications for larger-scale tissue engineering is still being continuously studied.

4.2 Alginate Applications

Alginate is a naturally sourced polymer extracted from brown seaweed, which consists of linear copolymers of α-D-mannuronic acid (M residue) and β-L-guluronic acid (G residue) linked by 1,4-glycosidic bonds (Tai et al. 2019). The distribution and length of the corresponding M and G residues would differ, attributing to the unique physiochemical properties of the alginate. Algal genera such as *Laminaria, Macrocystis, Ascophyllum*, and *Ecklonia* to name a few, are responsible for the formation of the unbranched polysaccharides in their cell walls (McCarthy et al. 2019a). This biopolymer is preferred over other polymers for its accessibility, low toxicity, slow biodegradability, and hydrogelation for 3D bioink composites. Alginate is one of the most popular polysaccharides used in 3D bioprinting, which requires a fast gelation process. This is achieved by alginate solutions forming ionic interchain bridges in the presence of multivalent cations. The hydrogel formation of alginate with the addition of calcium or magnesium ions is said to form an "egg box" structure where the ions bind guluronic acid and mannuronic acid blocks (Wan et al. 2009). Researchers have exploited the simple structure of alginate through the extraction and organization of these blocks to engineer alginate for use in specialized applications. Blocks composed of consecutive G residues and M residues intertwine with alternating M and G residues varying in length and sequence. G blocks increase the gel-forming potential while MG and M blocks contribute to the flexibility of the biomaterial (Lee and Mooney 2012).

Depending on the bioprinting method used, bioink suitable polymers require a certain rheology and degradative profile for the successful culture and proliferation of seeded cells. Molecular weight of the alginate, alginate concentration, and cell density would contribute to the overall rheology of the composite. An increase in molecular weight can lead to a highly viscous alginate solution, which is undesirable and difficult to process. More importantly, high viscosity can lead to damaging cells or proteins embedded in the solution due to shear forces while extruding through a

nozzle. A study by Freeman et al. (2017) demonstrated that by maintaining a low viscosity while achieving a desirable elastic modulus can be done by mixing both high and low molecular weight alginate polymers (Fig. 13). Relative to other polymers, alginate has a facile printing and handling method while providing a layer of protection around the cells it encapsulates.

Furthermore, a factor that influenced the mechanical properties of the alginate bioink post-print was the young's modulus where a study found that with an increase in cross-linker used (i.e., $CaSO_4$, $CaCO_3$, $CaCl_2$) a positive correlation was observed (Fig. 13). Varying alginate to cross-linker ratios showed an increasing trend in stiffness and mechanical integrity. This addressed the problem of pure alginate solutions failing to maintain structural integrity after printing layer-by-layer scale-ups because of the low phase changing temperature and shear thinning characteristic of the polymer.

A plethora of studies have proven the necessity to mix alginate with other polymers to form composites that would meet the prerequisites of bioink status. Table 2 describes the various bioink formulations that catered to specific applications, which allowed for the successful proliferation and viability of cells post-print. Despite alginate hydrogels' ease of use, the polymer had an additional downside including its limited degradation. The degradation rate of the polymers would contribute to the overall integrity and mechanical strength of the printed 3D constructs. This would also have a factor in the cell spreading, adhesion and proliferation of cells seeded in the constructs. A group of researchers found that when collagen or agarose was incorporated into the alginate solution, the mechanical strength and, therefore, bioactivity of the bioinks improved (Yang et al. 2018).

Similarly, this was reaffirmed by Luo et al. (2020) in their study on gelatin-alginate composites modified by cellulose nanofiber, resulting in thermal-responsive prints and observable ECM accumulation applicable for cartilage tissue applications.

Formulated bioinks are not only applicable to either vascular, bone, or cartilage tissue, but are tunable and versatile in the addition of growth factors and bioactive compounds. Alginate-based materials continuously evolve in complexity by the enhancement of the marine-sourced polymer through chemical modification and conjugation of its structure. Alginate used along with other polymers only widens the different possibilities and resulting effects of the combined features the materials bring. Together with the fast-paced development of 3D bioprinting technologies, the versatile material is said to pave the way for revolutionized bioink-ready polymers.

4.3 Other Examples

Although bioinks composed of cellulose and alginate have proven to be the most popular in recent bioprinting studies – a plethora of naturally occurring polysaccharides have yet to be fully studied in their potential as a bioink component. Polysaccharides such as xanthan, gellan, dextran, and hyaluronic acid are just a few of the many bacterial polymers employed by researchers today. With the larger array of

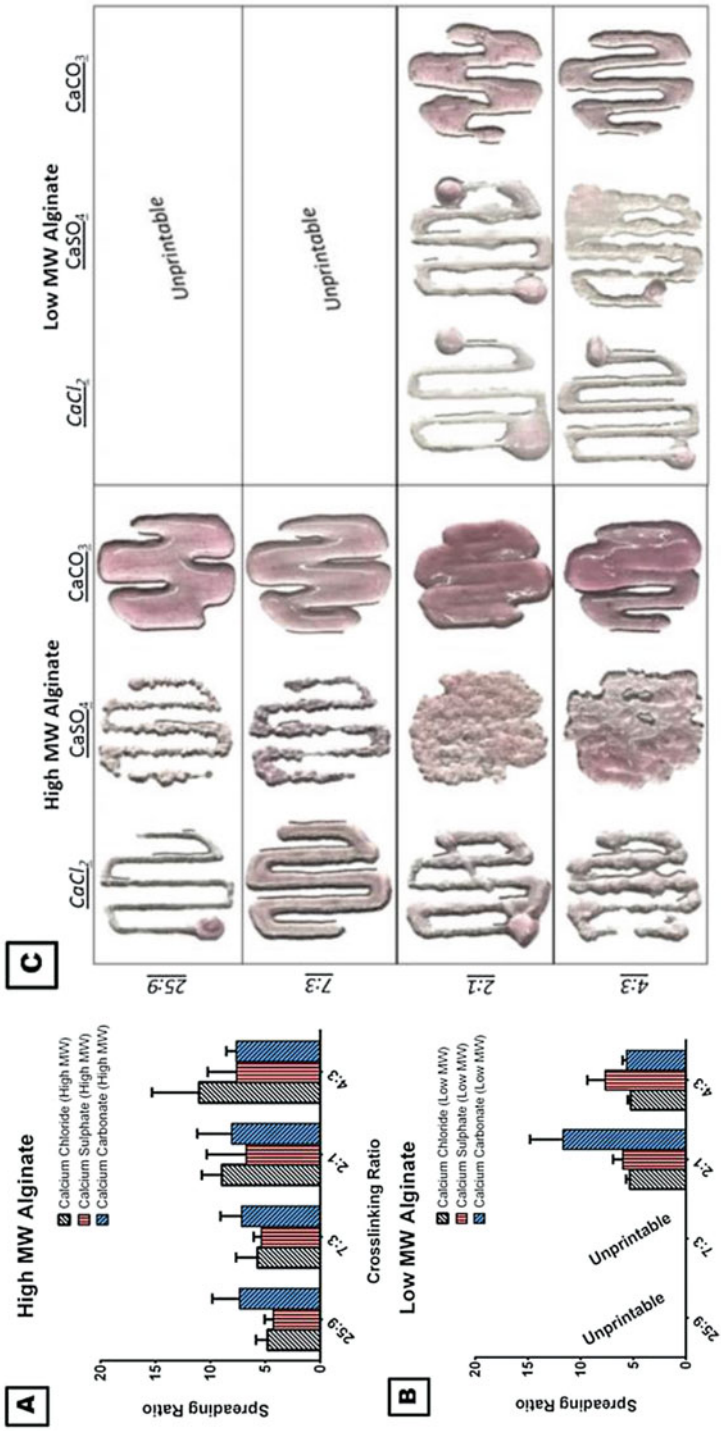


Fig. 13 Examining the printability of (**c**) alginate-based bioinks. Graphs comparing ratios of (**a**) high molecular weight alginate and (**b**) low molecular weight alginate to cross-linkers. (Source: Reproduced from Freeman et al. (2017))

Table 2 Alginate-incorporated bioink for 3D bioprinting applications

Cross-linking technique	Composition	Cells used	Effect	Reference
	Alginate-gelatin	Human corneal epithelial cells (hCECs)	High printing resolution, cell viability, good mechanical properties	Wu et al. (2016)
Ionic $CaCl_2$,$CaCO_3$ $CaSO_4$	Alginate-gelatin mixture with cellulose nanofiber (CNF)	Rabbit fibrochondrocyte cells (rFCs)	Cell viability maintained post print, ECM accumulated	Luo et al. (2020)
	Nanofibrillated cellulose with alginate	Induced pluripotent cells (iPS)	High survivability of cells post-print	Nguyen et al. (2017)
	Alginate with collagen (type I) or agarose	Chondrocytes	Improved mechanical strength	Yang et al. (2018)
	Alginate with chitosan; double cross-linked with HCl solution	Human corneal epithelial cells (hCECs)	Mechanically strong, higher strain in compression tests	Liu et al. (2018)
Covalent	Methacrylated-alginate /PEG	(mouse) Calvaraia osteoblast precursor cells (MC_3T_3)	Enhanced proliferation, cell attachment improved	Samorezov et al. (2015)
UV	Norbornene methylamine conjugated alginate/LAP	Adriamycin-resistant cells (L929)	Alginate printable at low conc., range of mechanical properties	Ooi et al. (2018)

materials to choose from and a diverse set of cross-linking mechanisms, the possible formulations of bioink are exponential.

Xanthan gum (XG) is an exopolysaccharide of the microorganism *Xanthomonas campestris*. Found to be soluble in cold water, xanthan gum solutions exhibit unique pseudoplastic behavior and synergistic interactions with a class of compounds called galactomannans. As described above, XG is made up of a cellobiose backbone with repeating unit and side chains of a trisaccharide: D-mannose (β-1,4), D-glucuronic acid (β-1,2), and D-mannose. These side chains are attached to alternating glucose residues through an α-1,3-linkage. The branched structure of the gum polymer is capable of building not only physical networks, but also chemical networks. XG is a suitable bioink composite due to its range of cross-linkers with itself using adipic acidic dihydrazide or citric acid, for example. These cross-linking mechanisms compensate for the lack of form and shape fidelity in stand-alone solutions. Xanthan gum also offers "softness" and flexibility allowing for the elasticity and spatial requirement for cells to proliferate. Although not as popular as its counterparts, xanthan gum has been increasingly studied due to its interactions with other

polymers to provide potential microenvironments for cells to grow and differentiate. Not only is this material versatile in its in vivo applications but is also stable under hydrolytic and enzymatic conditions (Petri DFS 2015).

Gellan gum (GG) is a water-soluble anionic polysaccharide excreted by the microorganism *Sphingomonas elodea* also known as *Pseudomonas elodea*. At an industrial scale, the polymer is fermented and can result in two forms: one with high acyl content and one with low acyl content. Like alginate, gellan is nontoxic and if injected into tissues, immunogenic. Despite its weak structural integrity, it is also used for cell encapsulation and drug delivery systems. In terms of bioprinting, the inclusion of gellan in a bioink composite is found to be more biocompatible and highly favorable for the recognition, adhesion, and eventual proliferation of cells. In a study by Koivisto et al. (2019), a bioink composite made up of gellan gum and gelatin was used to mimic the biochemical attributes of the extracellular matrix. GG can provide the flexibility, structural support, and hydrogelation properties while gelatin allowed for cell attachment to occur. These GG-gelatin hydrogels were found to be successful through the "beating" of the human cardiomyocytes measured over 24 h using a phase contrast video software.

Dextran is a bacterial hydrophilic polysaccharide composed of α-1,6-linked D-glucopyranose widely used in the pharmaceutical and biomedical field due to its biocompatible and nontoxic features. Like other polysaccharides, dextran can be chemically modified to improve the degree of networking within a construct. A study by Pescosolido et al. (2011) demonstrated that by combining the viscoelastic and favorable biochemical features of hyaluronic acid and a chemically modified dextran derivative, a bioink with the correct viscosity resulted in a printed scaffold that retained the desired shape and design. The scaffold was also found to have high porosity and tunable fiber spacing/orientation. High porosity would permit the diffusion of nutrients and waste products as cells undergo their usual biochemical pathways to proliferate.

Glycosaminoglycans (GAGs) are no strangers to the biomaterial field. This class of naturally sourced polymers is reputable for its role in significantly increasing bioreactivity and biocompatibility as an additive in bioink composites. One GAG is **hyaluronic acid (HA)** also known as hyaluronan. It is a polysaccharide composed of D-glucuronic acid and *N*-acetyl-D-glucosamine. Found in the ECM of cells, HA is known to exhibit distinguishable biocompatibility and biodegradability properties. The polymer can also be degraded in the presence of enzymes such as hyaluronidase, β-glucuronidase, and β-*N*-acetyl-glycosaminidase into smaller molecular weight compounds. HA can be used as an additive in bioprinting to control the viscosity and bioreactivity of the bioink. Although HA is known to be highly water-soluble leading to printed constructs with weak infrastructure, researchers have found ways to make HA more hydrophilic by conjugating hydrophobic moieties along the chain using cross-linking techniques. Another method is to reinforce HA (Fig. 14) by conjugating methacrylate groups, added in the presence of a photo initiator, followed by UV-exposure that would cause for a denser network to form (Poldervaart et al. 2017).

Recent studies require even more research on the effects of these anionic polysaccharides due to their valuable inherent characteristics that play important

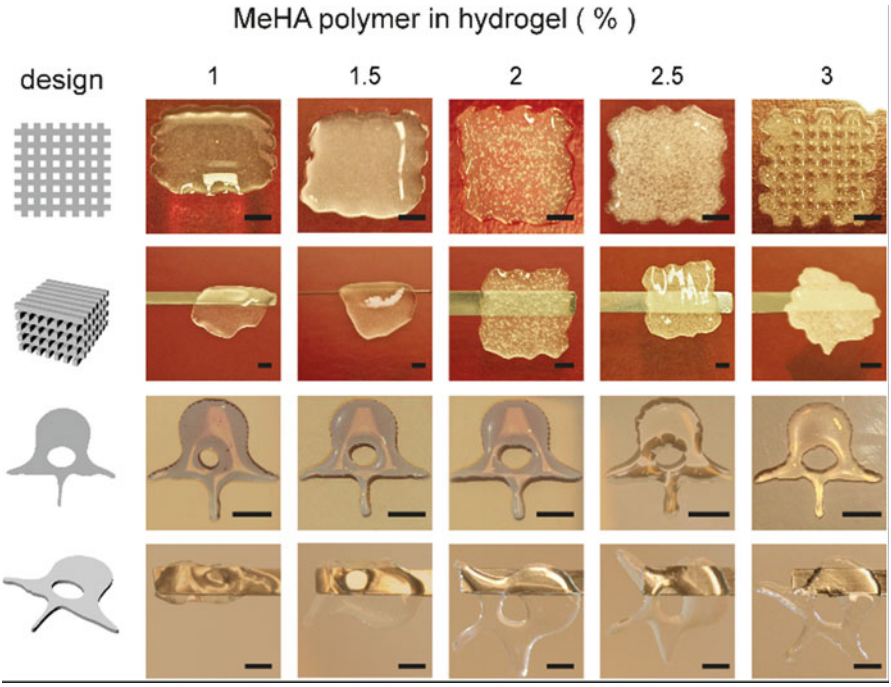


Fig. 14 Images of MeHA (methacrylated hyaluronic acid) printed constructs followed by UV-treatment; porous cubes (first and second row) and non-porous vertebrae shapes (third and fourth row) using designs on the left pane (scale bar = 500 μm). (Source: Borrowed from Poldervaart et al. (2017) with the permission of PLOS ONE)

roles in influencing angiogenesis and/or inducing biochemical processes to occur. Despite the shortcomings, studies carried out have proven that with modifications to the side chains of these polymers, the material is built more suitably as a component in a bioink formulation successfully meeting the delicate balance between tolerance of bioprinter shear strains and biochemical adaptiveness for the nurturing of stem cells.

5 Conclusion

The use of microbial polysaccharides in 3D printing applications and notably in regenerative medicine and food engineering revealed their potential in literature but not really in industry where these applications are still primitive. The development of « intelligent polysaccharides » with specific properties such as antibiofilm or other biological activities as that of printing technologies will probably open the way to a commercialization of microbial polysaccharide-based biomaterials in high value markets of tissue engineering and regenerative medicine (Tai et al. 2019). Indeed, the requirements of regenerative medicine for biocompatible hydrogels is probably

the greatest opportunity for 3D printing of microbial polysaccharides (McCarthy et al. 2019a,b; Tai et al. 2019). Very recently, Tai et al. (2019) described the high potential of anionic microbial exopolysaccharides such as β-(1,4)-polyglucuronic acid (glucuronan) from *Sinorhizobium meliloti* M5N1CS. This full anionic cellulose mimetic with high molecular weight (more than 500 kDa) has very good gelling properties allowing the production of thermostable hydrogels in the presence of divalent cations (Ca^{2+}, Cu^{2+}, and Ba^{2+}) with potential in skin regeneration (Tai et al. 2019). Consequently, as frequently performed with alginate, this anionic polysaccharide (glucuronan) and derivatives could constitute the new generation of bioink for 3D bioprinting development in regenerative medicine.

Moreover the development of the molecular gastronomy always at the research of new textures after physical and chemical transformations of ingredients is a good opportunity for microbial polysaccharides having unique rheological properties and, for some of them such as gellan, curdlan, or xanthan, the status of food additives.

Another future opportunity for microbial polysaccharides could be in a next future the emergence of 4D printing technology. Four-dimensional printing was introduced in 2013 in several research areas including biomedical applications and notably tissue and organ regeneration (Ge et al. 2013). It consists of building 3D structures incorporating in them materials able to self-transform after printing depending on external stimuli and physicochemical environment parameters, leading to memory materials. The controlled degradation of 3D printing architectures could be, in this way, classified also as a 4D effect. Recently, a hydrogel composite ink composed of stiff cellulose and acrylamide was successfully printed and led to a biocompatible hydrogel swelling readily in water. The swelling behavior was under the control of cellulose fibrils alignment. This material can be assimilated to a plant cell wall-inspired architecture changing shape depending on immersion in water (Gladman et al. 2016). Native or modified microbial polysaccharides are well known for their ability to be used as smart materials and several studies have been published focusing on this subject. Their implementation in 4D printing strategies could easily lead to the obtaining of controlled biodegradable hydrogels sensitive to external stimulus including magnetic fields, humidity, temperature, pH, light, and others (Tamay et al. 2019). Some of these polysaccharides have been always described and their production is under control. For example, a family of smart water-soluble pullulan, xanthan, and hyaluronan with (thermo)-associative properties influencing by polysaccharide concentration, or salinity, have been described (Hamcerencu et al. 2011; Mocanu et al. 2011; Niang et al. 2016).

References

Azeredo HMC, Barud H, Farinas CS, et al. Bacterial cellulose as a raw material for food and food packaging applications. Front Sustain Food Sys. 2019;18:1–14.

Baran EH, Erbil HY. Surface modification of 3d printed PLA objects by fused deposition modeling: a review. Colloids Interfaces. 2019;3(2):43.

Berteau O, Mulloy B. Sulfated fucans, fresh perspectives: structures, functions, and biological properties of sulphated fucans and an overview of enzymes active toward this class of polysaccharide. Glycobiology. 2003;13:29–40.

Börjesson M, Westman G. Crystalline nanocellulose- preparation, modification and properties. IntechOpen; 2014. p. 159–82.

Chen P, Yu H, Liu Y, Chen W, Wang X, Ouyang M. Concentration effects on the isolation and dynamic rheological behavior of cellulose nanofibers via ultrasonic processing. Cellulose. 2013;20:149–157.

Cote GL, Ahlgren JA. Microbial polysaccharides. In: Kirk-Othmer, editor. Encyclopedia of chemical technology. New York: Wiley; 2000. p. 578–611.

Cruz D, Vasconcelos VMV, Pierre G, et al. Exopolysaccharides from cyanobacteria: strategies for bioprocess development. Appl Sci. 2020;10:3763.

Della Giustina G, Gandin A, Brigo L, et al. Polysaccharide hydrogels for multiscale 3D printing of pullulan scaffolds. Mater Design. 2019;165:107566–75.

Dizon JRC, Espera AH, Chen Q, et al. Mechanical characterization of 3d-printed polymers. Addit Manuf. 2018;20:44–67.

Florea M, Hagemann H, Santosa G, et al. Engineering control of bacterial cellulose production using genetic toolkit and a new cellulose-producing strain. PNAS. 2016;113:E3431–40.

Freeman FE, Kelly DJ. Tuning alginate bioink stiffness and composition for controlled growth factor delivery and to spatially direct MSC fate within bioprinted tissues. Sci Rep. 2017;7:1–12.

Gaignard C, Laroche C, Pierre G, et al. Screening of marine microalgae: investigation of new exopolysaccharide producers. Algal Res. 2019;44:101711.

Garcıa-Ochoa F, Santos VE, Casas JA, et al. Xanthan gum: production, recovery, and properties. Biotechnol Adv. 2000;18:549–79.

Ge Q, Qi HJ, Dunn ML. Active materials by four-dimension printing. Appl Phys Lett. 2013;103:131901.

Giavasis I, Harvey L, McNeil B. Gellan gum. Crit Rev Biotechnol. 2000;20:177–211.

Gibson I, Rosen DW, Stucker B. Sheet lamination processes. In: Additive manufacturing technologies: rapid prototyping to direct digital manufacturing. Boston: Springer US; 2010.

Gibson I, Rosen D, Stucker B. Binder jetting. In: Additive manufacturing technologies: 3D printing, rapid prototyping, and direct digital manufacturing. Springer, New York; 2015a.

Gibson I, Rosen D, Stucker B. Directed energy deposition processes. In: Additive manufacturing technologies: 3D printing, rapid prototyping, and direct digital manufacturing. New York: Springer; 2015b.

Gibson I, Rosen D, Stucker B. Vat photopolymerization processes. In: Additive manufacturing technologies: 3D printing, rapid prototyping, and direct digital manufacturing. New York: Springer; 2015c.

Gladman AS, Matsumoto EA, Nuzzo RG, et al. Biomimetic 4D printing. Nat Mater. 2016;15:413–8.

Godoi FC, Bhandari BR, Prakash S, et al. Chapter 1: An introduction to the principles of 3D food printing. In: Fundamentals of 3D food printing and applications. London: Academic; 2019.

González-Henríquez CM, Sarabia-Vallejos MA, Rodriguez-Hernandez J. Polymers for additive manufacturing and 4d-printing: materials, methodologies, and biomedical applications. Prog Polym Sci. 2019;94(1):57–116.

Hamcerencu M, Desbrières J, Khoukh A, et al. Thermodynamic investigation of xanthan-poly (N-isopropylacrylamide) hydrogels. Polym Int. 2011;60:1527–34.

Hirai A, Tsuji M, Horii F. TEM study of band-like cellulose assemblies produced by *Acetobacter xylinum* at 4°C. Cellulose. 2002;9:105–13.

Holland S, Foster T, Tuck C. Chapter 9: Creation of food structures through binder jetting. In: Fundamentals of 3D food printing and applications. London: Academic; 2019.

Huang L, Du X, Fan S, et al. Bacterial cellulose nanofibers promote stress and fidelity of 3D printed silk-based hydrogel scaffold with hierarchal pores. Carbohydr Polym. 2019;221:146–56.

ISO/ASTM. Additive manufacturing – general principles – terminology (iso/astm 52900:2015). 2020(Jun 24). 2015. https://www.iso.org/standard/69669.html

Jessop ZM, Al-Sabah A, Gao N, et al. Printability of pulp derived crystal, fibril and blend nanocellulose-alginate bioinks for extrusion 3D bioprinting. Biofabrication. 2019;11:1–17.
Jiménez M, Romero L, Domínguez IA, et al. Additive manufacturing technologies: an overview about 3D printing methods and future prospects. Complexity. 2019;2019(1):30.
King WE, Anderson AT, Ferencz RM, et al. Laser powder bed fusion additive manufacturing of metals; physics, computational, and materials challenges. Appl Phys Rev. 2015;2(4):041304.
Kirchmajer DM, Gorkin Iii R, In Het Panhuis M. An overview of the suitability of hydrogel-forming polymers for extrusion-based 3d-printing. J Mater Chem B. 2015;3(20):4105–17.
Kodama H. Automatic method for fabricating a three-dimensional plastic model with photo-hardening polymer. Rev Sci Instrum. 1981;52:1770–3.
Koivisto JT, Erring C, Karvinen J, et al. Mechanically biomimetic gelatin-gellan gum hydrogels for 3D culture of beating human cardiomyocytes. ACS Appl Mater Interfaces. 2019;11(23):20580–602.
Kothari D, Das D, Patel S, et al. Dextran and food application. In: Ramawat KG, Mérillon JM, editors. Polysaccharides: bioactivity and biotechnology. 1st ed. Cham: Springer; 2014. p. 735–52.
Lee KY, Mooney DJ. Alginate: properties and biomedical applications. Prog Polym Sci. 2012;37 (1):106–26.
Lee J-Y, An J, Chua CK. Fundamentals and applications of 3d printing for novel materials. Appl Mater Today. 2017;7(1):120–33.
Lehninger AL. In: Nelson DL, Cox MM, editors. Principles of biochemistry. 5th ed. New York: W.H. Freeman; 2008, 1158 pp.
Liu Q, Li Q, Xu S, et al. Preparation and properties of 3D printed alginate-chitosan polyion complex hydrogels for tissue engineering. Polymers. 2018;10(664):1–13.
Liu J, Sun L, Xu W, et al. Current advances and future perspectives of 3D printing natural-derived biopolymers. Carbohydr Polym. 2019;207:297–316.
Luo W, Song Z, Wang Z, et al. Printability optimization of gelatin-alginate bioinks by cellulose nanofiber modification for potential meniscus bioprinting. J Nanomater. 2020;2020:1–13.
MacDonald E, Wicker R. Multiprocess 3D printing for increasing component functionality. Science. 2016;353(6307):2093.
Markstedt K, Mantas A, Tournier I, et al. 3D bioprinting human chondrocytes with nanocellulose – alginate bioink for cartilage tissue engineering applications. Biomacromolecules. 2015;16:1489–96.
McCarthy RR, Ullah MW, Booth P, et al. The use of bacterial polysaccharides in bioprinting. Biotechnol Adv. 2019a;37(8):107448.
McCarthy RR, Ullah MW, Pei E, et al. Antimicrobial inks: the anti-infective applications of bioprinted bacterial polysaccharides. Trends Biotechnol. 2019b;37(11):1155–9.
Mocanu G, Mihai D, Dulong V, et al. Anionic polysaccharide hydrogels with thermosensitive properties. Carbohydr Polym. 2011;83:52–9.
Mohammed JS. Applications of 3D printing technologies in oceanography. Methods Oceanogr. 2016;17:97–117.
Mondschein RJ, Kanitkar A, Williams CB, et al. Polymer structure-property requirements for stereolithographic 3D printing of soft tissue engineering scaffolds. Biomaterials. 2017;140(1):170–88.
Moniri M, Moghaddam AB, Azizi S, et al. Production and status of bacterial cellulose in biomedical engineering. Nanomaterials. 2017;7:257.
Morris G, Harding S. Polysaccharides, microbial. In: Encyclopedia of microbiology. 3rd ed. Amsterdam: Elsevier; 2009.
Morris ER, Nishinari K, Rinaudo M. Gelation of gellan – a review. Food Hydrocoll. 2012;28: 373–411.
Müller M, Öztürk E, Arlov Ø, et al. Alginate sulfate nanocellulose bioinks for cartilage bioprinting applications. Ann Biomed Eng. 2017;45:210–23.
Nguyen D, Hägg DA, Forsman A, et al. Cartilage tissue engineering by 3D bioprinting of iPS cells in a nanocellulose/alginate bioink. Sci Rep. 2017;7(658):1–10.
Niang PM, Huang Z, Dulong V, et al. Thermo-controlled rheology of electro-assembled poly-anionic polysaccharide (alginate) and polycationic thermo-sensitive polymers. Carbohydr Polym. 2016;139:67–74.

Ooi HW, Mota C, Cate ATT, et al. Thiol-ene alginate hydrogels as versatile bioinks for bioprinting. Biomacromolecules. 2018;19(8):3390–400.
Park JK, Khan T. Chapter 21: Other microbial polysaccharides: pullulan, scleroglucan, elsinan, levan, alternant, dextran. In: Philipps GO, Williams PA, editors. Handbook of hydrocolloids. 2nd ed. Duxford: Woodhead Publishing; 2009. p. 592–614.
Paul F, Morin A, Monsan P. Microbial polysaccharides with actual potential industrial applications. Biotechnol Adv. 1986;4:245–59.
Pescosolido L, Schuurman W, Malda J, et al. Hyaluronic acid and dextran-based semi-IPN hydrogels as biomaterials for bioprinting. Biomacromolecules. 2011;2011(12):1831–8.
Petri DFS. Xanthan gum: a versatile biopolymer for biomedical and technological applications. App Polymer. 2015;132(23):1–13.
Poldervaart MT, Goversen B, De Ruijter M, et al. 3D bioprinting of methacrylated hyaluronic acid (MeHA) hydrogel with intrinsic osteogenicity. PLoS One. 2017;12(6):e0177628.
Samorezov JE, Morlock CM, Alsberg E. Dual iconic and photo-crosslinked alginate hydrogels for micropatterned spatial control of material properties and cell behavior. Bioconjug Chem. 2015;26(7):1339–47.
Severini C, Derossi A, Ricci I, et al. Printing a blend of fruit and vegetables. New advances on critical variables and shelf life of 3D edible objects. J Food Eng. 2018;220(1):89–100.
Shin S, Park S, Park M, et al. Cellulose nanofibers for the enhancement of printability of low viscosity gelatin derivatives. Bioresources. 2017;12:2941–54.
Stanton M, Trichet-Paredes C, Sánchez S. Applications of three-dimensional (3D) printing for microswimmers and bio-hybrid robotics. Lab Chip. 2015;15:1634–7.
Sun J, Zhou W, Huang D, et al. An overview of 3D printing technologies for food fabrication. Food Bioprocess Technol. 2015;8:1605–15.
Sutherland IW. Bacterial exopolysaccharides. In: Boons G-J, Kamerling JP, Lee YC, Suzuki A, Taniguchi N, Voragen AGJ, editors. Comprehensive glycoscience: from chemistry to system biology, vol. 2. Oxford: Elsevier B.V.; 2007. p. 521–57.
Tai C, Bouissil S, Gantumur E, et al. Use of anionic polysaccharides in the development of 3D bioprinting technology. Appl Sci. 2019;9(13):2596.
Tamay DG, Usal TD, Alagoz AS, et al. 3D and 4D printing of polymers for tissue engineering applications. Front Bioeng Biotech. 2019;7:164.
Wan LQ, Jiang J, Arnold DE, et al. Calcium concentration effects on the mechanical and biochemical properties of chondrocyte-alginate constructs. Cell Mol Bioeng. 2009;1(1):93–102.
Wu Z, Su X, Xu Y, et al. Bioprinting three-dimensional cell-laden tissue constructs with controllable degradation. Sci Rep. 2016;6(2016):1–10.
Wu Y, Lin ZY, Wenger AC, et al. 3D bioprinting of liver-mimetic construct with alginate/cellulose nanocrystal hybrid bioink. Bioprinting. 2018;9:1–6.
Yanagawa F, Sugiura S, Kanamori T. Hydrogel microfabrication technology toward three dimensional tissue engineering. Regen. Ther. 2016;3:45–57.
Yang X, Lu Z, Wu K, et al. Collagen-alginate as bioink for three-dimensional (3D) cell printing based cartilage tissue engineering. Mater Sci Eng C. 2018;83(2018):195–201.
Yap YL, Wang C, Sing SL, et al. Material jetting additive manufacturing: An experimental study using designed metrological benchmarks. Precis Eng. 2017;50(1):275–85.
Zhang W, Ullah I, Shi L, et al. Fabrication and characterization of porous polycaprolactone scaffold via extrusion-based cryogenic 3D printing for tissue engineering. Mater Des. 2019;180:107946.
Zhang H, Zhang F, Yuan R. Applications of natural polymer-based hydrogels in the food industry. In: Chen Y, editor. Hydrogels based on natural polymers. 1st ed. Cambridge: Elsevier; 2020. p. 357–410.
Ziaee M, Crane NB. Binder jetting: a review of process, materials, and methods. Addit Manuf. 2019;28(1):781–801.

Chitosan-Based Gels for Regenerative Medicine Applications

51

Deepti Bharti, Bikash Pradhan, Sarika Verma, Subhas C. Kundu, Joaquim Miguel Oliveira, Indranil Banerjee, and Kunal Pal

Contents

D. Bharti · B. Pradhan · K. Pal (✉)
Department of Biotechnology and Medical Engineering, NIT, Rourkela, India

S. Verma
Council of Scientific and Industrial Research Advanced Materials and Processes Research Institute (CSIR-AMPRI), Bhopal, India

S. C. Kundu · J. M. Oliveira
3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Zona Industrial da Gandra, Barco, Guimarães, Portugal

ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães, Portugal
e-mail: kundu@i3bs.uminho.pt; miguel.oliveira@i3bs.uminho.pt

I. Banerjee
Department of Bioscience and Bioengineering, IIT, Jodhpur, India

J. M. Oliveira et al. (eds.), *Polysaccharides of Microbial Origin*,
https://doi.org/10.1007/978-3-030-42215-8_65

Abstract

The tissue repair and regeneration process often require implants that can help in the healing of damaged tissue. A naturally obtained polysaccharide chitosan has properties like processability, biocompatibility, biodegradability, and non-immunogenicity. The presence of an amino group and hydroxyl group provides the scope of many functionalizations to the chitosan. Chitosan gels (nano, micro, and macro) can be prepared either by physical, ionic interactions, or chemical methods. Poor solubility of chitosan is sometimes held responsible for its limited application. Many strategies have been adapted for the fabrication of gels made up of chitosan and its derivative to overcome this limitation. Chitosan gels have been explored by researchers for several applications in the arena of regenerative medicines. The gels of chitosan are having pH sensitivity, temperature sensitivity, and ion-sensitivity behavior. They have been used as a carrier for bioactive molecules, proteins, peptides, and living cells which can aid in the healing of damaged tissues and have been successfully tested in the in vitro and in vivo models. Chitosan gels have the potential to provide an appropriate microenvironment for the living cell to grow and proliferate. A vast number of chitosan-based gels, viz., nanogels, microgels, and macrogels, and their gelation mechanism have been discovered for bone, cartilage, skin, tissue regeneration, which has been discussed in detail in the chapter.

Keywords

Chitosan · Crosslinking · Nanogel · Microgel · Macrogel · Regeneration

1 Introduction

The use of gel is extensive and growing rapidly in various filed at the present time. The growing application of gels in the field of regenerative medicine is appreciable. In this regard, the gels can act as a substitute of extracellular matrix (ECM) for a variety of tissue repair and regeneration and can facilitate targeted therapeutic delivery. Based upon the unit size of network, gels can be classified as nano (1–100 nm)-, micro (0.1–700 μm)-, and macro (>1 mm)-gels. Micro- and nanogels have been explored for drug delivery in quite many cases. A few of the crucial requirements of the biomaterials used for any such application will be biocompatibility, biodegradability, and nonimmunogenicity. Since most of the naturally derived macromolecules possess all these features, researchers' attention in exploring different natural polymers is rising with time. Starch, alginate, cellulose, and chitosan are a few of the commonly utilized polysaccharides obtained from plants, microorganisms, and insects (Takada and Kadokawa 2015). These naturally occurring polysaccharides usually act as an energy provider and structural component for functional bio-based materials. The intra- as well as intermolecular hydrogen bonds present among the hydroxyl group of saccharides are often responsible for their low

solubility, further assisting in gelation. Chitosan, chemically (1-4)-2-amino-2-deoxy-β-d-glucan, is one of the most explored naturally obtained linear polysaccharides. Chitosan can be derived from chitin's deacetylation, which is an abundant polysaccharide and is extensively found in the exoskeleton of crustaceans. Apart from crustaceans' shells, chitosan can also be obtained from fungi and insects' cell walls (Fig. 1). The deacetylation can occur either by enzymatic reaction or under the hot alkali treatment (Pal et al. 2013).

Structurally, chitin is made from the glycosidic linkage of N-acetyl-glucosamine (NAG) and N-glucosamine. The period of alkali deacetylation governs the degree of deacetylation plus molecular mass of chitosan. Therefore, chitosan can be defined as a shared term used for de-N-acetylated chitin with diverse degrees of deacetylation, molecular weight, and viscosity. The percentage deacetylation of the commercially available chitosan is nearly 60–100%. The broad scope of functionalization of chitosan comes from the three reactive groups present in them, that is, an amino group, a primary hydroxyl group, and a secondary hydroxyl group (Fig. 2). These groups are located at second, third, and sixth carbon positions, respectively. By these functional groups, chitosan can undergo many modifications like acylation, alkylation, Schiff base formation, carboxymethylation, and others (Shi et al. 2006). Due to the free amino group's ionization at lower pH, chitosan's dissolution is possible at the pH value lower than its pKa (~6.5). The presence of an amino group makes

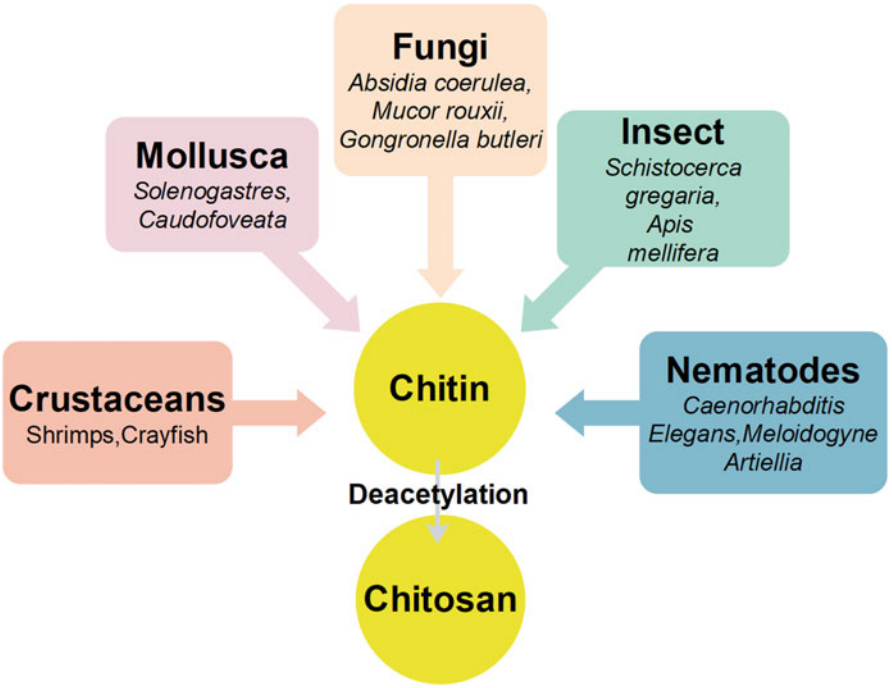


Fig. 1 The schematic diagram indicates the different sources of chitosan in nature

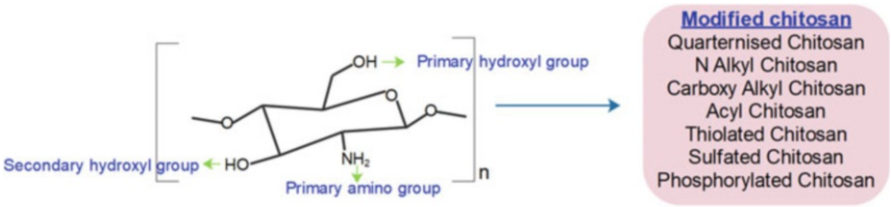


Fig. 2 Functional group responsible for chitosan modification

chitosan a cationic polyelectrolyte. The possibility for chitosan to interact with polyanions is widely used to develop macro-, micro-, and nanogels. Similarly, the chitosan's mucoadhesive property is contributed by the free amino group by which they form bonds with mucin(negatively charged), present in the mucosal layer of the humans. The cationic nature of chitosan gives an opportunity to interact with anionic glycosaminoglycans (GAG) existing in the extracellular matrix of human tissues (Iovene et al. 2021). Along with its cationic characteristics, nontoxicity, biodegradability, biocompatibility, antibacterial, anti-inflammatory, and antimicrobial activity are a few other associated properties of chitosan (Kravanja et al. 2019).

Chitosan gels have shown much successful application in the field of regenerative medicines and tissue engineering. They act as carriers for various bioactive/pharmacological molecules at the same type as crucial scaffold components for successful tissue regeneration. The biodegradability products of chitosan have shown safe interaction in in vivo and in vitro conditions (Pang et al. 2017). Chitosan gels can be added as a scaffold to modify the inner structure, texture, and mechanical property, which are acute for cell growth and proliferation. These gels are reported to have special self-healing features, pH sensitivity, temperature sensitivity, and ionic sensitivity. The application and benefits of chitosan gels offer a broad possibility of tissue regeneration which is witnessed in wound healing, gene therapy, and tissue engineering (Sultankulov et al. 2019; Shi et al. 2006). Similarly, a group of pH/thermoresponsive injectable gels of chitosan has been majorly studied for the regeneration of bone and dental tissue (Tang et al. 2020). This chapter has summarized the gelling concepts of gels made up of chitosan and its derivatives. Further, the application of these gels in regenerative medicine as promising vehicles for drug delivery, cell therapy, and tissue engineering has been discussed in detail.

2 Mechanism of Chitosan Gelation

In recent times, different methodologies for the development of chitosan gels have been adapted. These methods can be classified as physical gelation, self-assembly, ionic gelation, polymerization, coacervation, and cryogelation. The methodology for the fabrication of chitosan gels will differ based on mechanism, reaction conditions, and characteristics which are being discussed in the subsequent section.

2.1 Physical Gelation

The physical gelation method of synthesizing chitosan gels is interesting because it does not involve using an emulsifier, crosslinker, or chemical for inducing gelation. The conversion from solution to gel is a highly tunable process that can be achieved by altering the physical parameters such as pH, temperature, and ionic strength. The van der Waals force and electrostatic interactions are the two vital forces that are involved with the physical gelation of chitosan. One of the approaches to form a physical gel with physical gelation is to perform controlled re-acetylation of a fully deacetylated chitosan (Sacco et al. 2018). The formation of chitosan gels through this method occurs in a series of steps. The multifaceted approach and the need for highly specialized equipment have led to a limited number of studies where chitosan gels have been prepared using this method. The re-N-acetylation occurs by using acetic anhydride, which is responsible causes acetylation of chitosan at amino and hydroxyl groups. Here, the proposed gelation mechanism is the molecular aggregation among the acetylated chitosans, which forms a rigid, colorless, and transparent gel. Studies suggest that an increase in the temperature, the concentration of acetic anhydride, and chitosan aid in gelation (Montembault et al. 2005). The formation of gels can also be enhanced in few other possible ways. Firstly, an alteration in the solubility of chitosan can promote polymer-polymer interactions. This can be possible if the repulsive interaction between the chitosan chains can be condensed and achieved by lowering the charge density of chitosan. It has been proposed the application of gaseous ammonia that raises the pH of chitosan solution to 9, which decreases the charge density of the chitosan chains by the deprotonation of amino groups. This phenomenon results in the initiation of the gelation of chitosan molecules (Wang et al. 2017). The chitosan gels, formed from gaseous ammonia to induce physical gelation, have shown regenerative potential in the case of myocardial infarction (Fiamingo et al. 2016). These gels were found to be proper for cardiac tissue engineering and did not display any toxicity. Secondly, the concentration of the chitosan should always be greater than the chain entanglement concentration. The presence of the chitosan molecules above this critical concentration promotes improved networking of the polymeric chitosan chains (Brunel et al. 2008). An extension to the above work was performed by Brunel et al. (2013). They have successfully loaded copper in chitosan gel using the above technique. The chitosan gel thus fabricated was explored for the antimicrobial role (Brunel et al. 2013). In the typical pH range of 4 and 6, chitosan behaves like polyelectrolytes where they can make bonds with oppositely charged small ions like Cu^{2+}. Altogether, chitosan physical gels are an attractive system due to their low toxicity and easy tunability.

2.2 Self-Assembly

Gels formed by the self-assembly of chitosan have shown great potential as delivery systems with better encapsulation and precise release of bioactive compounds (Debele et al. 2016). Self-assembly of chitosan occurs by forces like ionic bonding,

hydrogen bonding, van der Waals interaction, and covalent interaction. Researchers have explored many organic acids, proteins (e.g., bovine serum albumin), peptides, and salts to form self-assembled chitosan structures. As mentioned above, chitosan readily interacts with the acids like ethylenediaminetetraacetic acid, benzoic acid, caffeic acid, and methacrylic acid by creating an amide group in the middle of the amine group of chitosan and the carboxylic group of acids (Wang et al. 2017). Likewise, peptides and larger globular proteins can form self-assembled chitosan nanogel via electrostatic interaction. The significant parameters of consideration during the formation of these gel are the concentration of chitosan, surface charge density, particle size distribution, and turbidity (Rusu et al. 2020). Therefore, these factors are also known as quantitative functional parameters of the formed gel. The fabrication of chitosan gel via this method is more straightforward and convenient. Simultaneously, unlike the physical gelation of chitosan, there is no dedicated step required for the separation of formed gel in this case. A pH-sensitive gel was prepared with the self-assembly of chitosan and ovalbumin (Yu et al. 2006). The core of the nanogel is formed owing to the crosslinking of chitosan with ovalbumin. Even though the gels, fabricated using this method, have come forward as a sound delivery system, their clinical usefulness must be explored thoroughly. The use of self-assembly has been evaluated with luminescent gold (Au) nanocrystals placed in chitosan gel that can be applied in developing sensors and biomedical devices. This method eases the accessibility of control and targeted release of drugs to specific environment. The photoluminescence of the gels can be improved by the use of Au nano crystal of dissimilar emission color and different thiolate ligands which is known to form complex with metal nano crystals (Goswami et al. 2016). The thiolate ligand provides the negative charge of its carboxylic group to interact with the positive amine group of the chitosan.

2.3 Radical Polymerization

A chemical process of transformation of small molecules into larger ones is called polymerization. As a prerequisite for radical polymerization, chitosan is usually modified to gain polymerizable groups. This can be achieved through the process of styrenation and methacryaltion. Styrenation involves combining chitosan with monomer like alpha-methyl-styrene. However, methacryaltion involves the modification of glycol chitosan with glycidyl methacrylate (GMA) to obtain *N*-methacrylate glycol chitosan (MGC). MGC is a water-soluble chitosan derivative. The polymerization can be done either by increasing temperature or exposing the chitosan solution to UV light (Brunel et al. 2013). Photopolymerization can occur by the exposure of modified chitosan to UV light in the existence of a photoinitiator (Hu et al. 2012). Despite the disadvantage arises from the use of inorganic chemicals as initiators or crosslinker in this process, it has still shown successful application in regenerative medicines and drug delivery.

The process of photopolymerization starts with the free radical generated by the photoinitiator molecules when the reaction mixture is irradiated with light. The free

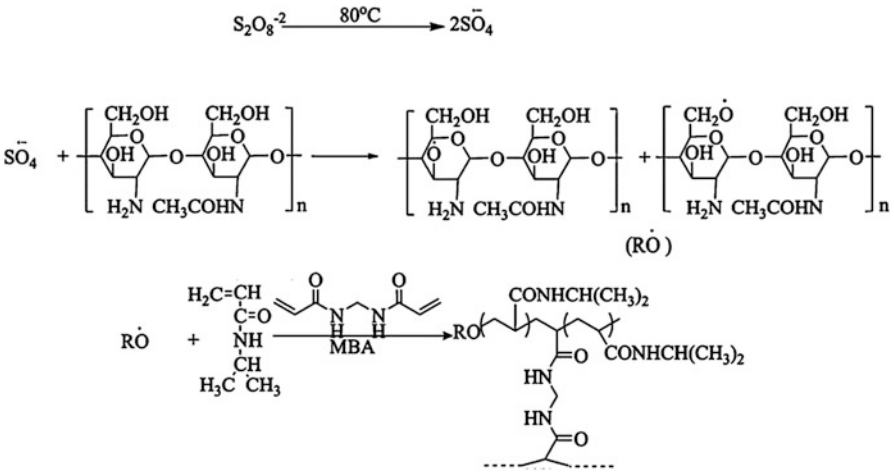


Fig. 3 Radical copolymerization for the formation of chitosan nanogel. (Reproduced with permission from Duan et al. 2011a; License number: 5052591486565)

radical transforms the double bond of the polymerization functional groups of chitosan into radicals. This is marked as the beginning of the chain reaction, which propagates to form a crosslinked network. MGC can crosslink upon UV exposure and has been successfully explored for cell encapsulation and drug delivery studies (Amsden et al. 2007). Poly (*N*-isopropyl acrylamide) (PNiPAm) interacts with acrylic acid-functionalized chitosan derivatives via radical polymerization to form pH or thermos-responsive gel. PNiPAm has gained much attention recently as the lowest critical solution temperature (LCST) value of PNiPAm is close to the body temperature (Peniche et al. 1999). The hydrophobic interaction of PNiPAm is dominant at a temperature above LCST. At this temperature, crosslinking occurs using ammonium persulfate as an initiator of the reaction and *N*, *N*-methylenebisacrylamide as a crosslinking agent (Fig. 3) (Duan et al. 2011a). The initiator molecule helps forming sulfate anion, which interacts with the hydroxyl group of chitosan to form alkoxy radicals. In the presence of the crosslinking agent, these alkoxy radicals start the copolymerization of PNiPAm onto chitosan. Apart from the temperature and pH sensitivity characteristics, these chitosan nanogels also enhance drug activity and improve the target release. The chitosan gel developed with this method holds many applications in drug delivery and regenerative medicines.

2.4 Ionic Gelation

One of the commonly adapted fabrication methods for chitosan gel having high water content is ionic gelation. Herein, the gelation occurs between chitosan and other polymers (e.g., alginate, hydroxyapatite) decorated with functional groups of opposite charges. Several parameters, including temperature, pH, time, molecular

mass, molar ratio, flow rate, and mixing speed, can help in the precise ruling of the dimensions of the formed gel. The technique of ionic gelation to form chitosan gel has been applied to polymer-drug interaction, the structure and function studies in tissue-specific regeneration, and the absorption/release mechanism of pharmacological compounds. Polyphosphate is often used due to its nontoxicity. Its multivalent nature readily forms ionic gels with chitosan (Pant and Negi 2018). The polyphosphate is dissolved in an acidic solution where polyanionic polyphosphate is added dropwise to obtain cationic charge chitosan (Debnath 2011). The interaction takes place amid the positive charge amino group of chitosan and negatively charged phosphate. The size of the gel can be controlled by regulating various parameters such as concentration of polyphosphate, pH, and degree of deacetylation of chitosan (Huang and Lapitsky 2017). These chitosan gels are recognized for their ability to promote the bioavailability and stability of pharmacological compounds. Chitosan and polyphosphate system is one of the most broadly explored gel systems, studied for drug delivery and tissue regeneration. These gels have been extensively studied along with other polymers and additives like alginate, polysorbate, mannose, BSA, glucosamine, etc. (Wang et al. 2017).

The ionic gelation method is also examined to look for the effect of the surface charge of the gel upon absorption at the intestinal segments. Insulin is a commonly used treatment modality in the case of diabetes mellitus. A regular injection of insulin in patients results in a reduction in the quality of life. Since charged particles are quickly taken up by cells, insulin-incorporated chitosan and carboxy-methyl chitosan (CMCS) gels have been explored for this purpose (Fig. 4) (Wang et al. 2016). The weight ratio of CMC-to-chitosan is used to maintain different surface charges.

2.5 Coacervation

Coacervation is a method of phase separation between the two liquid phases. This is one of the most adapted techniques for the microencapsulation of drugs and bioactive agents. The gel size and homogeneity prepared from this method can be modified by altering the molecular weight and amount of the polymer used. The basic principle behind coacervation is the difference in the ionic forces of the polymer that can be adjusted with the knowledge of isoelectric point (pI). The process depends upon the involvement of the number of polymers and is majorly categorized into two sets: simple and complex coacervation.

2.5.1 Simple Coacervation

The simple coacervation involves the use of only one polymer. In this process, the induction of phase separation of the polymer occurs by the addition of salts that have less affinity towards the polymer than the water. This can even be achieved with an alteration in the environmental conditions (e.g., pH and temperature) that can promote aggregation of the polymer chains. Simple coacervation is used to prepare

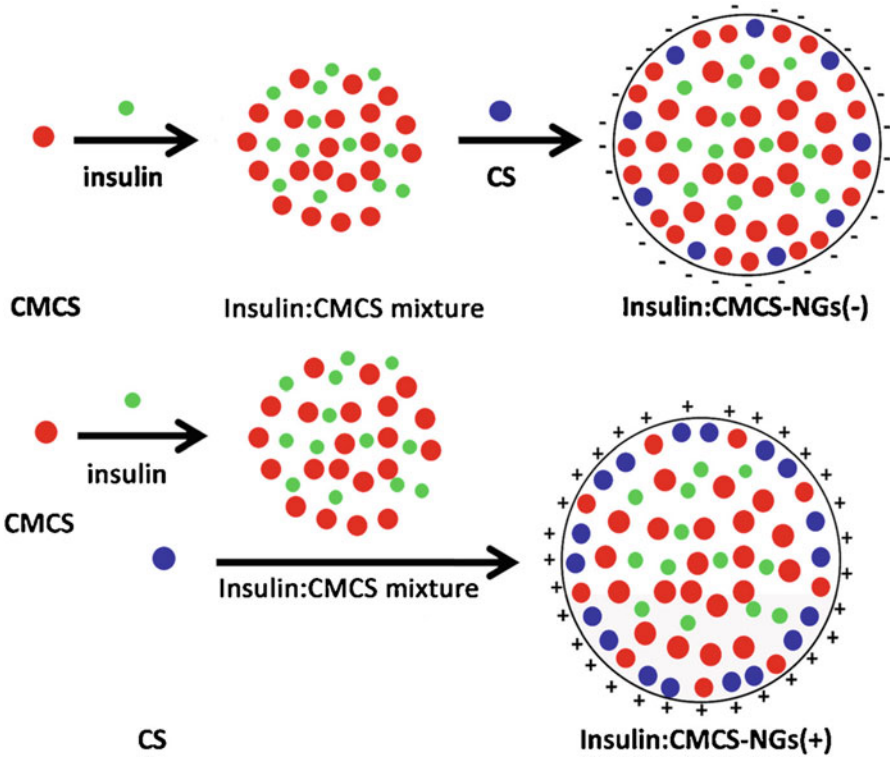


Fig. 4 The schematic representation of chitosan(CS)/CMCs nanogel loaded with insulin. (Reproduced with permission from Wang et al. 2016; License number: 5053671234643)

crosslinked chitosan gels using epichlorohydrin or glutaraldehyde that are known to react with the amine group present in chitosan (Gonçalves et al. 2005).

2.5.2 Complex Coacervation

In a system that is made up of mixed polymers, thermodynamic compatibility and incompatibility are the two possible interactions that can take place among the polymers. At a high concentration and high ionic strength, when polymers carry similar charges, a repulsive force arises between them. This is a scenario of thermodynamic incompatibility, which gives rise to phase separation. On the contrary, low concentration and low ionic strength polymers are thermodynamically compatible due to the presence of a net opposite charge. This state of thermodynamic compatibility is also called complex coacervation. As discussed earlier, the cationic nature of chitosan permits it to form an association with anionic polymers like hyaluronan, alginate, pectins, carrageenans, etc. One of the interesting chitosan coacervation systems is formed with hyaluronic acid (HA). In that case, the gelation is built on electrostatic interaction among the oppositely charged polysaccharides and hydrogen bonding (De La Fuente et al. 2008). Chitosan/HA gels, formed with such type of method,

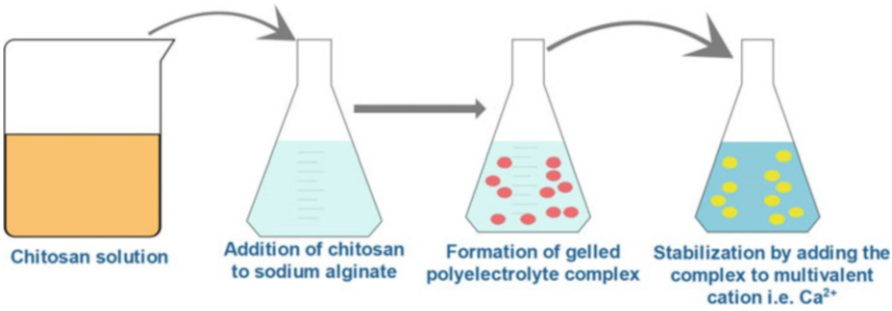


Fig. 5 Formation mechanism of chitosan gel using complex coacervation. (Modified from Pal et al. 2013)

have shown excellent potential to deliver drugs, therapeutic protein, and growth factors in pulmonary ailments (Oyarzun-Ampuero et al. 2009). The coacervated system developed using chitosan and alginate is depicted in Fig. 5 (Pal et al. 2013).

2.6 Cryogelation

Cryogels are fabricated at a cryogenic temperature (semi-frozen conditions), usually below the crystallization point of the solvent used for preparing polymeric solutions. The cryogenic treatment follows some basic steps that include freezing the polymer solutions, maintenance of the frozen state for a definite time, followed by defrosting. The frozen polymeric solutions are heterogeneous. This can be ascribed to the fact that the frozen solvent also consists of unfrozen liquid microphase (UFLMP) (Lozinsky et al. 2003). This phase is usually rich in gel-forming agents and thus intensely promotes the formation of the gels. At a lower temperature, the solvent (water) molecules form ice crystals that grow and combine over time. When melted at room temperature, they leave macropores that are scattered in between the polymeric chains. The three-dimensional supra and macroporous crosslinked matrix of cryogels provide structural and chemical stability to the system. A study in this regard has tried fabricating cryogels using chitosan and HA using glutaraldehyde for crosslinking in the cryogenic mixture (Fig. 6) (Kutlusoy et al. 2017). The mixture was allowed to freeze at −12 °C for gel formation. The chitosan cryogel, when co-polymerized with HA, has shown improvised cell penetration. Controlling the freezing temperature is an efficient way to modulate the pores that are formed in chitosan cryogels. Thus, altering the freezing temperature can help to improvise the physiochemical properties of the prepared gels (Zhang et al. 2019). Another approach to synthesize chitosan cryogel was made by modifying the chitosan (Evans et al. 2021). Through imine bonding of chitosan's amine group, an alkyl chain was introduced into the chitosan backbone. It helped in the improvisation of the hydrophobicity of the chitosan gel. This enhanced hydrophobicity in the cryogel

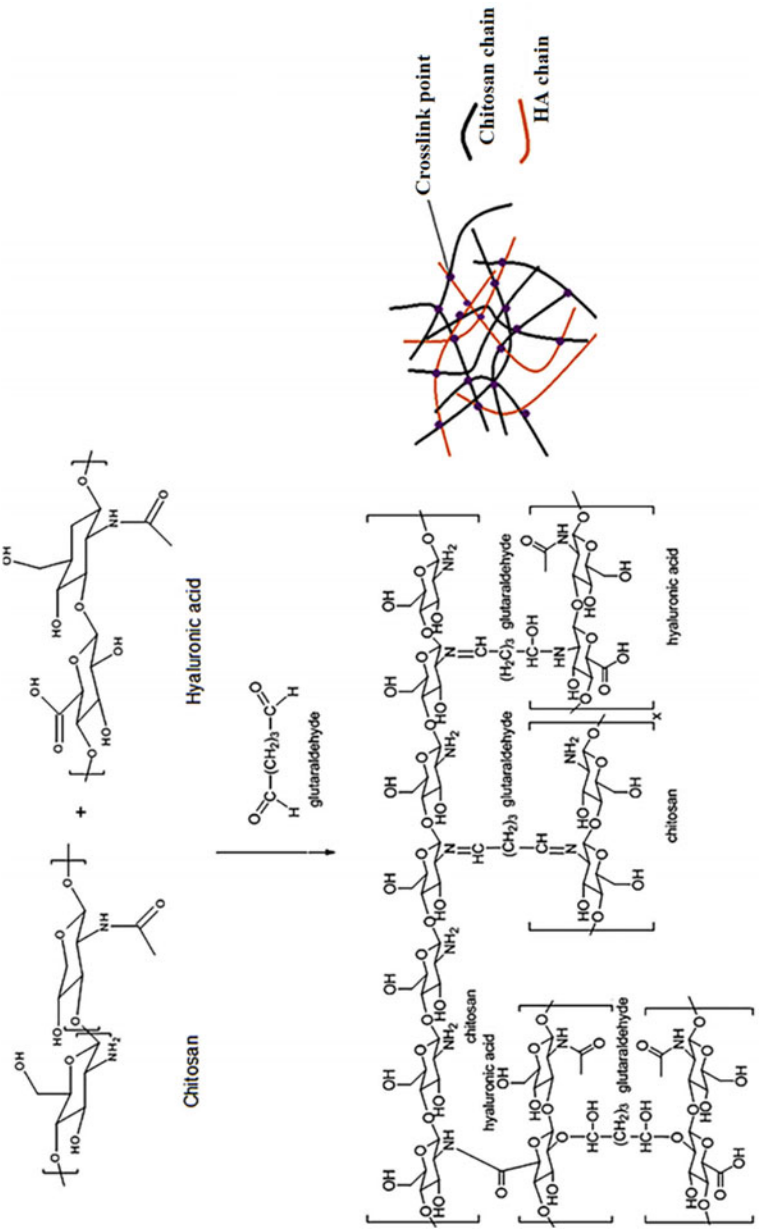


Fig. 6 Fabrication mechanism of chitosan/HA cryogel. (Reproduced with permission from Kutlusoy et al. 2017; License number: 5058181315646)

was useful in the adsorption of various anticancer medicines, which are hydrophobic in nature.

3 Application of Chitosan Gel

3.1 Chitosan Nanogels

Nanoparticles as a delivery vehicle in the pharmaceutical industry have gained enormous attention in the current time. With the advanced investigation in the field of nanotechnology, the importance of nano-sized distribution systems is rising rapidly in the field of regenerative medicines. These delivery systems have beneficial therapeutic effects because of their ability to upsurge the concentration of the pharmacological compounds at the damaged/ target site. As a result, the pharmaceutical compound's systemic contact gets reduced. Further, the varied administration routes of nanoparticles, including parenteral, oral, transdermal, and pulmonary routes, support its wide usage (Islam et al. 2017). Nanogels are 3-dimensional hydrogels formed by the networking of crosslinked polymers (Pathak et al. 2019). Thus, nanogels have a combined property of nanoparticle and hydrogel. Nanogels can retain a large volume of water and can also display high surface energy. The diameter of <200 nm is ideal for cellular uptake of nanogels via receptor-mediated endocytosis. Altogether, nanogels are suitable carriers for chemotherapeutic drugs, different peptides, and siRNA and are advantageous in regenerative medicines. In this regard specifically, chitosan nanogels have widely been researched due to their excellent biological properties. Chitosan nanogels are innovative biomaterials that are used as delivery systems in regenerative medicines and tissue engineering. The chitosan nanogel possesses a greater surface area that allows multiple bioconjugations and provides beneficial loading efficiency of the bioactive agents. Various therapeutic drugs, proteins, and peptides have shown efficient delivery to the target site when delivered through chitosan nanogels. The chemical modification of the chitosan gels can be employed to progress the properties of the nanogels. Among the various chitosan modifying agents, cinnamic acid (CA) is the one with vast biological activities (de Carvalho et al. 2021). The CA-induced modification in chitosan has been explored for the preparation of nanogel. Such nanogels are having a high affinity towards lipophilic substances like essential oils. The essential oil-containing nanogels of chitosan have been reported for improvised antibacterial and antifungal properties.

The nanogel can act as a niche for the growth and proliferation of living cells. Chitosan/TPP nanogel have shown appreciable cellular uptake and metabolic cell activity in the case of a wide variety of cell line (Schütz et al. 2011). Chitosan nanogel has been displayed as a suitable system for wound healing by a wide variety of researchers. Wound healing is a multifaceted biological process involving a cascade of events for skin remodeling. Although it is a desirable process, the treatment through antibiotics is limited in covering all the physiological and biological aspects of the wound. A nanodressing formed by chitosan, pectin, and titanium

oxide was used for wound healing applications (Archana et al. 2013). The NAG existing in chitosan is also a key component of human dermal tissue and thus is crucial for the repair and regeneration of wounds (Singh and Ray 2000). Similarly, the positive surface charge of chitosan encourages cell growth and induces thrombosis and blood coagulation. The gelling properties of pectin and mechanical stability and the antibacterial effect of titanium oxide provided a synergistic effect for the in vitro and in vivo wound healing process. Another moving idea is the inclusion of probiotics or microorganisms that assure health benefits into the chitosan gel system. The probiotics inclusion can modify the immune system to reduce inflammation and faster healing. Chitosan/TPP nanogel include with *Lactobacillus fermentum, Lactobacillus fermentum,* and *Bacillus subtilis* have shown significant wound healing rates in animal models (Ashoori et al. 2020).

A wide range of bone disorders arises due to accidental damage, aging, obesity, or trauma. A traditional method involving a substitute through autografts, allografts, or xenografts is often chosen as the treatment method in such events. However, the increased cost, the possibility of immune rejection, and the availability of grafts always remain a potential limitation of this procedure. The use of nanogels, loaded with factors and bioactive agents, promotes bone regeneration (Stevens 2008). BMP-2 is a vital factor for the investigation of bone repair. For effective repair and regeneration, a sustained release of this factor must occur at the point of damage. Though the positive charge of BMP-2 allows it to bind to surfaces like GAG, surface attachment does not govern for sustained release of proteins. To restore tissue structure and function, growth factors and proteins need to reside in a suitable microenvironment for cell growth and differentiation. TPP crosslinked chitosan gel, prepared after functionalization polycaprolactone fiber, resulted in efficient binding and sustained release of BMP-2 (Sundermann et al. 2021). The application of collagen II and chondroitin sulfate in cartilage regeneration is commendable as they are the chief component of the extracellular matrix of cartilaginous tissue. However, the direct application of these components for cartilage repair is limited because of the possibility of degradation. Therefore, researchers have tried fabricating hydrogel using MGC, further enhanced by collagen II and chondroitin sulfate for cartilage regeneration (Choi et al. 2014). In addition to cartilage tissue repair, chitosan nanogel has also shown a beneficial role in osteochondral defects. A genipin crosslinked nanogel prepared from glycol chitosan and an anionic polysaccharide fucoidan has been used to deliver anti-inflammatory peptides to the in vivo osteoarthritis model (Li et al. 2021). The in vitro study showed downregulation of inflammatory aspects like interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) along with an upregulation in the chondrogenic markers.

Abundant regenerative therapy based on small interfering RNA (siRNA) undergoes difficulty due to their larger size and repulsion from the plasma membrane. Nanogels of glycol-chitosan have been shown to act as a gene (including siRNA) carrier to intracellular compartments (Wang et al. 2017). This was possible as chitosan nanogels form a stable structure with nucleic acids. Additionally, hydrogel prepared from chitosan and bioglass (e.g., containing silica, calcium, and phosphate ions) was used as a plugging material for adequate blood clotting at the injured

site. Gel placed at the site of injury in the liver showed a significant reduction in the mass of blood loss. This can be attributed to the synergistic effect of chitosan and bioglass on the blood clot process. Figure 7 represents the photographic images of the surgical procedure involved in the placement and activity of gel (Sundaram et al. 2019). One potential application of chitosan nanogel has also been observed in periodontal regeneration. The regeneration of tissue (cementum, alveolar bone) supporting the tooth is referred to as periodontal regeneration (Cortellini and Tonetti 2000). Chitosan nanogel has been reported to provide an apposite microenvironment for the differentiation of progenitor cells into preosteoblasts and precementoblasts

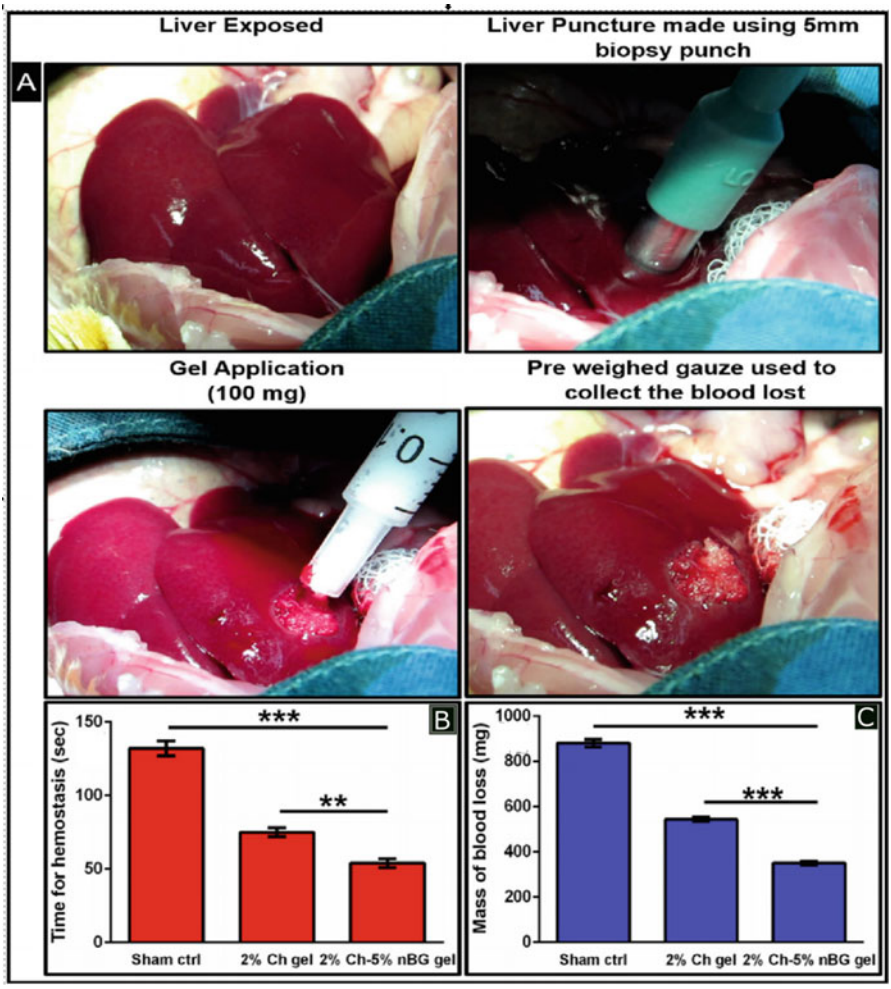


Fig. 7 (**a**) Chitosan(Ch)-bioglass(BG) hydrogel application at the liver site. (**b**, **c**) Quantitative representation of the time required for blood clots and mass of blood loss. (Reproduced with permission from Sundaram et al. 2019; License number: 5073500798929)

Table 1 Fabrication method and application of chitosan nanogel

Composition	Method of fabrication	Potential benefits	References
CMC, Genipin (crosslinking agent)	Ionic gelation followed by chemical cross linking	Enhanced osteoblastic differentiation An increased course of antibacterial efficiency	Meng et al. (2014)
CMC, N,N-Bis(acryloyl) cystamine, Glutaraldehyde (crosslinker)	Covalent crosslinking	Antiprotein adsorption ability Brilliant nonfouling behavior	Zhang et al. (2016)
Chitosan, NIPAM	Self-assembly	Active liver targeting pH-sensitive delivery	Duan et al. (2011b)
Chitosan, gelatin	Ionic-crosslinking	Higher new bone formation Increase expression of osteogenic and angiogenic markers	Oryan et al. (2017)
Chitosan, Recombinant human collagen-peptides	Chemical crosslinking	Improved gelation and mechanical strength Accelerated wound healing	Deng et al. (2021)

(Xu et al. 2012). Table 1 is a brief summary of a number of chitosan nanogel used in tissue repair and regeneration.

3.2 Chitosan Microgel

Microgels are a class of hydrogels that can be fabricated into different sizes and forms, having diameters fluctuating from tens of nanometers to micrometers (Wang et al. 2019). The majority of the therapeutic application requires the smallest amount of bioactive agent and growth factors. However, in a bulk hydrogel, there is a lack of control over the microenvironment in which cells are placed (Alzanbaki et al. 2021). Microgels with tunable size and shape are beneficial in this scenario. Potential features like injectability, porosity, mechanical strength, and size tunability have a crucial role in increasing the demand for microgels in biomedical, tissue engineering, and regenerative medicines. Among the various natural polysaccharides, chitosan has been extensively used to fabricate microgels that can be used for cell encapsulation and placement at the damaged tissue. The many displayed characteristics of chitosan microgel depend upon the size, shape, and mechanical stability of the fabricated microgel. A few of the very recent applications of chitosan microgels have been discussed in the next section.

Many study groups have discussed the use of chitosan microgel as the delivery system for bioactive cues. However, their poor solubility at physiological pH and the need for a powerful purification method limit their application as a cell carrier. Several groups have fabricated chitosan microgel in combination with other

polymers (e.g., alginate, collagen, and dextran) or additives (e.g., hydroxyapatite) to overcome this problem. Chitosan-lactate microgels have been fabricated recently using the approach of microfluidics for better control over the size and a simultaneous encapsulation of cells (Mora-Boza et al. 2021). The in situ gelation occurred in the presence of crosslinkers like glycerylphytate and TPP inside a flow-focusing microfluidic device. The role of glycerylphytate was to improve the biological activity by lowering the cytotoxicity and providing antioxidant property to the microgel. The formed microgel was efficient in MSC encapsulation and showed upregulation of paracrine signaling, which further assists in the remodeling of tissue. However, in large and complex injuries of tissues like bone, there occurs a loss of function and poor regeneration. Among the various regenerative potential of MSC, their ability for osteogenic differentiation is appreciable. Considering the beneficial impacts of chitosan and the availability of cell adhesion motifs in collagen, they were combined to fabricate microgel (60–100 μm diameter) incorporated with MSC (Annamalai et al. 2019). Microgels of chitosan and collagen have shown the potential for providing a specific microenvironment for MSC, and its conformal filling at the damaged site accelerated the bone repair.

Genipin-crosslinked chitosan microgel is yet another polymeric system that has been exhaustively studied for bone, cartilage, and cardiac tissue repair (Yu et al. 2021; Muzzarelli et al. 2015). Researchers have adapted an emulsion-free and solvent-free method for the crosslinking of chitosan with genipin (Erickson et al. 2021). The formed microgel has been tested for growth plate injuries. The growth plate is a type of cartilage tissue that is located at the end of long bones. Since chitosan as biomaterials have been reported for the improvement of chondrogenesis, it has been interpreted that chitosan microgel can aid the growth plate injury and thus can replace the use of surgical procedures. In another study, when microgels were loaded with endothelial cells and mesenchymal cells, they have shown improved angiogenic potential in mice models (Torres et al. 2020). This study is suggestive of the broad application of microgels in tissue repair mechanisms. Further, magnetic chitosan (modified with GMA) microgels have been fabricated to treat gastric wounds (Pellá et al. 2020). The magnetic receptiveness of these microgels improves the concentration of the drug in the gastrointestinal tract. Magnetic field assists in holding the microgel at the desired location where the drug needs to be absorbed. The Cobalt ferrite ($CoFe_2O_4$) nanoparticles provided the magnetic properties to the gel.

As discussed above, it is pretty clear that chitosan microgels are capable of supporting cellular growth and differentiation by providing a suitable microenvironment to the cells. However, one study has even reported the swelling behavior in these gels (Anirudhan et al. 2016). A UV crosslinkable n-pentyl(PTL) group along with a hydrophobic alkyl chain was introduced in chitosan to design a nonswelling microgel. This was possible due to the hydrophobic chain, which extruded water from the gel. The formed gel has shown excellent biocompatibility in both in vivo and in vitro models (Ding et al. 2021). The biological evaluation and desired in vitro and in vivo results of this study have been displayed in Fig. 8. Table 2 overviews the microgels prepared from variety fabrication techniques. Such microgels have shown potential outcomes and thus are explored for a variety of applications.

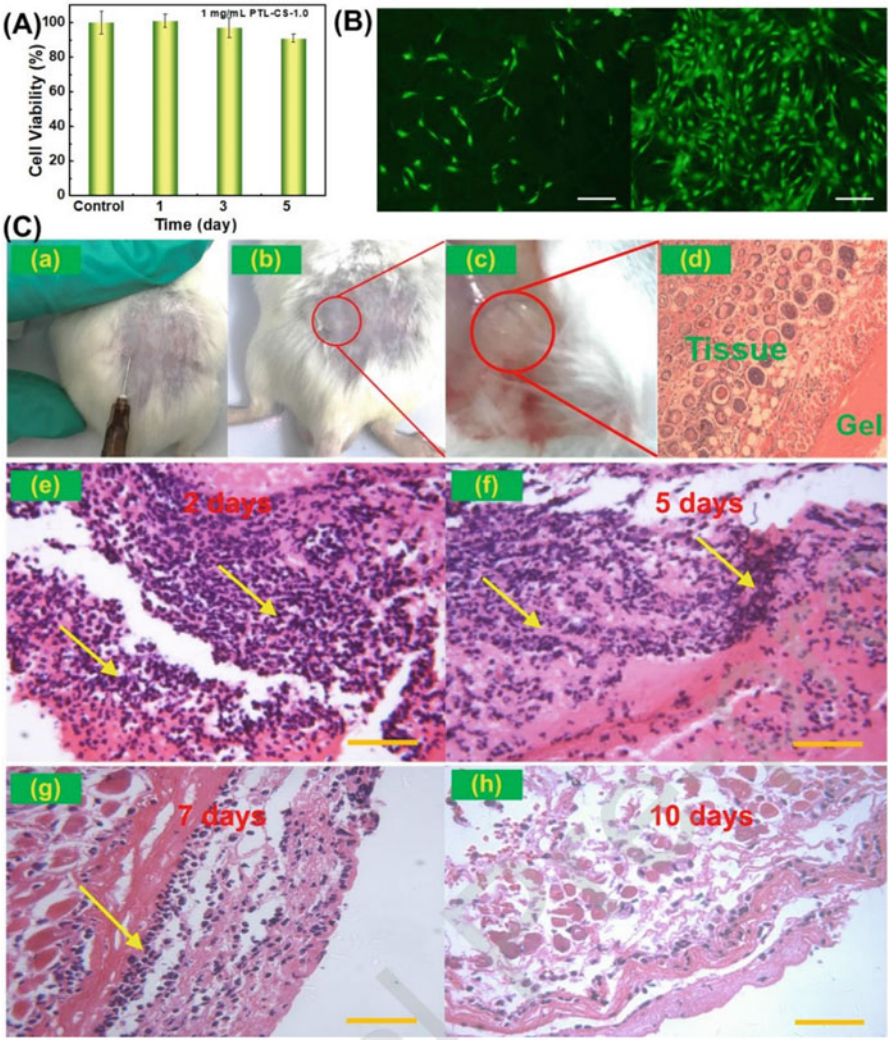


Fig. 8 (**A**) In vitro cell viability and (**B**) live dead assay of pentyl chitosan (PTL-CS) gel. (**C**) in vivo studies using PTL-CS microgel; (**a**–**d**) injection of microgel in the mouse model, (**e**–**h**) histological images where yellow arrow represents the inflammation. (Reproduced with permission from Ding et al. 2021; License number: 5073090252862)

3.3 Chitosan Macrogel

Gels having a particle size greater than 100 μm are categorized under macrogel (de Lima et al. 2020). The ability of the macrogel to swell without compromising the mechanical strength is desirable for the regeneration of many tissues of the human body like muscles, tendons, cartilages, etc. Chitosan macrogel shows good

Table 2 Application of chitosan microgel in tissue repair and regeneration

Composition	Method of fabrication	Potential benefits	References
N-[(2-hydroxy-3-trimethylammonium)propyl]chitosan chloride, TPP(crosslinker)	Ionic gelation	Faster release of chemothrepeutics Cell internalization via receptor-mediated endocytosis	Zhang et al. (2006)
Chitosan	Simple coacervation	Antifungal effect Pronounced encapsulation efficiency	Yuen et al. (2012)
Chitosan, gelatin	Complex coacervation	pH-dependent release of FITC-dextran	Kang et al. (2012)
Chitosan	Simple coacervation	Efficient delivery at the wound site Regeneration in grade-II burn	(Sharifi et al. (2021)

biocompatibility with blood, body fluids, and other tissue, which makes them ideal for their placement in tissue repair.

Chitosan-based macrogel has been extensively used as a biomaterial to promote bone regeneration. Macrogel prepared from the chitosan, glycerophosphate, and graphene oxide (GO) was explored for bone tissue repair (Saravanan et al. 2018). The inclusion of GO in the hydrogel improvised properties like protein adsorption and gelation time. The macrogel helped in promoting osteogenic differentiation of MSC, which was supported by the expression of different osteogenic markers. Hydroxyapatite (HAp) is a mineral constituent of natural bone and thus supports osteoconductive and osteoinductive properties. Therefore, the idea of including HAp in macrogel comprised of chitosan and glycerophosphate was done to improvise the stiffness, strength, and osteogenic potential of the hydrogel (Fig. 9). The in vivo experiment through the HAp incorporated hydrogel showed improvement in the bone defect by promoting bone formation (Dhivya et al. 2015).

Further cartilage regeneration through physically crosslinked chitosan macrogel, whose mechanical properties and strength were enhanced by polycaprolactone, has been evaluated for in vitro studies. Kartogenin, a chondro-inductive biomolecule, was immobilized onto the chitosan macrogel to promote the chondrogenesis of MSC. The in vitro studies using this hydrogel showed an elevation in the chondrogenic differentiation marker, indicating their potential application in osteo-arthritis (Baharlou Houreh et al. 2021). Similar to nanogel and microgel, the role of macrogel in wound healing is also appreciable. Scientists have tried using practical approaches to load drugs, bioactive molecules, proteins, and cell-derived materials in chitosan hydrogel in case of wound healing. One such cell-derived component is exosomes which are referred to as the membrane vesicles, which are derived due to the physiological state of the cell. Their ability to enhance intercellular communication can prove beneficial for skin tissue repair. Chitosan hydrogel prepared from the ionic interaction between the positive charge of chitosan ($-NH_2$ group) and

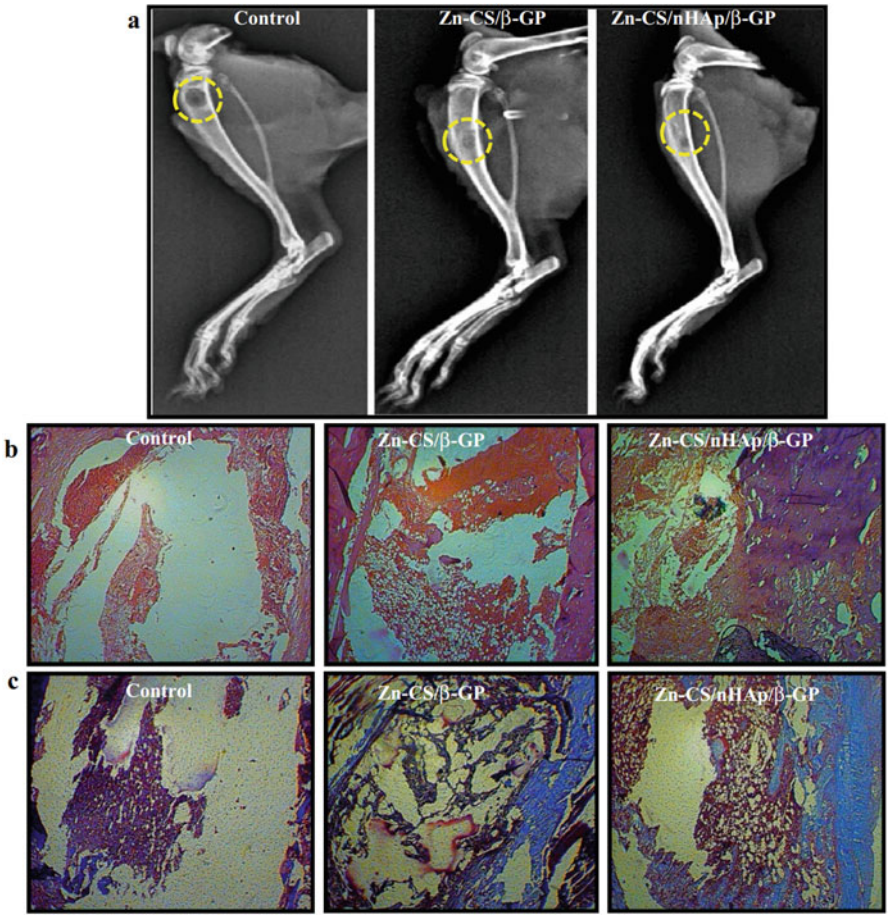


Fig. 9 In vivo bone healing by the application of zinc doped chitosan/nano-hydroxyapatite/β-glycerophosphate (Zn-CS/nHAp/β-GP) (**a**) radiographs of rat tibial defect and repair, (**b**, **c**) hematoxylin and eosin staining and collagen staining, respectively. (Reproduced from Dhivya et al. 2015 under Creative Commons license)

negative glycerol (-OH group) and hydrogen bonding of chitosan chains has been evaluated for this purpose (Nooshabadi et al. 2020). Chitosan/glycerol hydrogel has been previously reported for hemostatic potential and has shown re-epithelization for the progression in wound healing (Alizadeh et al. 2019). Photo-crosslinkable chitosan (Az-CH-LA) containing azide group (photo-reactive) and lactose moieties are capable of forming hydrogel that has shown to be effective in tissue adhesion and sealing in case of wounds (Ishihara et al. 2006). The hydrogel was loaded with fibroblast growth factor (FGF-2) and was applied to the wounded area of two different in vivo models. Chitosan is reported to protect FGF-2 from inactivation that can occur through heat or acid or simply degradation caused by proteases (Salmivirta et al. 1996). Interestingly, all the loaded FGF-2 retained inside the

Table 3 Application of chitosan macrogel in tissue repair and regeneration

Composition	Method of fabrication	Potential benefits	References
Glycol chitosan, HA	Photo-crosslinking	High cell viability Increased deposition of cartilaginous extra cellular matrix	Park et al. (2013)
Chitosan, pectin, cellulose nanocrystal	Chemical crosslinking	Improved mechanical properties Stiffer hydrogels	Ghorbani et al. (2020)
Chitosan, collagen, β-glycerophosphate	Chemical crosslinking	Osteogenic differentiation of MSC Minimally invasive delivery	Wang et al. (2013)
Chitosan, cellulose nanofiber	Physical linkage	Improvement of mechanical properties Restoration and regeneration of intervertebral disc	Doench et al. (2019)

hydrogel, and their release was gradual upon the biodegradation of hydrogel (Ishihara et al. 2006). The hydrogel, due to its adhesiveness and controlled release, can be used as a wound dressing, especially in the case where immediate hemostasis is required. A physically crosslinked chitosan hydrogel has been discovered as an implant material for periodontal regeneration (Yan et al. 2015). For this purpose, periodontal ligament cells (PDLCs) were selected and populated in the hydrogel. The formed hydrogel displayed more significant development in the ligament structure in the rat model. The successful formation of ligament structure can be quantified by the collagen amount and fiber arrangement of newly formed collagen. This was shown to be effective for periodontal regeneration in the animal model. Table 3 summarizes the main applications of chitosan macrogel with improved mechanical and biological properties.

4 Conclusion

Tissue repair and regeneration are the major fields that focus on the development of implants or substitutes for the damaged tissues. In this regard, researchers across the globe are exploring the potential applications of chitosan gels because of their enormous beneficial properties. This includes biocompatibility, biodegradability, antifungal, antibacterial, bioadhesiveness, and encapsulation capacity. At present, much research is going on improvising the fabrication techniques of chitosan gels for exploring their potential application in regenerative medicines. Different approaches to synthesize chitosan-based gels can yield gels with a wide variety of stiffness, mechanical strength, adhesion, as well as cell proliferation potential. These parameters need precise optimization for tissue-specific regeneration. The poor solubility of chitosan at physiological pHs bounds its use in some applications. The chemical modification done to introduce a hydrophilic group on chitosan is often helpful for increasing its solubility. Additionally, the mechanical properties of chitosan gel can be improvised by the accumulation of physical/chemical crosslinkers like glutaraldehyde, and genipin which helps in the formation of interpenetrating polymer

networks of chitosan. Based on the size, these gels are categorized as nano-, micro-, and macrogels. Their potential use in tissue regeneration has been observed due to their ability to provide a suitable microenvironment to the progenitor cells that can differentiate into cells specific to a tissue. The enhancement in the tissue formation by chitosan gel is possible because of their structural resemblance to the extracellular matrix of tissues like bone and cartilage. The success of repair or regeneration of any damaged tissue will depend upon the cell-carrier material. Therefore, chitosan gels have shown successful application in the repair and regeneration of bone, cartilage, periodontal tissue, and skin. Additionally, it can serve as a scaffold/implant for cellular growth and differentiation. However, the clear idea of the involvement of different molecular signals in such pathways needs to be explored even more. The understanding of such a mechanism will be helpful in tissue pathogenesis, and thus, based upon that, new approaches can be adapted for using chitosan gels in regenerative medicines.

References

Alizadeh R, et al. Differentiation of human mesenchymal stem cells (MSC) to dopaminergic neurons: a comparison between Wharton's Jelly and olfactory mucosa as sources of MSCs. J Chem Neuroanat. 2019;96:126–33. https://doi.org/10.1016/j.jchemneu.2019.01.003.

Alzanbaki H, Moretti M, Hauser CAE. Engineered microgels – their manufacturing and biomedical applications. Micromachines. 2021;12(1) MDPI AG:1–19. https://doi.org/10.3390/mi12010045.

Amsden BG, Sukarto A, Knight DK, Shapka SN. Methacrylated glycol chitosan as a photopolymerizable biomaterial. Biomacromolecules. 2007;8(12):3758–66. https://doi.org/10.1021/bm700691e.

Anirudhan TS, Divya PL, Nima J. Synthesis and characterization of novel drug delivery system using modified chitosan based hydrogel grafted with cyclodextrin. Chem Eng J. 2016;284:1259–69. https://doi.org/10.1016/j.cej.2015.09.057.

Annamalai RT, Hong X, Schott NG, Tiruchinapally G, Levi B, Stegemann JP. Injectable osteogenic microtissues containing mesenchymal stromal cells conformally fill and repair critical-size defects. Biomaterials. 2019;208:32–44. https://doi.org/10.1016/j.biomaterials.2019.04.001.

Archana D, Dutta J, Dutta PK. Evaluation of chitosan nano dressing for wound healing: characterization, in vitro and in vivo studies. Int J Biol Macromol. 2013;57:193–203. https://doi.org/10.1016/j.ijbiomac.2013.03.002.

Ashoori Y, et al. Development and in vivo characterization of probiotic lysate-treated chitosan nanogel as a novel biocompatible formulation for wound healing. Biomed Res Int. 2020;2020. https://doi.org/10.1155/2020/8868618.

Baharlou Houreh A, Masaeli E, Nasr-Esfahani MH. Chitosan/polycaprolactone multilayer hydrogel: a sustained Kartogenin delivery model for cartilage regeneration. Int J Biol Macromol. 2021;177:589–600. https://doi.org/10.1016/j.ijbiomac.2021.02.122.

Brunel F, Véron L, David L, Domard A, Delair T. A novel synthesis of chitosan nanoparticles in reverse emulsion. Langmuir. 2008;24(20):11370–7. https://doi.org/10.1021/la801917a.

Brunel F, El Gueddari NE, Moerschbacher BM. Complexation of copper(II) with chitosan nanogels: toward control of microbial growth. Carbohydr Polym. 2013;92(2):1348–56. https://doi.org/10.1016/j.carbpol.2012.10.025.

Choi B, Kim S, Lin B, Wu BM, Lee M. Cartilaginous extracellular matrix-modified chitosan hydrogels for cartilage tissue engineering. ACS Appl Mater Interfaces. 2014;6(22):20110–21. https://doi.org/10.1021/am505723k.

Cortellini P, Tonetti MS. Focus on intrabony defects: guided tissue regeneration. Periodontol. 2000;22(1):104–32. https://doi.org/10.1034/j.1600-0757.2000.2220108.x.

de Carvalho SYB, et al. Encapsulation of essential oils using cinnamic acid grafted chitosan nanogel: preparation, characterization and antifungal activity. Int J Biol Macromol. 2021;166:902–12. https://doi.org/10.1016/j.ijbiomac.2020.10.247.

De La Fuente M, Seijo B, Alonso MJ. Novel hyaluronic acid-chitosan nanoparticles for ocular gene therapy. Investig Ophthalmol Vis Sci. 2008;49(5):2016–24. https://doi.org/10.1167/iovs.07-1077.

de Lima CSA, et al. An updated review of macro, micro, and nanostructured hydrogels for biomedical and pharmaceutical applications. Pharmaceutics. 2020;12(10) MDPI AG:1–28. https://doi.org/10.3390/pharmaceutics12100970.

Debele TA, Mekuria SL, Tsai HC. Polysaccharide based nanogels in the drug delivery system: application as the carrier of pharmaceutical agents. Mater Sci Eng C. 2016;68. Elsevier Ltd:964–81. https://doi.org/10.1016/j.msec.2016.05.121.

Debnath S. (PDF) Ionotropic gelation – a novel method to prepare chitosan nanoparticles. 2011. https://www.researchgate.net/publication/288354069_Ionotropic_gelation_-_A_novel_method_to_prepare_chitosan_nanoparticles. Accessed 21 Apr 2021.

Deng A, et al. Preparation of a recombinant collagen-peptide (RHC)-conjugated chitosan thermosensitive hydrogel for wound healing. Mater Sci Eng C. 2021;119:111555. https://doi.org/10.1016/j.msec.2020.111555.

Dhivya S, Saravanan S, Sastry TP, Selvamurugan N. Nanohydroxyapatite-reinforced chitosan composite hydrogel for bone tissue repair in vitro and in vivo. J Nanobiotechnology. 2015;13(1). https://doi.org/10.1186/s12951-015-0099-z.

Ding H, et al. Nonswelling injectable chitosan hydrogel via UV crosslinking induced hydrophobic effect for minimally invasive tissue engineering. Carbohydr Polym. 2021;252:117143. https://doi.org/10.1016/j.carbpol.2020.117143.

Doench I, et al. Cellulose nanofiber-reinforced chitosan hydrogel composites for intervertebral disc tissue repair. Biomimetics. 2019;4(1):19. https://doi.org/10.3390/biomimetics4010019.

Duan C, et al. Chitosan-g-poly(N-isopropylacrylamide) based nanogels for tumor extracellular targeting. Int J Pharm. 2011a;409(1–2):252–9. https://doi.org/10.1016/j.ijpharm.2011.02.050.

Duan C, et al. Galactose-decorated PH-responsive nanogels for hepatoma- targeted delivery of oridonin. Biomacromolecules. 2011b;12(12):4335–43. https://doi.org/10.1021/bm201270m.

Erickson C, Stager M, Riederer M, Payne KA, Krebs M. Emulsion-free chitosan–genipin microgels for growth plate cartilage regeneration. J Biomater Appl. 2021:088532822199989. https://doi.org/10.1177/0885328221999894.

Evans C, Morimitsu Y, Hisadome T, Inomoto F, Yoshida M, Takei T. Optimized hydrophobically modified chitosan cryogels for strength and drug delivery systems. J Biosci Bioeng. 2021;132(1):81–7. https://doi.org/10.1016/j.jbiosc.2021.03.008.

Fiamingo A, et al. Chitosan hydrogels for the regeneration of infarcted myocardium: preparation, physicochemical characterization, and biological evaluation. Biomacromolecules. 2016;17(5):1662–72. https://doi.org/10.1021/acs.biomac.6b00075.

Ghorbani M, Roshangar L, Soleimani Rad J. Development of reinforced chitosan/pectin scaffold by using the cellulose nanocrystals as nanofillers: an injectable hydrogel for tissue engineering. Eur Polym J. 2020;130. https://doi.org/10.1016/j.eurpolymj.2020.109697.

Gonçalves VL, Laranjeira MCM, Fávere VT, Pedrosa RC. Effect of crosslinking agents on chitosan microspheres in controlled release of diclofenac sodium. Polímeros. 2005;15(1):6–12. https://doi.org/10.1590/s0104-14282005000100005.

Goswami N, Lin F, Liu Y, Leong DT, Xie J. Highly luminescent thiolated gold nanoclusters impregnated in nanogel. Chem Mater. 2016;28(11):4009–16. https://doi.org/10.1021/acs.chemmater.6b01431.

Hu J, et al. Visible light crosslinkable chitosan hydrogels for tissue engineering. Acta Biomater. 2012;8(5):1730–8. https://doi.org/10.1016/j.actbio.2012.01.029.

Huang Y, Lapitsky Y. On the kinetics of chitosan/tripolyphosphate micro- and nanogel aggregation and their effects on particle polydispersity. J Colloid Interface Sci. 2017;486:27–37. https://doi.org/10.1016/j.jcis.2016.09.050.

Iovene A, Zhao Y, Wang S, Amoako K. Bioactive polymeric materials for the advancement of regenerative medicine. J Funct Biomater. 2021;12(1):14. https://doi.org/10.3390/jfb12010014.

Ishihara M, et al. Controlled releases of FGF-2 and paclitaxel from chitosan hydrogels and their subsequent effects on wound repair, angiogenesis, and tumor growth. Curr Drug Deliv. 2006;3(4):351–8. https://doi.org/10.2174/156720106778559047.

Islam P, Water JJ, Bohr A, Rantanen J. Chitosan-based nano-embedded microparticles: impact of Nanogel composition on physicochemical properties. Pharmaceutics. 2017;9(1):1. https://doi.org/10.3390/pharmaceutics9010001.

Kang MK, Dai J, Kim JC. Ethylcellulose microparticles containing chitosan and gelatin: PH-dependent release caused by complex coacervation. J Ind Eng Chem. 2012;18(1):355–9. https://doi.org/10.1016/j.jiec.2011.11.099.

Kravanja G, Primožič M, Knez Ž, Leitgeb M. Chitosan-based (nano)materials for novel biomedical applications. Molecules. 2019;24(10) MDPI AG:1960. https://doi.org/10.3390/molecules24101960.

Kutlusoy T, Oktay B, Apohan NK, Süleymanoğlu M, Kuruca SE. Chitosan-co-hyaluronic acid porous cryogels and their application in tissue engineering. Int J Biol Macromol. 2017;103:366–78. https://doi.org/10.1016/j.ijbiomac.2017.05.067.

Li T, et al. Intra-articular injection of anti-inflammatory peptide-loaded glycol chitosan/fucoidan nanogels to inhibit inflammation and attenuate osteoarthritis progression. Int J Biol Macromol. 2021;170:469–78. https://doi.org/10.1016/j.ijbiomac.2020.12.158.

Lozinsky VI, Galaev IY, Plieva FM, Savina IN, Jungvid H, Mattiasson B. Polymeric cryogels as promising materials of biotechnological interest. Trends Biotechnol. 2003;21(10) Elsevier Current Trends:445–51. https://doi.org/10.1016/j.tibtech.2003.08.002.

Meng G, He J, Wu Y, Wu F, Gu Z. Antibiotic-loaded chitosan hydrogel with superior dual functions: antibacterial efficacy and osteoblastic cell responses. ACS Appl Mater Interfaces. 2014;6(13):10005–13. https://doi.org/10.1021/am502537k.

Montembault A, Viton C, Domard A. Rheometric study of the gelation of chitosan in aqueous solution without cross-linking agent. Biomacromolecules. 2005;6(2):653–62. https://doi.org/10.1021/bm049593m.

Mora-Boza A, et al. Microfluidics generation of chitosan microgels containing glycerylphytate crosslinker for in situ human mesenchymal stem cells encapsulation. Mater Sci Eng C. 2021;120:111716. https://doi.org/10.1016/j.msec.2020.111716.

Muzzarelli RAA, El Mehtedi M, Bottegoni C, Aquili A, Gigante A. Genipin-crosslinked chitosan gels and scaffolds for tissue engineering and regeneration of cartilage and bone. Mar Drugs. 2015;13(12) MDPI AG:7314–38. https://doi.org/10.3390/md13127068.

Nooshabadi VT, et al. Impact of exosome-loaded chitosan hydrogel in wound repair and layered dermal reconstitution in mice animal model. J Biomed Mater Res Part A. 2020;108(11):2138–49. https://doi.org/10.1002/jbm.a.36959.

Oryan A, Alidadi S, Bigham-Sadegh A, Moshiri A, Kamali A. Effectiveness of tissue engineered chitosan-gelatin composite scaffold loaded with human platelet gel in regeneration of critical sized radial bone defect in rat. J Control Release. 2017;254:65–74. https://doi.org/10.1016/j.jconrel.2017.03.040.

Oyarzun-Ampuero FA, Brea J, Loza MI, Torres D, Alonso MJ. Chitosan-hyaluronic acid nanoparticles loaded with heparin for the treatment of asthma. Int J Pharm. 2009;381(2):122–9. https://doi.org/10.1016/j.ijpharm.2009.04.009.

Pal K, Behera B, Roy S, Sekhar Ray S, Thakur G. Chitosan based delivery systems on a length scale: nano to macro. Soft Mater. 2013;11(2):125–42. https://doi.org/10.1080/1539445X.2011.586082.

Pang Y, et al. Biodegradable and biocompatible high elastic chitosan scaffold is cell-friendly both *in vitro* and *in vivo*. Oncotarget. 2017;8(22):35583–91. https://doi.org/10.18632/oncotarget.14709.

Pant A, Negi JS. Novel controlled ionic gelation strategy for chitosan nanoparticles preparation using TPP-β-CD inclusion complex. Eur J Pharm Sci. 2018;112:180–5. https://doi.org/10.1016/j.ejps.2017.11.020.

Park H, Choi B, Hu J, Lee M. Injectable chitosan hyaluronic acid hydrogels for cartilage tissue engineering. Acta Biomater. 2013;9(1):4779–86. https://doi.org/10.1016/j.actbio.2012.08.033.

Pathak C, Vaidya FU, Pandey SM. Mechanism for development of Nanobased drug delivery system. In: Applications of targeted nano drugs and delivery systems. Elsevier; 2019. p. 35–67.

Pellá MCG, et al. Magnetic chitosan microgels: synthesis, characterization, and evaluation of magnetic field effect over the drug release behavior. Carbohydr Polym. 2020;250:116879. https://doi.org/10.1016/j.carbpol.2020.116879.

Peniche C, Argüelles-Monal W, Davidenko N, Sastre R, Gallardo A, San Román J. Self-curing membranes of chitosan/PAA IPNs obtained by radical polymerization: preparation, characterization and interpolymer complexation. Biomaterials. 1999;20(20):1869–78. https://doi.org/10.1016/S0142-9612(99)00048-4.

Rusu AG, Chiriac AP, Nita LE, Rosca I, Rusu D, Neamtu I. Self-assembled nanocarriers based on modified chitosan for biomedical applications: preparation and characterization. Polymers (Basel). 2020;12(11):2593. https://doi.org/10.3390/polym12112593.

Sacco P, Furlani F, de Marzo G, Marsich E, Paoletti S, Donati I. Concepts for developing physical gels of chitosan and of chitosan derivatives. Gels. 2018;4(3):67. https://doi.org/10.3390/gels4030067.

Salmivirta M, Lidholt K, Lindahl U. Heparan sulfate: a piece of information. FASEB J. 1996;10(11):1270–9. https://doi.org/10.1096/fasebj.10.11.8836040.

Saravanan S, Vimalraj S, Anuradha D. Chitosan based thermoresponsive hydrogel containing graphene oxide for bone tissue repair. Biomed Pharmacother. 2018;107:908–17. https://doi.org/10.1016/j.biopha.2018.08.072.

Schütz CA, Juillerat-Jeanneret L, Käuper P, Wandrey C. Cell response to the exposure to chitosan-TPP//alginate nanogels. Biomacromolecules. 2011;12(11):4153–61. https://doi.org/10.1021/bm201231x.

Sharifi E, Chehelgerdi M, Fatahian-Kelishadrokhi A, Yazdani-Nafchi F, Ashrafi-Dehkordi K. Comparison of therapeutic effects of encapsulated Mesenchymal stem cells in Aloe vera gel and Chitosan-based gel in healing of grade-II burn injuries. Regen Ther. 2021;18:30–7. https://doi.org/10.1016/j.reth.2021.02.007.

Shi C, Zhu Y, Ran X, Wang M, Su Y, Cheng T. Therapeutic potential of chitosan and its derivatives in regenerative medicine1 1 this work was supported by '973' programs on severe trauma (NO. 1999054205 and NO. 2005CB522605) from the Ministry of Science and Technology of China. J Surg Res. 2006;133(2):185–92. https://doi.org/10.1016/j.jss.2005.12.013.

Singh DK, Ray AR. Biomedical applications of chitin, chitosan, and their derivatives. J Macromol Sci – Polym Rev. 2000;40(1):69–83. https://doi.org/10.1081/MC-100100579.

Stevens MM. Biomaterials for bone tissue engineering. Mater Today. 2008;11(5) Elsevier:18–25. https://doi.org/10.1016/S1369-7021(08)70086-5.

Sultankulov B, Berillo D, Sultankulova K, Tokay T, Saparov A. Progress in the development of chitosan-based biomaterials for tissue engineering and regenerative medicine. Biomol Ther. 2019;9(9) MDPI AG:470. https://doi.org/10.3390/biom9090470.

Sundaram MN, Amirthalingam S, Mony U, Varma PK, Jayakumar R. Injectable chitosan-nano bioglass composite hemostatic hydrogel for effective bleeding control. Int J Biol Macromol. 2019;129:936–43. https://doi.org/10.1016/j.ijbiomac.2019.01.220.

Sundermann J, et al. Varying the sustained release of BMP-2 from chitosan nanogel-functionalized polycaprolactone fiber mats by different polycaprolactone surface modifications. J Biomed Mater Res – Part A. 2021;109(5):600–14. https://doi.org/10.1002/jbm.a.37045.

Takada A, Kadokawa JI. Fabrication and characterization of polysaccharide ion gels with ionic liquids and their further conversion into value-added sustainable materials. Biomol Ther. 2015;5(1) MDPI AG:244–62. https://doi.org/10.3390/biom5010244.

Tang G, et al. Recent advances of chitosan-based injectable hydrogels for bone and dental tissue regeneration. Front Bioeng Biotechnol. 2020;8. Frontiers Media S.A:1084. https://doi.org/10.3389/fbioe.2020.587658.

Torres AL, et al. Microvascular engineering: dynamic changes in microgel-entrapped vascular cells correlates with higher vasculogenic/angiogenic potential. Biomaterials. 2020;228:119554. https://doi.org/10.1016/j.biomaterials.2019.119554.

Wang L, Rao RR, Stegemann JP. Delivery of mesenchymal stem cells in chitosan/collagen microbeads for orthopedic tissue repair. Cells Tissues Organs. 2013;197:333–43. https://doi.org/10.1159/000348359.

Wang J, et al. Positive/negative surface charge of chitosan based nanogels and its potential influence on oral insulin delivery. Carbohydr Polym. 2016;136:867–74. https://doi.org/10.1016/j.carbpol.2015.09.103.

Wang H, Qian J, Ding F. Recent advances in engineered chitosan-based nanogels for biomedical applications. J Mater Chem B. 2017;5(34) Royal Society of Chemistry:6986–7007. https://doi.org/10.1039/c7tb01624g.

Wang Y, Guo L, Dong S, Cui J, Hao J. Microgels in biomaterials and nanomedicines. Adv Colloid Interface Sci. 2019;266. Elsevier B.V:1–20. https://doi.org/10.1016/j.cis.2019.01.005.

Xu C, Lei C, Meng L, Wang C, Song Y. Chitosan as a barrier membrane material in periodontal tissue regeneration. J Biomed Mater Res Part B Appl Biomater. 2012;100B(5):1435–43. https://doi.org/10.1002/jbm.b.32662.

Yan XZ, Van Den Beucken JJJP, Cai X, Yu N, Jansen JA, Yang F. Periodontal tissue regeneration using enzymatically solidified chitosan hydrogels with or without cell loading. Tissue Eng – Part A. 2015;21(5–6):1066–76. https://doi.org/10.1089/ten.tea.2014.0319.

Yu S, Hu J, Pan X, Yao P, Jiang M. Stable and pH-sensitive nanogels prepared by self-assembly of chitosan and ovalbumin. 2006. https://doi.org/10.1021/la053158b.

Yu Y, Xu S, Li S, Pan H. Genipin-cross-linked hydrogels based on biomaterials for drug delivery: a review. Biomater Sci. 2021;9(5) Royal Society of Chemistry:1583–97. https://doi.org/10.1039/d0bm01403f.

Yuen CWM, et al. Development of miconazole nitrate containing chitosan microcapsules and their anti-Aspergillus niger activity. J Microencapsul. 2012;29(5):505–10. https://doi.org/10.3109/02652048.2011.642017.

Zhang H, Mardyani S, Chan WCW, Kumacheva E. Design of biocompatible chitosan microgels for targeted pH-mediated intracellular release of cancer therapeutics. Biomacromolecules. 2006;7(5):1568–72. https://doi.org/10.1021/bm050912z.

Zhang L, Xi L, Shi G, Zhu C, Ni C. Reduction-responsive zwitterionic nanogels based on carboxymethyl chitosan for enhancing cellular uptake in drug release. Colloid Polym Sci. 2016;294(3):629–37. https://doi.org/10.1007/s00396-015-3822-2.

Zhang H, Liu C, Chen L, Dai B. Control of ice crystal growth and its effect on porous structure of chitosan cryogels. Chem Eng Sci. 2019;201:50–7. https://doi.org/10.1016/j.ces.2019.02.026.

Index

J. M. Oliveira et al. (eds.), *Polysaccharides of Microbial Origin*,
https://doi.org/10.1007/978-3-030-42215-8