Advances in Material Research and Technology

Md Saquib Hasnain
Amit Kumar Nayak
Saad Alkahtani *Editors*

Polymeric and Natural Composites

Materials, Manufacturing and Biomedical Applications

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Advances in Material Research and Technology

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Editors
Md Saquib Hasnain
Department of Pharmacy
Palamau Institute of Pharmacy
Daltonganj, Jharkhand, India

Amit Kumar Nayak
Department of Pharmaceutics, Seemanta
Institute of Pharmaceutical Sciences
Mayurbhanj, Odisha, India

Saad Alkahtani
Department of Zoology, College
of Science, King Saud University
Riyadh, Saudi Arabia

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Preface

Polymeric composites are known as engineered materials of high performance and versatility. In general, polymeric composites are designed by employing a combination of materials containing different phases, at least one of which, normally the matrix, is a polymer and the resultant composites possess advantageous mechanical and thermal characteristics, which are insufficient to be accomplished by a single polymer. In addition, polymeric composites are capable to facilitate excellent friction and wear performances after being modified with functional fillers and reinforcements. The recent trend is to explore naturally derived raw materials from various renewable resources because of growing environmental concerns over the sustainability of raw materials extracted from synthetic resources for the threat to the ecosystem and well-being. Various naturally derived raw materials are also becoming more commercialized due to their biodegradability and sustainable production/extraction from the renewable natural resources. Recently, these natural materials are employed as useful raw materials for the manufacturing of polymeric composites, which makes these more flexible for the uses in many industrial applications including biomedical uses. In this context, raw materials, their manufacturing and biomedical applications (i.e. in drug delivery, growth factor delivery, orthopaedics, dentistry, wound dressing, etc.) of different polymeric and natural composites need to be thoroughly understood.

The current book highlights the overview and extensive knowledge of the up-to-date research and developments of various types of polymeric and natural composites. Exclusively, it covers the different aspects of raw materials, manufacturing and biomedical applications of polymeric and natural composites by prominent researchers in academia and industry as well as government/private research laboratories across the world. Overall, this book *Polymeric and Natural Composites—Materials, Manufacturing and Biomedical Applications* will serve as a holistic reference source suitable for professionals, students and researchers from different disciplines.

The current book is a collection of totally 14 chapters presenting different key topics by the academicians and researchers across the world. A concise account on the contents of each chapter has been described to provide a glimpse of the book to the readers.

The first chapter entitled "Natural Polymers-Based Biocomposites: State of Art, New Challenges, and Opportunities" describes the role of different carbohydrates and protein-based natural polymers along with their physical, chemical and biological properties.

The second chapter entitled "Natural Fibre-Reinforced Polymer Composites: Manufacturing and Biomedical Applications" presents the most relevant and more recent advances in natural fibre-reinforced polymer composites, their manufacturing and uses in biomedical applications.

The third chapter entitled "Polymeric Biocomposites from Renewable and Sustainable Natural Resources" focuses on the use of biocomposites in tissue engineering and analytical applications. The studied materials include polysaccharides such as chitosan, cellulose and alginate, as well as polyhydroxyalcanoates as matrixes, and fillers like nanoparticles, carbon nanotubes or polymers, among other combinations.

The fourth chapter entitled "Polymer/Carbon Nanocomposites for Biomedical Applications" presents a comprehensive survey of the existing and current literature on different aspects of CNTs, their NCs with polymeric materials and their biomedical applications. This chapter also highlights a variety of methods used to produce CNT polymer nanocomposites, along with their characterization techniques. Polymer nanocomposites (PNCs) based on CNTs offer remarkably improved mechanical, electrical and sensing properties. All this justifies the emergent interest in both academia and industrial development. Likewise, the present status and upcoming possibilities of CNT/PNCs are examined in general along with appropriate examples drawn from the existing literature.

The fifth chapter entitled "Molecularly Imprinted Polymer—Carbon Dot Composites for Biomedical Application" deals with the biomedical application of molecularly imprinted polymer functionalized carbon dots. The brief characterization of carbon dot synthetic approaches together with summarized overview of imprinting process and its limitations followed by detailed discussion of the current state of the art of the carbon dot molecularly imprinted polymer conjugates for biomedicine will provide insight into the future prospects of those advanced materials.

The sixth chapter entitled "Magnetic Polymer Nanocomposites: Manufacturing and Biomedical Applications" discusses the current explanation of magnetic nanocomposites from basic science to the latest innovations as given. Starting with the introduction of magnetism and magnetic materials, characterization of magnetic biomaterials, synthesis techniques, production methods and application areas were studied. An easy way to understand new techniques emerging in this field is presented to the reader. In addition, more current processes and practices are briefly mentioned.

The seventh chapter entitled "Jackfruit Seed Starch-Based Composite Beads for Controlled Drug Release" presents a comprehensive review of various JSS-based composite beads for controlled sustained releasing of encapsulated drugs.

The eighth chapter entitled "Polymeric Nanocomposites for Cancer-Targeted Drug Delivery" deals with the polymeric nanocomposites for cancer-targeted drug delivery, their efficacy and impact on cancer therapy and multiscale molecular simulation studies for nanostructured polymer systems.

The nineth chapter entitled "Biopolymeric-Inorganic Composites for Drug Delivery Applications" focuses on the use of biopolymeric–inorganic composites in the preparation of drug delivery systems. The types of biopolymeric and inorganic materials that can be combined into composite materials and their characteristics are summarized herein. The given materials are just examples for the composite materials of interest, and many other composites can be synthesized from different types of inorganic and biopolymeric materials.

The tenth chapter entitled "Natural Polymeric-Based Composites for Delivery of Growth Factors" deals with the different natural polymer-based composites for growth factor delivery.

The eleventh chapter entitled "Biopolymers/Ceramic-Based Nanocomposite Scaffolds for Drug Delivery in Bone Tissue Engineering" presents a distinct variety of biopolymer–ceramic-based nanocomposite scaffolds for drug delivery in bone tissue engineering.

The twelfth chapter entitled "Biopolymeric Nanocomposites for Orthopedic Applications" summarizes the recent research results on the development and applications of various types of biopolymeric nanocomposites utilized in prosthetic devices to bone grafts, for cell delivery, with a special focus on material type, formulations, current design and performance in bone tissue engineering. Important challenges related to the degradation of biopolymeric nanocomposite scaffolds, wide range of properties and benefits for bone healing are addressed.

The thirteenth chapter entitled "Natural Polymer-Based Composite Wound Dressings" scrutinizes the evolution of natural polymers in wound dressing from traditional to modern-day treatment methods. The major property of a natural polymer which is widely utilized as biomaterials is presented. Properties of composite material with peculiar heed on their applications in the skin tissue repair field are discussed. Finally, the unmet needs and developmental prospectives of the new generations of environmentally friendly, naturally derived, smart wound dressings are addressed in the light of future research.

The fourteenth chapter entitled "A View on Polymer-Based Composite Materials for Smart Wound Dressings" presents an overview on the challenges and complexity of a chronic wound, exploring the event of a wound infection and discussing the large range of polymer-based composite materials and products in use for each specific wound condition, taking into account the key decision aspects defined by the clinicians. Different tissue engineering strategies are also herein addressed with varied reported clinical success, ranging from non-cellularized to considerably sophisticated cellularized products, reproducing the compositional complexity of both dermis and epidermis. Recent advances in smart dressings and sensors are also brought to discussion as sensing the wound can give us new insights about the series of complex biochemical events related to the healing and regeneration process, while contributing for a better wound assessment.

We would like to convey our sincere thanks to all the authors of the chapters for providing timely and valuable contributions. We thank the publisher—Springer Nature. We specially thank Dr. Shadia Jamil Ikhmayies (Series Editor, *Advances in Material Research and Technology*, Springer Nature), Mayra Castro and

Boopalan Renu for their invaluable support in organization of the editing process right through the beginning to finishing point of this book. We gratefully acknowledge the permissions to reproduce copyright materials from various sources. Finally, we would like to thank our family members, all respected teachers, friends, colleagues and dear students for their continuous encouragements, inspirations and moral supports during the preparation of the current book. Together with our contributing authors and the publishers, we will be extremely pleased if our endeavour fulfils the needs of academicians, researchers, students, biomedical experts, pharmaceutical students and drug delivery formulators. In a nutshell, it will also help the health professionals in academia as well as in the industries.

Jharpokharia, India Dr. Amit Kumar Nayak
Daltonganj, India Dr. Md Saquib Hasnain
Riyadh, Saudi Arabia Dr. Saad Alkahtani

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About the Editors

Dr. Md Saquib Hasnain is currently working as Professor in the Department of Pharmacy, Palamau Institute of Pharmacy, Jharkhand, India. He has over 9 years of research experience in the field of drug delivery and pharmaceutical formulation analyses, especially systematic development and characterization of diverse nanostructured drug delivery systems, controlled release drug delivery systems, bioenhanced drug delivery systems, nanomaterials and nanocomposites employing quality by design approaches and many more. Till date, he has authored over 50 publications in various high-impact peer-reviewed journals and 100 chapters to his credit. In addition, he has authored 2 books and edited 9 books published by International Publisher(s). He is also serving as Reviewer of several prestigious journals. Overall, he has earned highly impressive publishing and cited record in Google Scholar (H-Index: 26). He has also participated and presented his research work at over ten conferences in India and abroad. He was also Member of scientific societies, i.e. Royal Society of Chemistry, Great Britain; International Association of Environmental and Analytical Chemistry, Switzerland; and Swiss Chemical Society, Switzerland.

Dr. Amit Kumar Nayak is currently working as Associate Professor at Seemanta Institute of Pharmaceutical Sciences, Odisha, India. He earned his Ph.D. in pharmaceutical sciences from IFTM University, India. He worked as Senior Research Associate at IIT Kanpur in a CSIR-sponsored project. He has over 12 years of research experiences in the field of pharmaceutics, especially in the development and characterization of novel biopolymeric and nanostructured drug delivery systems made of natural biopolymers. He has authored over 130 research and review articles in various high-impact peer-reviewed journals and 102 chapters in various international books. In addition, he has authored 2 books and edited 10 books published by International Publisher(s). He has received University Foundation Day Research Award-2019 by Biju Patnaik University of Technology, Odisha. He serves as Reviewer for several reputed journals and is Life Member of Association of Pharmaceutical Teachers of India (APTI).

Dr. Saad Alkahtani has over 26 years of research and teaching experience in the field of cell biology and genetics, especially isolation and cDNA synthesis, isolation, purification, cloning, sequencing, electrophoresis and RFLP analysis, conventional drug delivery systems and nanostructured drug delivery systems, controlled release drug delivery systems, bioenhanced drug delivery systems, nanomaterials and nanocomposites and many more. Till date, he has authored over 187 publications in various high-impact peer-reviewed journals and 3 books to his credit. He is also serving as Reviewer of several prestigious journals. Overall, he has earned highly impressive publishing and cited record in Google Scholar (*H*-index: 20). He has also participated and presented his research work at over 25 conferences.

Natural Polymers-Based Biocomposites: State of Art, New Challenges, and Opportunities

Laxmikant Gautam, Anamika Jain, Priya Shrivastava, Sonal Vyas, and Suresh P. Vyas

Abstract In the present scenario, in the development of the novel drug delivery system, the role of the natural polymer will be more preferential as compared to the other derivative. The biocompatible and biodegradable nature of the natural polymer is the aim of current research. Along with that natural polymer can be worked as a site-directed ligand that can specifically bind with the cell receptor and target the diseased cell/tissues. The role of different carbohydrates and protein-based natural polymers were incorporated in this chapter along with their physical, chemical, and biological properties. The role of these natural polymers in the pharmaceutical and biomedical applications also are incorporated.

Keywords Carbohydrates · Protein · Drug delivery system · Ligand

1 Introduction

Natural polymers are obtained from animals and plant sources. Chitin, starch, cellulose, casein, alginates, soy protein, polyhydroxyalkanoates, hemicelluloses alginates, and polylactic acid are some of the examples of natural polymers. Various attractive features of natural polymers have drawn the attention of many researchers for their pharmaceutical application. They are natural, biodegradable, renewable, abundant, and biocompatible. Nowadays research is focused on the development of advanced polymeric materials such as nanocomposites, blends, and composites by combining natural polymers with other polymers and fillers [1]. Natural polymers have been explored for the delivery of drugs and bioactive molecules. They can be easy modified for drug delivery; exhibit specific interaction with biomolecules and undergo controlled enzyme degradation. Natural polymers can be used for the delivery of

L. Gautam · A. Jain · P. Shrivastava · S. P. Vyas (✉)
Drug Delivery Research Laboratory, Department of Pharmaceutical Sciences, Dr. Harisingh Gour Vishwavidyalaya, Sagar 470003, India

S. Vyas
Bundelkhand Medical College, Sagar 470002, India

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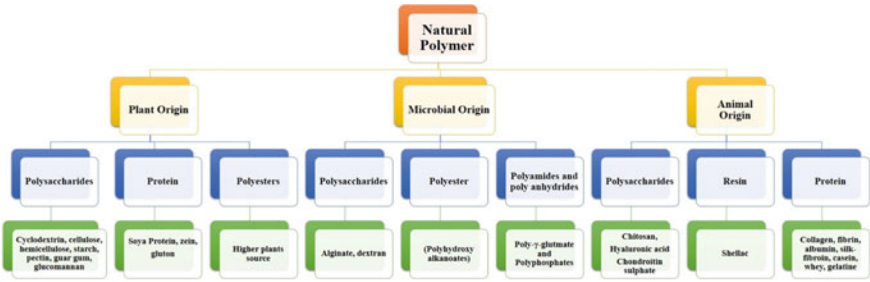


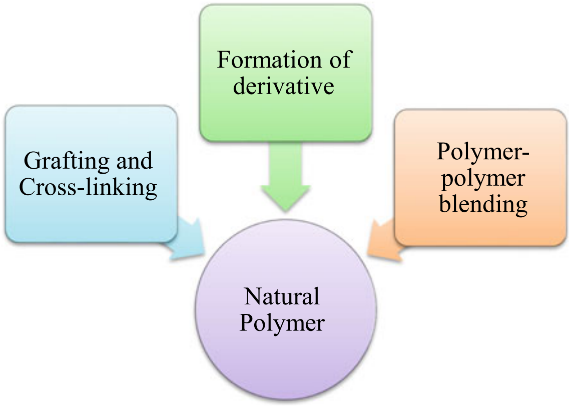
Fig. 1 Biodegradable natural polymer applied in drug delivery

proteins, DNA, as well as tissue engineering, apart from small molecular weight drugs, wound healing, , and anticancer drugs. Various chemical and morphological modifications are also carried out to enable them target orientation, stimuli sensitive etc. As compared to other synthetic polymers, natural polymers offer various advantages including accessibility, modification, and biocompatibility. Natural polymers possess various reactive groups, thus activity-specific functional groups can be introduced, with various altered however desirable physico-chemical properties [2] (Fig. 1).

1.1 Natural Polymers: An Insight

Natural polymers have been extensively explored as a carrier for the delivery of various bioactives. Most of the natural polymers are used as an excipient in pharmaceutical preparations because they are generally considered safe in vivo. Polysaccharidesexhibit various superior characteristics such as biocompatibility, enzyme degradation characteristics, non-toxic, highly stable, harmless, gel-forming ability, and as per need can be altered chemically and biochemically [3]. Natural polymers largely include polysaccharides such as dextran, chitosan, agarose, hyaluronic acid, alginate, cyclodextrin, and carrageenan and protein-based polymers which include collagen, albumin, soy, gelatin, etc. These polymers exhibit broad molecular distribution and batch-to-batch variability, which impose various challenges. Chitosan is one of the most widely used polymers due to its easy surface modification, non-immunogenic properties, lower toxicity, and blending property with other polymers. Chitosan and alginate have widely been explored as compared to other polymers. Several preclinical trials suggest successful use of some biodegradable polymers, however, only a few have been accepted for further clinical trials and none of them enter the post clinical trials. Thus there is a scope to explored new polymers for the targeted delivery and sustained drug release [4].

Fig. 2 Modifications in natural polymers



1.2 Modifications of Natural Polymers

Modification of natural polymers imparts versatility and drug release and delivery properties. Other properties such as solubility, viscosity, and microbial degradation can be further modified to overcome the drawbacks. The modification should not alter their biological properties. Various modification methods include polymer–polymer blending, grafting, crosslinking, and formation of derivative [5] (Fig. 2).

1.3 Grafting and Cross-Linking

Mittal and coworkers chemically grafted Barley husk (BH) following its copolymerization with palmitic acid. Urea formaldehyde cross-linked PVA/Starch composite films, along with natural BH, poly vinyl alcohol/starch and, grafted BH were used to prepare a blend grafted film. The effect of content of BH, urea/starch ratio, and grafted BH on the water uptake (%), mechanical properties and biodegradability of composite films was studied. Tensile strength of cross-linked film was increased by 40.23% as compared to the PVA/St film, when urea starch ratio increases from 0 to 0.5 in blend. Whilst the tensile strength was recorded to be enhanced by 72.4% as compared to PVA/St film in a grafted BH composite film. The degradation rate of PVA/St film was shorter than natural BH composite film. Nevertheless, film properties can be modulated by varying the degree of cross-linking polymer network [6]. Astrain and coworkers designed some bionanocomposite hydrogels from furan modified gelatin by using maleimide-functionalized cellulose nanocrystals (CNCs) as multifunctional cross linkers. Functionalization of the nanocrystals with maleimide component was confirmed by X-ray photoelectron spectroscopy. Functionalized cellulose nanocrystals were used as cross-linkers using Diels–Alder "click" reaction to furan modified gelatin. Swelling and rheological parameters were assessed to ascertain the role of CNCs as cross-linkers for gelatin. Changes in the swelling properties of the hybrid hydrogels clearly depict the additional cross-linking points resulted due to the presence of functionalized CNCs. Thus synthetic strategies seem to be of potential applications in the material design for use in pharmaceuticals [7].

1.4 Formation of Derivative

Physiochemical properties of the natural polymers such as solubility, drug release, hydrophilicity, swellability, targeting, film coating and stimuli-responsiveness can be ameliorated by polymers derivatization. Various derivatives are based on acetylation, phosphorylation, carboxymethylation, esterification, cyanoethylation etc. Koliada and coworkers synthesized collagen derivatives of porcine skin with polyvinyl acetate (PVAc) and polyvinyl alcohol (PVA) and studied the morphology of the prepared fibers. The distance between collector and syringe needle was 9–12 cm. Material obtained from the PVAc and PVA with the addition of gelatin and collage were the derivative fibers of diameter within the range of 0.502–0.894 μm for PVAc:CD and PVA:CD, respectively [8]. Chitosan is used as a polymer for concentration, recovery, and separation of metal ions as well as formation of a range of functional materials. Unmodified chitosan possesses unique complexing properties for various metal ions, although its selectivity and sorption capacity can be enhanced by means of chemical modification. Lewis basicity and chelating ability of chitosan can be adjusted through introduction of functional groups apart from the hydroxyl and amino groups [9].

1.5 Polymer–polymer Blending

Polymer-polymer blending is a convenient way to modify polymers without application of any chemical reaction or synthesis of new polymer. Van der Waals forces, London dispersion forces, and hydrogen bonding play an important role in polymer–polymer blending. Although some sort of chemical bonding also gets involved in some blending. Blending of xanthan gum, alginate and locust bean gum in the formation of microspheres increases the drug entrapment efficiency and reduces drug release as compared to locust bean, alginate and xanthan gum [5].

2 Recent Application of Natural Polymers in Nano-Drug Delivery

Nanomedicines are the new generation medicines unique due to their nano size features. After oral delivery, various drugs in macro or micro formulations exhibit poor pharmacokinetics and lesser bioavailability. Thus, formulation based on natural or synthetic biodegradable polymers have gained attention in the field of controlled and targeted drug delivery to enhance safety, biocompatibility, enhanced permeability, bioavailability, lesser toxicity, and greater retention time. Suitable biodegradable polymers can be chosen as drug carrier for sustained as well as targeted delivery [4]. Polymeric amphiphiles have a great potential to deliver anticancer drugs, possess minimum side effects, thus gained attention over the past decade. These polymers can be self-assembled in the micelles, with a hydrophilic corona and hydrophobic core. This structure possesses great potential in anticancer drug targeting including prolonged circulation. The hydrophilic outer layer act as a barrier, which decreases interaction with the other cells and hence results in prolonged circulation. The hydrophobic core is responsible for the pharmacokinetic properties, such as drug release and drug entrapment. Enhanced permeation and retention (EPR) effect can be achieved with a polymeric nanosized drug carrier, thus enhances localization of drug within tumor site [10]. Apart from the pharmaceutical application, natural polymers also exhibit great potential in the bio nanotechnology and nanomedical field. Biodegradable natural polymers possess great potential for targeted and site-specific drug delivery, especially for the development of artificial limbs, biosensing application and tissue engineering. Various natural polymers are suitable for bio nano technological applications, when blended, cross-linked, and functionalized for the designing of nano scaffolds. Several natural polymers are used as a scaffold for corneal repair including chitosan, alginate, cellulose, heparin, gelatin, silk fibroin etc. Chitosan promotes corneal wound healing, alginate improves viability of the corneal epithelial cells, cellulose improves mechanical and chemical properties of the ophthalmic formulation, gelatin provides transparency to the formulation and silk fibroin is compatible with limbal stem cell and promotes epithelial formation [11]. Polymeric drug delivery is also used for biomimetic, targeting polymeric drug delivery drug and also as free macromolecular therapeutics. Polymeric gene delivery systems, non-virial vectors, and virial vectors for gene delivery have extensively been studied in the past decade. The systems for virial vectors are RNA conjugates and DNA conjugates for gene delivery and for non-virial vectors are polyethyleneimine copolymers, polyethyleneimine derivative, and polyethyleneimine conjugated bioreducible polymers. Polymeric drug carriers that are based on the pathogen like viruses and bacteria are potentially immunogenic. Although they also have adjuvant ability, thus certain degree of immunogenicity can be expected from polymeric drug carriers. The polymeric drug delivery systems thus possess great potential in near future by combining biological and synthetic fields [12]. In Table 1 different carbohydrates and protein- based natural polymer are listed along with their properties and application.

Table 1 Different types of carbohydrates-protein -based natural polymer and their properties and pharmaceutical applications

S. No.	Polysaccharide	Monomer unit	Properties	Pharmaceutical application	References
1	Chitosan	Linear copolymer of β-(1 → 4)-linked 2-acetamido-2-deoxy-β-D-glucopyranose and 2-amino-2-deoxy-β-D-glucopyranose	Chitosan is less toxic, non-immunogenic, simplicity of modification, biocompatible, degradable by enzymes, high bioavailability	Chitosan act as a stabilizing agent, reducing agent	[12, 13]
2	Cellulose	Glucose linked via β-(1 → 4) glycosidic linkage	Excellent film forming nature, Conventional softener, low conductivity, and high resistivity	Mucoadhesive and bio-adhesive drug delivery system, ether and ester derivatives in solid dosages form coating process, osmotic drug delivery	[14]
3	Alginate	Linear copolymer composed of 2 monomeric units (D-mannuronic acid and L-guluronic acid)	Low toxic, biocompatible, mild gelation by addition of Ca^{2+}	Binder, disintegrant, taste masking agent, stabilizer, thickener, emulsifier, surface active agent, suspending and viscosity increasing agent	[15, 16]
4	Heparinara>	Sulfate repeating disaccharides units	Linear anionic polysaccharides having 2-O-sulfo-α-iduronic acid and 2—deoxy-2-sulfamino-6-O-sulfo-α-d-glucose unit	Anticoagulant, antithrombic	[17]
5	Guar Gum	Galactose and mannose units	high molecular weight polysaccharides composed of galactomannans consisting of a (1 → 4)-linked β-D-mannopyranose backbone with branch points from their 6-positopns linked to α-D-galactose (i.e. 1 → 6-linked α-D-galactopyranose)	Thickener, stabilizer, emulsifier, disintegrant, binder, bulking gent in laxatives	[18]

(continued)

Table 1 (continued)

S. No.	Polysaccharide	Monomer unit	Properties	Pharmaceutical application	References
6	Pectin	Galacturonic acid monomer units are linked via α-(1 → 4)-glycosidic bond	Exists as solid, freely soluble in water, and a weakly acidic compound	Biodegradable polymer, possess gelling properties, used as a matrix for the entrapment and/or delivery of a variety of drugs, proteins and cells	[19, 20]
7	Alginates	D-mannuronic acid and L-guluronic acid	Anionic polymer obtained from brown seaweed	Biocompatibility, relatively low toxicity, and cost-effective	[15]
9	Hyaluronic acid	D-glucuronic acid and N-acetyl-D-glucosamine, linked via alternating β-(1 → 4) and β-(1 → 3) glycosidic bonds	Anionic polymer	Biodegradability, biocompatibility, and non-immunogenicity	[21]
10	Carrageenan	D-galactose-2-sulfate (1, 3-linkage) and D-galactose-2,6- disulfate (1, 4-linkage)	Anionic polysaccharides	Used as gelling, thickening, emulsifying, and stabilizing agent in pharmaceutical formulations	[22]
11	Gelatin	18 different amino acids joined together in a chain	Cationic by character	Biodegradable, biocompatible, could be used in sustained and controlled release formulations	[23]
12	Collagen	3 proteins arranged in a triple helix with non-helical ends	Fibrous protein, great tensile strength, and elasticity	Sustained and controlled drug delivery, as controlling material for transdermal drug delivery	[24]

(continued)

Table 1 (continued)

S. No.	Polysaccharide	Monomer unit	Properties	Pharmaceutical application	References
13	Albumin	It is monomeric in nature	Globular protein	Plasma expander, drug delivery, pulmonary-based vaccines	[25]
14	Silk fiber	Sericin and fibroin	Smooth natural fiber, good moisture regains property	Nanocrystal and nanoparticle stabilization, solubility enhancement, and sustained and controlled release formulations	[26]
15	Chitin	β-(1–4)-linked D-glucosamine (deacetylated unit) and *N*-acetyl-D-glucosamine (acetylated unit)	Mucopolysaccharides, biodegradability, inert in nature and low toxicity	Used in mucoadhesive formulations, antioxidant, anticancer, antimicrobial activity, and immune-stimulating characteristics	[27, 28]

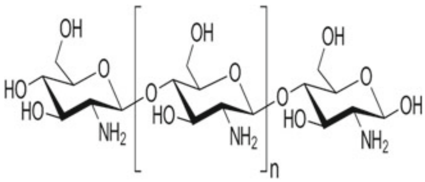
2.1 Polysaccharides Based Drug Delivery Systems

2.1.1 Chitosan-Based Nanocomposites

Environmental pollution such as water pollution has become a global concern in recent years. Various pollutants include dyes, phosphate, biodegradable waste, metals, nitrate, toxic chemicals, pharmaceuticals and radioactive pollutants. Among them, dyes are of main concern, especially crystal violet (CV), which belongs to basic dyes. Massoudinejad et al. have recovered CV dye using magnetic chitosan nano-composites (MCNCs) and studied process variables which effects dye recovery. These variables include CV concentration, pH, contact time, adsorbent dose, etc. via response surface methodology (RSM). Modeling-based results suggest that MCNCs dosage and contact time were effective process variables which showed their effect on adsorption efficiency. pH played an insignificant role in the adsorption uptake. Maximum adsorption efficiency (72%) of MCNCs was obtained, when adsorbent dosage, contact time, and initial concentration of CV were 1 g, 140 min, and 77 mg/L, respectively. Freundlich, Langmuir, and Temkin model has been used to evaluate the qualitative uptake of CV. Freundlich isotherm was well fitted with experimental results. Kinetic studies reveal the pseudo-first-order model fitted the experimental data, which shows adsorption rate of CV onto MCNC was time-dependent. Thus chitosan nanocomposite proves to be a potential adsorbent for dye-containing waste water treatment [29]. Ciprofloxacin (CIP) is widely used 2nd generation fluoroquinolone antibiotics. Although CIP possesses various benefits, but CIP residues can cause some serious health hazards. Thus, there is a need of developing effective and sensitive methodologies for the detection and management of CIP residues in food products. Hu et al. have developed novel electrochemical aptasensing transducer platform, which is based on the carbon nanotube (CNT)-V2O5-chitosan (CS) nanocomposites with modified screen-printed carbon electrode (SPCE) for measurement of CIP. Single-stranded DNA aptamer was immobilized within CNT-V_2O_5-CS/SPCE as basal electrode transducer platform. Electrochemical impedance spectroscopy (EIS) is used for the quantitative detection of CIP. This formulation combines film forming strength of CS, efficient electron transfer capacity of multiwalled CNTs, biocompatibility of V_2O_5, and portability of SPCE. The aptasensor possesses a dynamic range from 0.5 to 64.0 ng mL^{-1}, limit of detection was 0.5 ng mL^{-1} and linearity were obtained between 0.5 to 8.0 ng mL^{-1}. Thus these results show good selectivity to CIP [30] (Fig. 3).

2.1.2 Alginates-Based Nanocomposites

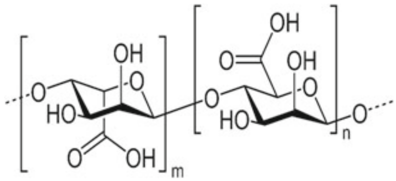
Alginate is obtained from the brown seaweeds including Laminaria japonica, Laminaria hyperborean, Ascophyllum nodosum, Laminaria digitata, and Macrocystis pyrifera. It is made up of two (1N4)-linked α-L-guluronate and β-D-mannuronate monomer. The proportion of monomers was from different sources. Alginate exhibits



Molecular Formula: $(C_{56}H_{103}N_9O_{39})$ n
Molecular wt.: 1526.5 g/mol
Synonyms: Poliglusam, Deacetylchitin, Chicol etc.

Fig. 3 Chemical structure of chitosan

high availability, biocompatible and low toxicity, which impart alginates widest biomedical applicability. Alginate is mainly used as a stabilizer in pharmaceutical formulation [31]. Alginate- based nanocomposites have been extensively studied drug delivery systems due to their good biocompatibility and biodegradability. Alginate is used to produce particles with various applications due to properties such as easy availability, cheap, natural origin, sol–gel transition, and versatility [32,33]. Auriemma et al. have prepared alginate--based gastro-retentive delivery system bearing piroxicam with a bimodal release profile in the gastrointestinal tract (GIT). Higher oral absorption and prolonged half-life shown by piroxicam, however its elimination is inconsistent in old age patients. Thus, to overcome these limitations floating gastro-retentive gel beads with sustained release profile were prepared using prilling techniques. Matrix of the beads prepared as a hollow/multipolymeric system, which is based on the alginate, hydroxypropyl methylcellulose. Various parameters such as floating properties, particle micromeritics, drug release profile, hollow inner structure in the GIT tract were studied. Thus, formulation provides desired bimodal drug release pattern with the controlled and delayed in vitro piroxicam drug release. As compared to standard piroxicam, in vivo anti-inflammatory activity of the floating beads achieved up to 48 h. Hence desired characteristics can be achieved in the elderly patient with chronic inflammatory disease, which required rapid onset of action followed by maintenance dose [34]. Llgin et al. have prepared alginate- based hydrogel as an oral drug carrier for the colon targeting. Hydrogel exhibited immense potential for clinical application owing to their antibacterial and pH-sensitive properties. Hydrogel was characterized by using Scanning electron microscopy (SEM), Fourier transform-infrared spectroscopy (FT-IR), and thermogravimetric analysis (TGA) analysis. Water absorption capacity of the hydrogel of various monomer compositions and effect of salt, temperature and pH was studied. Diclofenac sodium was used as a model drug to study in vitro drug release in gastric fluid and simulated intestinal fluid. Disc diffusion method was used to study antibacterial effects of hydrogels against gram-positive and gram-negative bacteria. The results of studies demonstrated that produced hydrogels could exhibit potential for developing pH-controlled drug delivery devices [35] (Fig. 4).

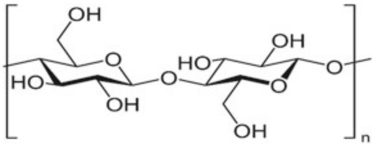


Molecular Formula: $(C_6H_7O_6)$ mn
Molecular wt.: 216.12 g/mol
Synonyms: Sodium alginate, D-galacturonic acid sodium salt, natrimglucuronate, alginic acid monosodium salt

Fig. 4 Chemical structure of alginates

2.1.3 Cellulose Based Nanocomposites

Cellulose is produced from the photosynthesis and is the most abundant polymer on earth. Cellulose nanomaterials are produced from the lignocellulos's biomass and inturn used to develop new materials for use in nanotechnology. Cellulose- based hydrogels have gain attention during the last decade due to their biodegradability, low toxicity, biocompatibility, and excellent mechanical stability. Hydrogels represent three-dimentional polymeric network, which has the capacity to absorb greater amount of water or biological fluid. Their ability to retain water confers similar properties as shown by natural living tissues. Du et al. have prepared cellulose nanofibrils (CNFs) and cellulose nanocrystals- (CNCs) based hydrogel. CNFs and CNCs based hydrogels are used for wound dressings, drug delivery and tissue engineering scaffolds. Thus cellulose- based nanocomposites possess great potential in various biomedical applications [36]. Self-healing hydrogels, which mimic human skin's function, have been studied in recent years. There is still a challenge to prepare an integrative conductive gel which exhibits self-healing as well as offers mechanical properties. Shao et al. have developed self-healing, tough and self-adhesive ionic gel by forming synergistic multiple coordination bonds between tannic acid-coated cellulose nanocrystals., poly (acrylic acid) chains, and metal ions within a covalent polymeric network. Excellent mechanical performance was obtained by the dynamic connected bridge within the porous network, in which multiple coordination bonds are present. Reversible coordination interactions are responsible for superior recovery property as well as reliable electrical and mechanical self-healing property without any aid of external stimuli. Catechol group of the tannic acid is responsible for the durable and reproducible adhesiveness, which could be adhered on skin without any irritation or inflammatory response. Apart from these ionic gels show a larger strain sensitivity, through which flexible strain sensors can be employed to monitor large motions (joint bending) and subtle motion (pulse and breath). Thus, data can be analyzed via programmable wireless transmission on the user interfaced with a smart phone. Thus this work provides a hope in near future to design biocompatible cellulose-based hydrogels, exhibiting self-adhesive, stretchable, self-healing, and strain-sensitive properties for wearable electronic sensors and healthcare monitoring [37] (Fig. 5).



Molecular Formula: $(C_6H_{10}O_5)$ n
Molecular wt.: 342.3 g/mol
Synonyms: Diethylaminoethyl cellulose, cellulose pulver etc.

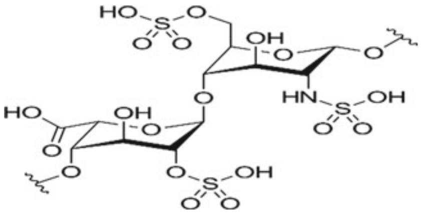
Fig. 5 Chemical structure of cellulose

2.1.4 Heparin Based Nanocomposites

Shi et al. have prepared Graphene based nanomaterials for drug delivery and photothermal therapy due to their unique physiochemical properties. However, biocompatibility issues and limited water solubility limit their further applications. Shi et al. have prepared smaller and uniform size grapheme oxide (GO) nanosheets (approx. 85 nm) using modified Hummers' method. They grafted unfractionated heparin (UFH) on GO covalently using adipic acid dihydrazide (ADH) as a linker to developed nanocomposites for the delivery of curcumin (Cur). The novel nanocomposites exhibit stronger photothermal effect. They were of small size (42 nm) and lateral dimension as compared to GO nanosheets. In vitro experiments conducted on cell lines revealed combined i.e., chemotherapeutic and photothermal effect of Cur-loaded vehicles. They exhibited cytotoxicity of Rgo-ufh/Cur against A549 and MCF-7 cell line. Retention time of Cur was enhanced in nanocomposites as compared to the free Cur solution. Thus it has a immense potential as drug delivery vehicles [38]. Ataei et al. have developed plasma-treated polyurethane/heparinized carbon nanotube (PU/HCNT) nanocomposite based thin films as polymeric heart valve. The nanocomposite films (PU/HCNT) were developed by solvent casting technique. Carbon nanotube was heparinized to overcome the dispersal and calcification resistance of CNTs and the polyurethane matrix. The dispersal of CNTs within matrix of produced films was analyzed by Transmission Electron Microscopy (TEM). Greater calcification resistance and storage modules were observed for nanocomposites. The nanocomposites were exposed to O_2 plasma treatment. The nanocomposites film surface was characterized by using SEM, ATR-FTIR, and EDXA, and water drop contact angle measurements. Cytotoxicity studies were performed on L929 fibroblast cells, wherein no cytotoxicity was observed. Platelets adhesion test revealed that the modified film was more blood compatible as compared to unmodified film [39] (Fig. 6).

2.1.5 Hyaluronic acid (HA) Based Nanocomposites

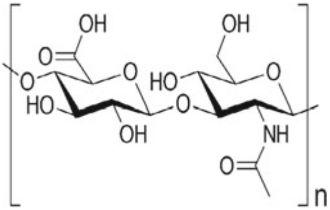
HA found in many tissues and fluids but abundantly present in articular cartilages and synovial fluids. HA is a naturally occurring non-sulfated glycosaminoglycan (GAG) with repeating units of β-1, 4-D-glucuronic acid and β-1, 3-N-acetylglucosamine units. The HA has binding affinity basically for receptors: (A) Intercellular adhesion molecule 1 (ICAM-1), (B) Hyaluronate mediated mobility receptors, and (C) most



Molecular Formula: $C_{26}H_{42}N_2O_{37}S_5$
Molecular wt.: 1134.9 g/mol
Synonyms: Enoxaparin, Nadroparine, Novohepaarin etc.

Fig. 6 Chemical structure of heparin sulfate

importantly membrane proteoglycans (CD44) which interact with the other ligands including osteopontin, collagen and matrix metalloproteinase (MMPs) [40]. Some of the formulation conjugated with the HA are described here with their different targetability and approaches in drug delivery system. Makvandi et al. have developed thermo sensitive and injectable hydrogels, which are composed of hyaluronic acid, β-tricalcium phosphate and corn silk extract nano silver for bone tissue regeneration engineering. Microwave-assisted green approach using CSE is used to synthesize spherical nanoparticles of silver. Rheological studies reveal that thermo sensitive hydrogels have edification temperature (T_{gel}), which is closed to the body temperature. The formulation exhibited antibacterial activity against gram-positive and gram-negative bacteria, without any cytotoxicity. Mesenchymal stem cells seeded within nanocomposites showed greater bone differentiation, which clearly depicts that they could be an ideal candidate for bone tissue regeneration [41]. Pandey and his co-worker synthesized novel pH responsive hyaluronic acid (HA)-lenalidomide nanoconjugates for glioblastoma. They prepared a HA modified drug delivery system by using carbodiimide chemistry of lenalidomide and characterized using different spectroscopic methods, i.e., FTIR, PXRD, SEM, TEM, AFM, etc. The Functionalization of HA result in the CD-44 mediated cellular uptake inside the tumor cells. The size and surface charge reported 128.5 ± 3.2 nm and 131.1 ± 2.5 nm, 20.5 ± 2.2 mV and 19.3 ± 2.1 mV respectively. The result showed that systems were cationic in nature having high efficiency of cell interaction and internalization. The expression of CD44 on blood-brain barrier (brain microvascular endothelial cells) involved into transport the conjugated systems. The cytokine assay showed reduced level of IL-6 and increased level of TNFα. The outcome of the research concluded that dual drug delivery can effectively work in the brain tumor [42]. In another research for the dermal treatment Faccendini and his group, synthesized the hyaluronic acid or chondroitin sulfate conjugated, norfloxacin loaded pullulan and chitosan nanocomposites by using electro spanning with fiber range of 500 nm. This system is shown affinity to decrease the bio-burden up to 100 folds and due to this the resultant drug loading effect showed that there is no effect on biological activity of norfloxacin. The formulation showed minimal swelling in aqueous environment and controlled release of norfloxacin [43]. The treatment of cancer the chemo photo-thermal therapy, based on gold nanorods functionalized with hyaluronic acid containing doxorubicinwere reported by Geo et al. They synthesized a system with responsive action to pH/ thermal sensitive release, EPR effect, deep tumor penetration



Molecular Formula: $C_{33}H_{54}N_2O_{23}$
Molecular wt.: 846.8 g/mol
Synonyms: Etamucine, Luronit, Amvisc, Biolon, Hyvisc, Amo Vitrax, Mucoitin, Sepracoat, SynviscSofast, Hylan G-F 20, HSDB 7240, Hyaluronate Sodium (hyaluronic acid) etc.

Fig. 7 Chemical structure of hyaluronic acid

affinity, targeting through receptor-mediated endocytosis, and synergistic therapeutic effects [44] (Fig. 7).

2.1.6 Carrageenan Based Nanocomposites

Carrageenan is extracted from natural polysaccharides red sea woods of Rhodophycea class. It is a gelling (viscosity↑) agent used in different drug delivery system, tissue engineering, and wound dressing. Carrageenan based silver nanoparticles/clay nanocomposites film developed by using solution casting method. The effect of Plasmonic on these particles was analyzed by UV spectroscopy at 420 nm, tensile strength of the composites increases 14–26 folds due to the nanofillers and water vapor permeability decreased up to 12–27 folds due to the carrageenan film. The result showed the characteristic gram-negative anti-microbial activity [45]. Tavakoli et al. developed the nanocomposites based hydrogel with Kappa carrageenan coated cellulose/starch nanofibers for the hemostatic application. Comparative research of different formulations showed 2 times higher mechanical strength of Kappa carrageenan coated hydrogels. The above results conclude the synergistic effects, good blood clotting ability, adjustable degradation rate, and good mechanical properties of Kappa carrageenan coated hydrogel formulations [46]. Currently, the use of carrageenan in the development of ecofriendly drug delivery system has been reported. Polat et al. prepared the formulation of triethylene glycol divinyl ether cross-linked agar/Kappa-carrageenan/montmorillonite hydrogel. Swelling properties were found to be 2523% under at 70 °C. Non-Fickian behavior of each formulation showed controlled swelling behavior of the hydrogel, thus suggests an effective carrier potential for the biomedical applications [47] (Fig. 8).

2.1.7 Pectin Based Nanocomposites

Pectin, is a polysaccharide of esterified D-glalacturonic acid that resides in α-(1–4) chain. It is also known as methoxy pectin/D-lyxose as a derivative of glucuronic acid derivative. Pectin is water soluble, physically solid by nature, on the basis of pKA it is a component with acidic characteristics. The presence of pectin is reported in all human tissues, specifically located in lysosomes. The role of pectin is reported

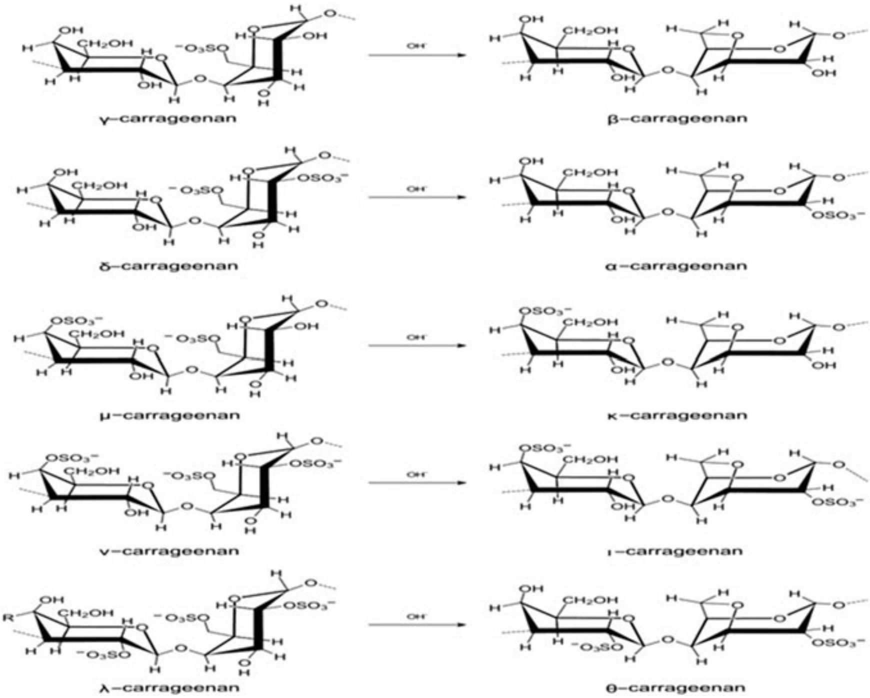


Fig. 8 Chemical structure and different types of Carrageenan structure

in colon specific drug delivery system. Wang et al. reported that pectin modified nano-carbon nanocomposites based gel films effectively worked in oral/colonic-specific delivery of the 5–fluorouracil. The entrapment efficiency was found to be 30.1–52.6%. The release pattern was better in comparison to the single pectin based system. The MTT assay was performed on A549 (Lung cancer), HeLa (Cervical cancer) and L 929 (Murine fibroblast) cell lines, A549 and HeLa effective cytotoxic effect were recorded [48]. In another scientific report, pH-responsive film based on pectin containing curcumin and sulfur nanoparticle exhibited effective antibacterial and antioxidant activity. The solution casting method was used for the development of formulation. The resultant film showed thermal stability as well as higher water contact angle. The antibacterial activity was checked on *E. Coli* and *L. monocytogenes* [49]. In Table 2 different extraction methods are reported (Fig. 9).

2.1.8 Guar Gum-Basednanocomposites

Galactomannan (galactose + mannose) is a polysaccharide extracted from guar beans also known as guaran. Pharmaceutically it is a viscosity enhancer, and stabilizing agent, also used in feed, food industries. In the drug delivery system guar gum grafted

Table 2 Natural pectin obtained from different raw materials using various extraction procedures [50]

Extraction method	Raw Materials
Hydrothermal-assisted extraction	Potato peel, orange peel, pomegranate peel, apple peel, cashew apple pulp, chamomile, durian rind, jackfruit peel, hibiscus, banana peel, passion fruit, tomato husk (All waste material)
Hydrodynamic cavitation	Orange peel waste
Enzyme-assisted extraction	Sisal waste, Artichoke
Microwave-assisted extraction	Tobacco waste
Ultrasound-assisted extraction	Mango peel waste, Sisal waste, Passion fruit, Custard apple peel waste
Subcritical water extraction	Cocoa pod husk waste

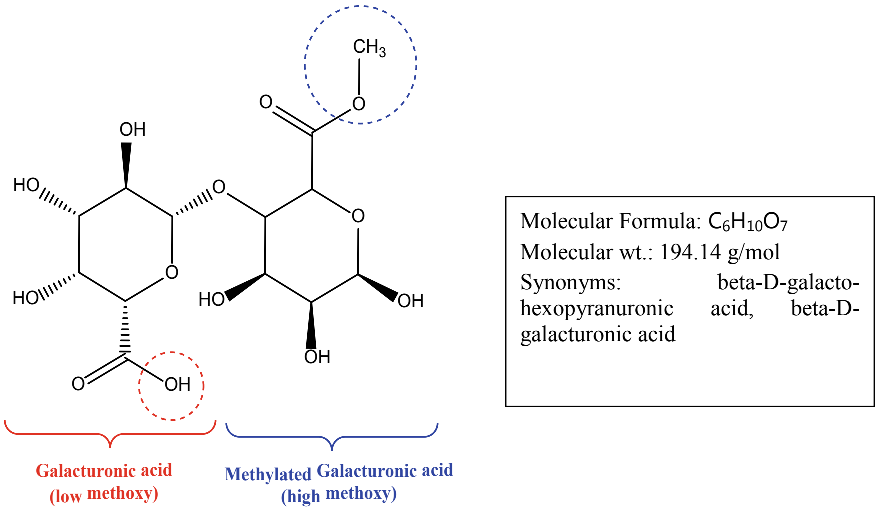


Fig. 9 Chemical structure of low and high methoxy pectin

nanoparticles were developed for the biomedical application**.** Palem et al., prepared silver nanocomposite hydrogel grid of grafted polymer of guar gum (polyacrylamidoglycolic acid) for the delivery of 5-fluorouracil. The designed system displayed effective antimicrobial activity against *B. Subtilis* and *S. ebony.* Thermal analysis confirm the higher stability of the formulation [51]. Another hydrogel formulation of guar gum/Al_2O_3 used as effective photocatalyst prepared by sol-gel method. The formulation in the presence of solar irradiation responds to 80 and 90% degradation of malachite green dye followed by coupled adsorption and photocalalysis [52] (Fig. 10).

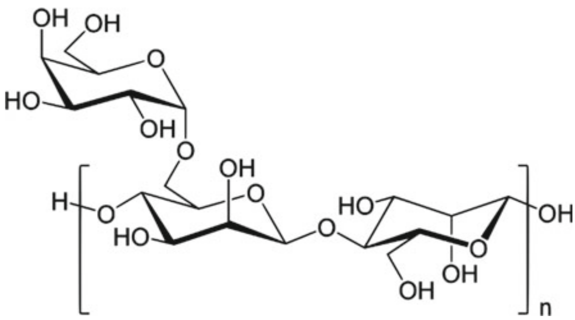


Fig. 10 Chemical structure of Guar gum

2.2 *Protein Based Drug Delivery Systems*

Besides carbohydrate based nanocomposites, protein-based drug delivery modules are also used as vehicles for the bioactive(s) delivery. In addition to the characteristics like biodegradability and biocompatibility, the surface of this protein based nanocomposites or nanoparticles could be easily functionalized owing functional groups into their primary structure. Moreover, charged proteins allow the facilitation of the loading of the bioactive(s) via electrostatic interactions [53, 54]. The nanoparticles which are based on natural polymers could be prepared under mild or aqueous conditions. The process of fabricating these nanoparticles is easy and safe as compared to those that are prepared by using synthetic polymers. Natural proteins possess the ability to uplift the cell retention and the toxicity caused by byproducts during degradation. Among proteins, core zein is generally employed to fabricate nanoparticles for the bioactive(s) delivery. This protein holds the potential for controlled and prolonged release of the bioactive(s) due to their hydrophobic nature [55]. Lai et al., formulated 5-fluorouracil loaded nanoparticles by using core zein protein. A standard phase separation procedure was employed to synthesize these nanoparticles. The size and % bioactive entrapment of these nanoparticles was measured to be 115 nm in diameter and 56.7% respectively. The author reported an initial burst release of the drug from the nanoparticles i.e., 22.4%. Animal studies revealed that the nanoparticles remained in systemic circulation for up to 24 h that is attributed to their high molecular weight prior to their localization in the liver [56]. Previously in literatures, it is reported that the core zein nanoparticles are also being employed for the sustained release of therapeutic proteins such as catalase and superoxide dismutase [57] and vitamin D3 [58]. In another study, Xu et al., fabricated hollow zein based nanoparticles. The manufacturing technique involves the blending of the protein i.e., core zein and sodium citrate based solutions. The core zein polymer is then deposited or collected in the vicinity of the sodium citrate crystals. It has resulted in the development of the nanoconstructs in which the core contains sodium citrate and the shell contains zein protein. These core–shell particles were then added to the aqueous phase i.e., water to produce hollow particles resulted in sodium citrate core dissolution. The size and % bioactive entrapment of these nanocomposites was measured to be < 100 nm and 30% respectively. Ex vivo studies revealed that these nanoconstructs were also successfully internalized by the cells upon incubation with 3T3 fibroblast cells [59]. Several other protein based drug delivery modules are also discussed here.

2.2.1 Gelatin Based Nanocomposites

Gelatin is generally procured from bones and skins of animals, connective tissues, and by breakdown and hydrolysis of collagen. In drug delivery, it is commonly referred to as a matrixing agent. Gautam et al., in there scientific research synthesized the mannosylated gelatin mesosphere's for the effective delivery of doxorubicin to the lung cancer. They were using steric stabilization process for the synthesis and prepared the insufflation formulation with the size range of 8.7 μm, zeta-potential 1.74 mV. The lung accumulation study of different system(s) have been performed and better result was found in the gelatin conjugated system. The report showed better target ability, effective drug release pattern, and effective cell viability [60]. Das et al., reported paclitaxel loaded nanoparticles consisted of gelatin mixed with montmorillonite (MMT). The solvent evaporation method was employed to produce these nanoparticles. The author reported that swelling was increased on increasing glutaraldehyde concentration and subsequently drug release up to a certain point. The further increase in the crosslinker concentration has resulted in enhanced swelling and bioactive release. The cumulative release of the bioactive was observed to be enhanced at higher pH. At pH 7.4, the release of the bioactive was observed to be 80% within 8 h whereas at pH 1.2 the drug release was less than 44% within 4 h. Drug release was enhanced by increasing the concentration of the loaded bioactive [61]. The protein based nanocomposites are often used in gene therapy. Both viral and non-viral vectors are frequently employed for the transfection of DNA into the cells. It is because, in the living tissues, the injection of naked DNA leads to the degradation of enzymes and decreased cell uptake because of the repulsion between the negatively charged DNA and cell membrane. Zwiorek et al., worked on gelatin nanoparticles and demonstrated its potential for effective and safe non-viral gene delivery. The nanoparticles were manufactured by using the two-step desolvation method. The cationized particles showed homogenous size distribution with a low polydispersity index, efficient in enabling gene expression, and possess less toxicity [62]. These gelatin based nanocomposites are promising and hold the potential in gene therapy and tissue engineering.

2.2.2 Collagen Based Nanocomposites

Collagen is another natural protein of animal origin that is extensively employed in the formulation of nanocomposites. The collagen possesses a triple helix structure comprising of amino acids like proline, hydroxyproline, and glycine molecules [63]. They are considered suitable materials and are largely employed in multiple sectors like pharmaceuticals, cosmetics, and medical sectors. In one of the studies, Thanikaivelan et al., developed collagen-SPION (superparamagnetic iron oxide nanoparticles) nanobiocomposites. The nanobiocomposites were fabricated by a simple method by using protein wastes from the leather industry. The nanobiocomposites showed magnetic tracking ability and selective oil absorption. Moreover, the author has also reported its oil removal applications. This strategy paves the new ways of converting bio-wastes into functional and effective nanocomposites [64]. Zhang et al., worked on nanocomposite hydrogel based on collagen to revitalize articular cartilage via stem cell therapy. The author developed aldehyde functionalized surface-modified cellulose nanocrystals by using facile one-pot oxidation. A nanocomposite was developed from the cellulose based nanocrystals and collagen hydrogel by applying Schiff base chemistry. The results demonstrated self-healing attributes of nanocomposites with fast shear thinning and increased elastic modulus. The author also reported the potential of developed nanocomposite in mesenchymal stem cell (MSC) delivery. MSCs loaded surface-modified cellulose nanocrystals/collagen hydrogel nanocomposite exhibited increased cell viability, higher cell retention, and enhanced implant integrity. In addition to cell protection during injection, the developed collagen based hydrogel would also fit into the inconsistent cartilage defect [65]. Therefore, the developed nanocomposite appears to be promising and holds enormous potential for the MSCs delivery for cartilage regeneration by using minimally invasive procedures.

2.2.3 Albumin Based Nanocomposites

Albumin as a natural carrier has been largely exploited in the design and development of drug delivery modules in cancer therapy. It is because it possesses and assures several advantages such as biocompatibility, easy availability, low toxicity, and other versatile characteristics. Therefore, for the diagnosis of cancer and its treatment, several kinds of albumin based nanoconstructs are being fabricated for the purpose of multiple imaging and therapeutic effectiveness [66]. Xu et al., fabricated albumin stabilized nanoconstructs which are based on manganese for the effective delivery of siRNA to brain tumors. In this study, bovine serum albumin functionalized with Arg-Gly-Asp (RGD) peptide was employed as a stabilizer to produce nanocomposites. The author reported that the developed nanocomposite improves tumor oxygenation and decreases endogenous H2O2 and acidity in the tumor microenvironment. Apart from these characteristics, nanocomposite showed good stability and excellent biocompatibility. In a glioblastoma (U87MG) orthotropic model, the developed nanocomposite exhibited strong contrast improvement at pH 6.5–6.9. In addition to this, imaging performance was enhanced inside the tumor region due to the disproportionation reaction of manganese in the weakly acidic environment [67]. Zhao et al., reported gold nanocomposite that is based on albumin. A moderate one-pot reduction route was employed to develop bovine serum albumin based gold nanoparticles using hydrazine hydrate as a reducer. The developed nanoconstruct was functionalized with hyaluronic acid. The mean diameter of the nanocomposites was 13.82 nm. The nanocomposites were reported to exhibit good colloidal stability, water dispersibility, low cytotoxicity, and hemocompatibility. Moreover, the nanocomplex exhibited enhanced contrast in CT scanning [68]. Therefore these albumin based nanoplatforms could be explored as a contrast agent in MRI and CT scanning and in other biomedical applications.

2.2.4 Silk-Fibres Based Nanocomposites

Recently, silk proteins or silk fibers are being widely exploited in the fabrication of biomaterials. By processing these nanobiomaterials such as nanoparticles, hydrogels, microspheres, nanofibres, nano devices into a diverse set of morphologies, the utilization of these silk protein based biomaterials have increased for future biomedical applications. Silk proteins/fibers are versatile by a character in terms of biocompatibility, controllable in vivo biodegradation rate, exceptionally robust mechanical characteristics, etc. These versatile properties of silk proteins have triggered a prompt interest of the scientists in the biomaterial field [69]. Kishimoto et al., developed silk fibroin based nanocomposites. Montmorillonite (MMT) was incorporated into silk fibers to improve its physical properties. High-resolution Transmission electron microscopy micrographs exhibited 1.2 nm thick MMT layers have interacted with spun silk nanofibres in an unknown way. The developed nanocomposite exhibited a circular cross-section and a three-dimensional high porosity structure. The silk fiber-based nanocomposites may be employed as a scaffold for several biomedical applications and tissue engineering like bone regeneration. It is because the nanocomposites consist of biocompatible and biodegradable silk protein and osteoinductive MMT. Additionally, these nanocomposites can be useful in cell culture owing to the especially high surface area [70].

3 Challenges and Opportunities of Using Natural Polymers in Nanodrug Delivery

Natural polymer based nanocomposites serve as frontier areas in the bioactive(s) delivery that has gained huge attention from both academia and industry. From the pharmaceutical standpoint, natural polymers are commonly employed as binders, diluents, disintegrant, and matrixing agents in solid oral dosage forms. Over the last few years, these polymers are being used in the development of nanotechnology based nanocomposites. By virtue of their versatile characteristics like biodegradability, biocompatibility, easy availability, low immunogenicity, sustained and targeted drug release, these polymers are effectively and extensively utilized in the drug and vaccine delivery. These polymers are also being studied for gene therapy and tissue engineering. Despite promising trends and huge potential for applicability, these biodegradable polymers of natural origin are also associated with some disadvantages. The disadvantages include such as poor mechanical properties, rapid degradation rate, and high hydrophilic capacity. Moreover, in some cases, some polymers exhibit poor mechanical properties in the presence of humid environments making their application unprofitable/unviable.

4 Conclusion

Conclusively, natural polymers play a pivotal role in advanced bioactive delivery because of their biodegradability, compatibility, and less toxicity. They are selected according to the pharmaceutical dosage form. So by understanding the chemical, physical, and pharmacological properties, one can select a particular polymer for a drug delivery system. Additionally, the use of natural polymer has increased in the present scenario. These natural polymers are also used as surface modifying agents (as a ligand) in different drug delivery systems such as solid lipid nanoparticles, nanorods, liposomal formulations, microspheres, mesospheres, immunological preparations, etc. Moreover, they are widely employed as drug delivery modules for the treatment of various diseases such as cancer, rheumatoid arthritis, infectious diseases, and so on. The natural polymer-based research now has reached the preclinical/clinical levels.

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Natural Fibre-Reinforced Polymer Composites: Manufacturing and Biomedical Applications

Tielidy A. de M. de Lima, Gabriel Goetten de Lima, and Michael J. D. Nugent

Currently, the use of natural fibres as a reinforcement in composites presents many attractive benefits, including the reduction of materials from non-renewable sources and reduction of environmental impact. Intensive research is being carried out to develop biocomposites which combine natural fibres with biodegradable polymers. One major advantages of these biocomposites is that they are totally degradable and sustainable. Additionally, such compounds exhibit a wide variety of properties and can compete with non-biodegradable polymers in different industrial fields.

One major aspect in the use of natural fibres is the reduction in the amount of polymeric material in the end application. The scope for using natural fibres is wide, ranging from traditional applications, in the textile industry, to the reinforcement of thermoplastic and thermoset polymer matrices. Natural fibres are less abrasive than inorganic fibres, are usually used as reinforcement, and thus, generate less wear on the equipment involved in their processing [1]. Natural fibres offer the possibility of delivering greater added value to the final product, due to the lower costs of manufacture, sustainability, and recyclability, especially in the automotive industry.

When selecting fibres for reinforcement in composites, it is essential to consider several factors such as: cost and availability, effect on the viscosity characteristics of the polymer, physical properties, thermal stability, chemical resistance, abrasiveness or wear, biodegradability, toxicity, recyclability, wettability, and compatibility with the polymer matrix [2–4]. A particularly important aspect, one should also consider is the possibility of incompatibility between the polymeric matrix and the fibre, given that the interfacial interaction is, in many cases, very weak. In this case, a third

T. A. de M. de Lima · G. G. de Lima · M. J. D. Nugent (✉)
Materials Research Institute, Athlone Institute of Technology, Athlone, Ireland
e-mail: MNugent@AIT.IE

G. G. de Lima
Programa de Pós-Graduação em Engenharia e Ciência dos Materiais – PIPE, Universidade Federal do Paraná, Curitiba, Paraná, Brazil

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component is used in the composite, reducing the interfacial tension and increasing the adhesion between the phases of the polymer blend: the compatibilizing agent. There are several compounds that have this function, such as copolymers, glycidyl methacrylate, maleic anhydride, and among others [5].

Natural fibres are the most widely used for fabrication of biocomposites and can be applied in several areas such as railway sleepers[6], automotive[1, 7, 8], for wind turbine blades[9], building/construction industry [10–12], and biomedical applications [13–17]. The focus of this chapter is the study of natural fibre-reinforced polymer composites, their manufacturing and biomedical applications.

The demand for new materials for cell therapy, regenerative medicine, and drug release is increasing due to the decrease in recovery time and the improvement in the quality of life of patients benefited by such systems. Systems for carrying and releasing drugs for permanent or temporary replacement of injured tissues are examples of growing applications in the biomedical area; in such cases, natural degradable polymers are shown potential in temporary tissue replacement. The development of new materials for applications in these areas has undergone major changes in recent decades; however, there is much to be explored in optimizing the end properties [18].

1 Natural Fibre

Natural fibres can be found in plants, animals, and minerals; occurring spontaneously in nature and/or grown in agricultural activities. Within these fibres, there are three main divisions namely,

1. Minerals—formed by elongated crystalline chains can be further categorized as amosite, crocidolite, tremolite, actinolite, and anthophyllite.
2. Vegetable fibres which have a cellulosic nature [19]. Plant-derived natural fibres can be classified according to the part, or type, of the plant from which they are extracted, as shown in Fig. 1. These fibres include lignocellulose fibres because the majority contains lignin in their structure, a natural polyphenol polymer [5–7, 20, 21].
3. Animal Fibres—generally comprise proteins; examples include silk, wool, horse hair, and alpaca hair.

1.1 Advantages and Disadvantages of Biocomposites

Biocomposites from natural fibres have multiple advantages and disadvantages as a reinforcement in conjugated materials (composites) and these are listed in Table 1 [6, 7, 22, 23].

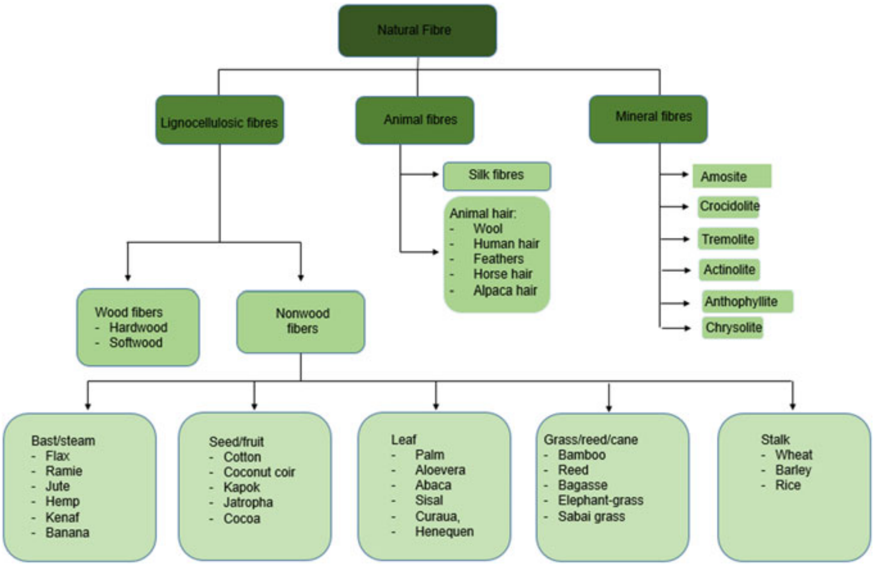

Fig. 1 Classification of the natural fibres

1.2 Chemical Compositions and Physical Properties of Natural Fibres

A natural fibre is a composite material consisting of cellulose, hemicelluloses, lignin, and further components. In this chapter, the focus will be concentrating on cellulose; however, we will briefly discuss each component present in natural fibre.

The chemical properties depend mainly on the content of cellulose and can vary; therefore, it is important to analyse each component present in the structure [6]. The physical properties of vegetable fibres are mainly determined by their chemical and physical composition, such as the fibre structure, cellulose, and degree of polymerization.

1.2.1 Cellulose

Cellulose is the essential component of all plants and this polymer exists abundantly in nature. Due to its chemical nature, it is capable of forming hydrogen bonds and is very hydrophilic. Between 40 and 50% of wood dry weight is in the form of cellulose [24]. Chemically, cellulose is a polysaccharide of molecular formula $(C_6H_{10}O_5)_n$ and composed from the union of ß -D-glucopyranose molecules through β-1,4-glycosidic bonds. Due to its high degree of crystallinity and polymerization, cellulose is usually more stable to degradation, be it mechanical, chemical, or thermal, when compared to non-cellulosic components co-forming these fibres.

Table 1 Advantages and disadvantages for natural fibres in biocomposites

Advantages
• Resources from renewable sources
• Abundant
• Desirable mechanical properties
• High specific strength
• Flexible behaviour
• Biodegradable
• Non-abrasive
• Low thermal conductivity
• Nonmagnetic and nonconductive
• Lightweight
• Environmentally safe and non-toxic
• Unique acoustic and thermal insulating properties
• Low cost
Disadvantages
• Low biological resistance—attacked by fungus and bacteria
• Usually requires low processing temperature in order to be shaped into desirable materials
• High thermal expansion coefficient
• Inconsistent physical properties for natural fibres vary with harvesting season and region
• Properties are dependent on the harvesting process, locality, and maturity of the plant

1.2.2 Hemicellulose

In addition to cellulose, hemicellulose also exists in natural fibres where 25–40% of the wood dry weight is in the form of hemicellulose [24]. The differences observed between cellulose and hemicellulose are associated with the chemical structure. Hemicelluloses are formed from various sugars with several branches that binds to cellulose microfibrils. The low crystallinity may be related to the branched and random structure of the hemicelluloses. In comparison with cellulose, hemicelluloses have shorter polymer chains and, therefore, low molecular weight. Hemicellulose is composed of pentoses, hexoses and is more susceptible to hydrolysis when compared to cellulose, since it has a lower degree of polymerization[24]. It occurs mainly in the primary cell wall with branched polymers containing five to six carbon sugars of varied chemical structures.

1.2.3 Lignin

Lignin is the third component fundamental to natural fibres, comprising between 15 and 35% of the wood dry weight. Lignin is a highly complex non-crystalline molecule comprised of a large number of phenyl-propane units [24]. Lignin is amorphous and has an aromatic structure with a high molecular weight.

1.2.4 Further Components

In addition to cellulose, hemicellulose, and lignin, we can find several other components in the structure of natural fibres. These compounds are in lesser quantity and can be turpins, waxes, acids, alcohols, proteins, inorganic material, among others. Chemical modification is usually required for natural fibres in order to remove surface impurities, and to eliminate the hydrophilic hydroxyl groups and, along with physical treatments, are widely used to modify its surface and structure to increase its performance [25, 26].

1.3 Modifications in the Surfaces of Natural Fibres

The interfacial bonding between both materials (matrix/fibre) in a biocomposite defines many of the composite properties. If the interface is clean, there will be a good bond between matrix and fibre which will help in effective load transfer. In the case of a lack of compatibility and adhesion between fibres and matrix, it could cause problem in processing and material performance [27]. Therefore, in order to achieve a better interaction, and to enhance the fibre/matrix compatibility, fibres are usually subjected to physical and chemical modifications [28]. Chemical and physical methods treat the surface of the fibre and optimize this interaction [29].

In chemical treatments, a variety of chemicals can be used, such as alkali treatments or coupling agents. These treatments can influence the cellulosic fibril, the degree of polymerization, the extraction of lignin and hemicellulose compounds, reduce the number of cellulose hydroxyl groups in the fibre-matrix interface, improve fibre-matrix adhesion, increase the strength of composite, decrease its water absorption and improve thermal stability [25, 30]. Abaca, coir, aspen, flax, hemp, ramie, sisal, and jute fibres have been studied using chemical treatments and the mechanical properties such as tensile strength, flexural modulus, and Young's modulus of the composites were increased significantly as well as the storage modulus [31–37].

Physical treatments can improve thermal stability, crystallinity, physical and mechanical properties, modification of the surface, polarity, and compatibility between hydrophilic fibres and the hydrophobic matrix [27, 38, 39]. As an example, thermal treatment (autoclave) as shown by Tavares et al. (2019) on açaí seeds (Euterpe oleracea) fibre were performed in order to use it as a composite reinforcement with a polypropylene (PP) matrix; which contributed to an increase of the fibre crystallinity and an increase in the fibre surface roughness, without compromising the thermal stability of the fibres. In addition, this treatment improved tensile strength but with a reduction in the tensile modulus [40]. Another useful treatment is the plasma treatment in which Sun (2016) demonstrated that surface modification of natural fibres can produce rougher and smoother surfaces. The treatment can provide many advantages, including altering surface properties of textiles and reducing the use of environmentally hazardous chemicals [41].

Recent works report the combined usage of using both chemical and physical methods in order to obtain materials with improved properties [42–45].

2 Composites/Biocomposites

Different combinations of metals, ceramics, and polymers can form composite materials. The composites can be classified on the basis of their structural components [3]:

1. Scale: nano-composites;
2. Reinforcement geometry: Fibre reinforced, particles reinforced, and sheet moulded;
3. Matrix material: Polymer matrix composites (PMCs), ceramic matrix composites (CMCs), and metal matrix composites (MMCs);
4. Biocomposites.

The focus of this chapter will be on the effect of natural fibres processed under various manufacturing processes in order to produce composites within various common polymetric matrices.

Natural fibres have been garnering considerable attention in composite applications because of advantages like reasonable mechanical properties, low density, renewability, resources from renewable sources, abundance, and economic feasibility [45]. The performance of natural fibre composites depends on numerous factors such as composition, structure, length, treatment of fibres, and fibre/matrix interface [29].

2.1 Composites Reinforced with Natural Fibres

Several studies incorporating natural fibres such as hemp [46–48], jute [48–50], banana [48, 51, 52], kenaf [48, 53–55], ramie[48], sisal [48, 56], coir [57], bamboo [52, 58–60], flax [61, 62], and Abaca [36, 49] have been reported, exploring their potential as a reinforcement into different polymer matrices.

2.2 Polymer Matrix Composites (PMCs)

A matrix is a binder material that is used to hold fibres and transfer external loads to internal reinforcements. A wide variety of thermoset and thermoplastic resins are used in polymer composites which have different chemical structures and undergo different reactions.

The polymeric matrix reinforced with natural fibres has higher resistance and this enables the interfacial bonding to maintain their chemical and mechanical identities. Fibres are the main members of the charge carriers, while the matrix retains them in place, and at a desired orientation [63]. In these composites, two types of matrices are commonly used, thermoplastic and thermoset, as shown in Table 2. A thermoset resin is cured by the application of heat and often by the addition of chemicals labelled as curing agents.

The mechanical properties of bast, fibrous material from a specific part of a plant—phloem—depend on the cellulose content and the angle of the microfibrils. Banana fibres have a complex structure with a high cellulose content (60–65%) and low microfibrillar angle. As such, banana fibre is emerging as one of the most important reinforcements due to its good specific strength and rotting resistance [28].

2.3 Advantages in Using Composites with Natural Fibres

Natural fibres are used as reinforcement in polymeric composites and depend on factors such as plant fibre structure, thermal stability, length, loading and orientation, presence of voids and moisture absorption of fibres [64]. The thermal behaviour is always affected by the moisture, additives, and other factors [137].

Varying the composite matrix, it is possible to have significant variations in the values of tensile strength and Young's modulus as seen in Fig. 2. Depending on the interfacial bonds and surface tension, it is possible that increasing the concentration of fibre will increase the mechanical properties values and make it stiffer. In addition, if more than one fibre is added and if they have similar polyvalences and charges, it is possible that these properties remains even more strong and a more resistant material is obtained. Finally, if these fibres are aligned towards the desired load, the resistance is also increased. Such behaviour has been reported extensively in the literature [138].

However, natural fibres may present different mechanical properties depending on the interfacial bond between the polymer, for example, the tensile modulus of jowar fibre composite is reported to be 11% greater than those of bamboo composites and 45% greater than those of sisal composites, respectively, at 0.40 volume fraction of fibre. The flexural strength of jowar composite is greater 4% than those of bamboo composites and also 35% greater than those of sisal composites with a flexural modulus 1.12 times and 2.16 times greater than those of bamboo and sisal composites, respectively. Jowar fibres as reinforcement in polyester matrix can be

Table 2 Thermoplastic and thermoset polymeric composites reinforced with different natural fibres [63, 64]

Thermoplastic	Characteristics	Vegetal fibres
Polypropylene (PP)	Lightweight material, good insulation properties, non-toxic, low moisture absorption, and low cost	Curaua [65], flax [66, 67], hemp [68], jute [69], palm, sisal [67], wheat straw [67], banana [70]
Polyethylene (PE)	Non-toxic, low moisture absorption, low cost, rigid, and moisture resistant	Banana [71], green coconut husks [72], rice husk [73], sisal
Nylon	High stability and adaptability	Kenaf, flax, hemp [74]
Cellulose acetate	Low toxicity, good stability, high permeability, high glass transition temperature (Tg), production of resistant films, biocompatibility with a series of additive agents, and ability to form micro and nanoparticles	Kenaf [75, 76], curauá [77], sisal [78]
Polystyrene (PS)	High resistance to alkalis and acids, low density and moisture absorption, low resistance to organic solvents, and heat	Agave [79], banana [80], hemp [80], sisal [80]
Polycarbonate (PC)	Excellent electrical insulating characteristics, strong, and rigid	Pineapple [81]
Polyvinyl chloride (PVC)	Resistant, waterproof, durable, innocuous, non-toxic	Areca [82], bamboo [83], straw [84], rice [85]
Acrylonitrille-butadiene-styrene (ABS)	Outstanding impact strength and high mechanical strength	Palm [86], kenaf [87]
High-density polyethylene (HDPE)	Good low-temperature impact resistance and excellent chemical resistance	Banana [88], curaua [89], sisal [90]
High impact polystyrene (HIPS)	Flexibility, impact resistance, easy machinability, and low cost	Green coconut husks [91], sisal [92], sugar cane bagasse [93]
Thermoset	*Characteristics*	*Vegetable fibres*
Polyester	Strong, flexible, dries quickly, resists wrinkles and shrinking	Bamboo [94], banana [95, 96], coconut [97, 98], curaua [99], flax [100],hemp [101, 102], jute [103, 104], pineapple [105], sisal [106–108]

(continued)

Table 2 (continued)

Thermoplastic	Characteristics	Vegetal fibres
Polyurethane (PU)	High abrasion resistance, good low-temperature capability, wide molecular structural variability, ambient curing possible, and low cost	Banana [109], coir [110], sisal [110–112], curaua [113]
Epoxy	Virtually no post-mould shrinkage, resistance to high impact and high temperatures, chemical resistant and fungus resistant	Banana [114–116], coir [117], cotton [118], flax [119, 120], hemp[120], Jute [121, 122], pineapple [123], sisal [116, 124–127]
Phenolic	Excellent dielectric strength, great mechanical strength and dimensional stability, resistant to high heat, wear resistant, low moisture absorption	Banana [128, 129], cotton [130] flax [131], jute [130], sisal [112, 132]
Urea–Formaldehyde	Very hard, scratch-resistant material with good chemical resistance, electrical qualities, and heat resistance	Allo [133], cotton [133], sisal [134], coir [135], straw [136]

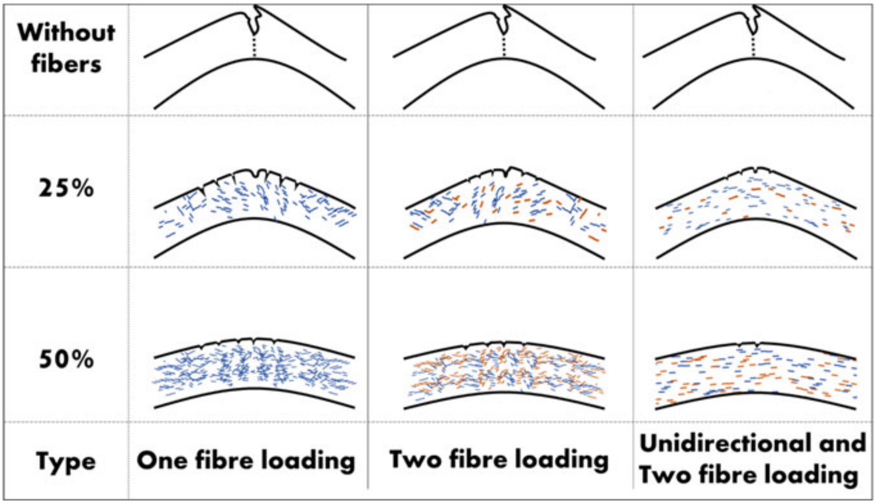

Fig. 2 Schematic representing the effect of adding different fibres, direction, and concentration in the resistance of the material

successfully developed as a composite material containing high strength and rigidity, with lightweight applications compared to conventional sisal and bamboo composites [139].

In the case of jute fibres, its usage can also improve the mechanical properties of composites, for example, the tensile strength obtained for PP laminate prepared from its own filaments was 23.24 MPa. However, when this same PP filament is synthesized with jute yarns, with a jute content of 19.34%, its tensile strength increased up to 31.21 MPa. On further increase the jute content to 37.1% and 55.89%, the tensile strength increased to 38.27 MPa and 53.06 MPa, respectively. Tensile modulus of composite containing jute yarn with matrix PP resulted, with fibre contents of 19.34%, 37.1%, and 55.89%, exhibits a tensile modulus increase in 23.7%, 50.42%, and 79.31%, respectively, compared with pure PP [69], as shown in Fig. 2.

The maximum tensile strength and tensile modulus of banana fibres incorporated into polypropylene composites (50%wt) are 70.82% and 67.60% higher than PP pure, respectively [70]. Using the same matrix, the variation in mechanical properties occurs by using different natural fibres, using flax fibre with epoxy resin increased 81.95% the tensile strength compared with banana fibre in the same matrix [116, 140]. In addition, hybrid composite with two or more fibre in the same matrix presents an improvement in tensile strength. Hemp composites with epoxy resin present a tensile strength of 36.48 MPa, a hybrid composites with Hemp/flax had 44.17 MPa and hybrids composites with hemp/flax/jute this value increased to 58.59 MPa. An increase of 21.08% and 60.60%, respectively [140].

3 Manufacturing Techniques

From ancient civilizations through to future innovation, composites have an important role. These materials offer many advantages such as: corrosion resistance, design flexibility, durability, lightweight ratios, and strength. Composites have been used for thousands of years in different areas. The first record was in 1500 BC when early Egyptians and Mesopotamian settlers used a mixture of mud and straw to create strong and durable buildings. This combination of mud and straw gives it a strong property against compression, torsion or bending [45]. In 1200 AD, the Mongols combined “animal glue”, bone, and wood in order to produce the first composite bow. In 1945, more than 3 million kg of glass fibres were used for various products, primarily for military applications. Composite materials continued to take off after the war and grew rapidly through the 1950s. In the following years, many records of the use of composites can be found in the most diverse areas. With the advance in the use of composites, processing techniques have been improved, and today, it is possible to develop diverse materials for applications ranging from aircraft turbines or advanced products in the biomedical field. Indeed, the composite industry is still developing, with much of the growth now focused on renewable energy and new manufacturing techniques. The following sections will discuss the main manufacturing techniques

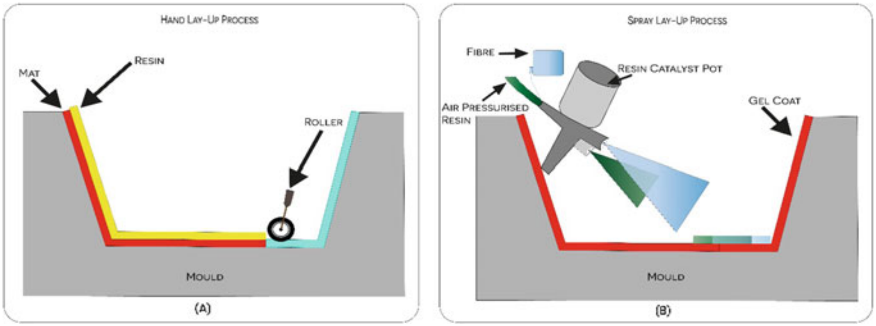

Fig. 3 **a** Hand lay-up process; **b** spray lay-up process

in the production of composites like hand lay-up, filament winding, compression moulding, injection moulding, and among others [141].

3.1 Open Moulding Technique

In this process, resin and fibres are cured in an open mould.

3.1.1 Hand Lay-up or Spray Lay-up

This technique is the simplest method of composite processing requiring minimal infrastructure [142]. Fibre reinforcements are placed by hand in a mould and resin is applied with a brush, roller, or spray, as shown in Fig. 3. There is no requirement of heat for the curing process.

However, there are quality issues using this technique, such as air entrapment can create a weak matrix and low-strength parts; the resin and catalyst should be accurately metered and thoroughly mixed for correct curing times; its toxicity and flammability of resin is an important safety issue, especially because of its high manual handling and the final application product; in addition, the surface roughness and surface detail could be acceptable on a moulded surface, but very poor in the opposite surface and shrinkage increases with higher resin volume fraction [143].

3.1.2 Filament Winding

Filament winding is an automated open moulding process that uses a rotating mandrel as the mould. The mould configuration produces a finished inner surface and a laminate surface on the outside diameter of the product as shown in Fig. 4. Various

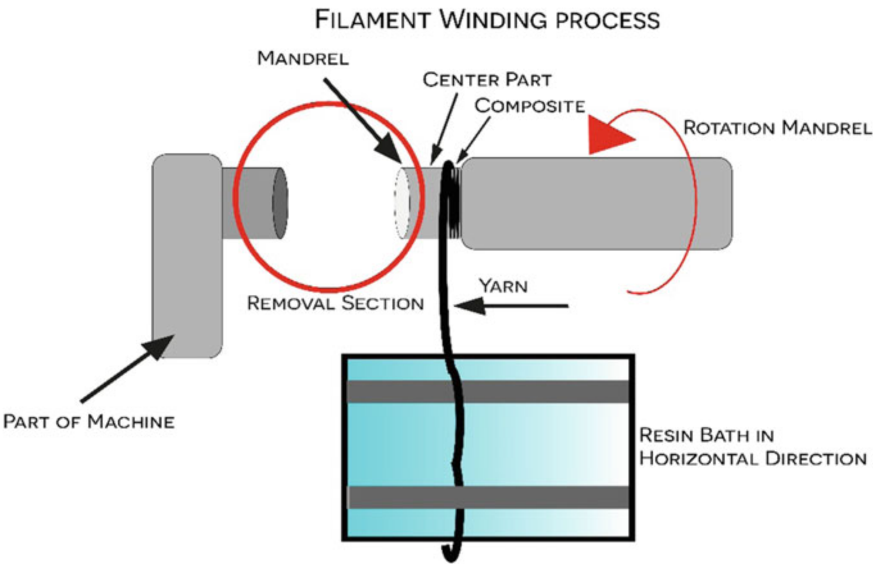

Fig. 4 Filament winding process

benefits to filament winding are evident such as short winding because of simplified tooling concept; short mandrel preparation time; availability of raw materials; relatively low cost of raw materials (matrices and reinforcements); relatively low tooling costs; polymers can easily be formulated, and the formulation can easily be changed according to individuals' needs; the process is reproducible or repetitive; continuous fibres can be used for the entire components; high fibre volume is achievable; fibres can be oriented in the loading direction; part size is not limited by oven-size; and process can be automated with cost savings. The main drawbacks of this process are that component must facilitate removal of mandrel; high cost of mandrel and complex; winding reverse curvature is not possible; difficulty in placing fibres parallel to the mandrel axis and need for external mandrel surface treatment for surface evenness [144, 145].

Ansari et al. (2019) studied the effect of winding speed on the mechanical properties of kenaf fibre reinforcement as geopolymer composites via filament winding technique. In this study, four speed winding (very low, low, high, very high) were used. The kenaf fibre was impregnated with resin by the means of a homemade impregnation machine. Compression tests in vertical axis results in values of 3.022, 7.328, 10.705, and 14.278 MPa for windings speeds from low to very high. The highest winding speed also resulted in highest strain, 9.69 mm/mm, while the lowest strain was for the lowest winding speed, 2.4 mm/mm. The maximum load to increase as well when increasing the speed, from 1.54 kN to 10.19 kN. Results of testing for the horizontal position were similar to vertical position. The speed of winding had affected the pattern of winding, additionally, it will also affect the thickness of filament wound product and the mechanical properties of the filament wound product [146].

3.2 Closed Moulding

In this process, resin and fibres are cured inside the mould.

3.2.1 Centrifugal Casting

Centrifugal casting, also called rotational moulding, rotomoulding, rotational casting, or corotational moulding. Fibre-reinforced composites can be produced by rotating a mixture of chopped strand and catalyzed resin inside a hollow mandrel as shown in Fig. 5. These composites can be less homogeneous than those produced by other techniques, because of differences in specific gravity [147]. Advantages of centrifugal casting include its ability to produce hollow parts with complex shapes; both mould and machine are simple and low cost; low-pressure process allows thin-walled low-strength moulds to be used; different sizes of parts can be produced simultaneously on the same machine; large metal inserts, graphics, surfaces, and textures can be moulded directly into parts with, usually, very low scrap as all the materials are consumed to make the part. On the other hand, this process has some disadvantages like the process is not suited for very large production runs of smaller parts; there is limited selection of material available for this process [141, 147]; cycle times are comparatively longer as the mould needs to be heated and then cooled, loading, and unloading of the mould could be labour intensive and large flat surfaces, bosses, ribs, and dimensional tolerances can be difficult to produce [141].

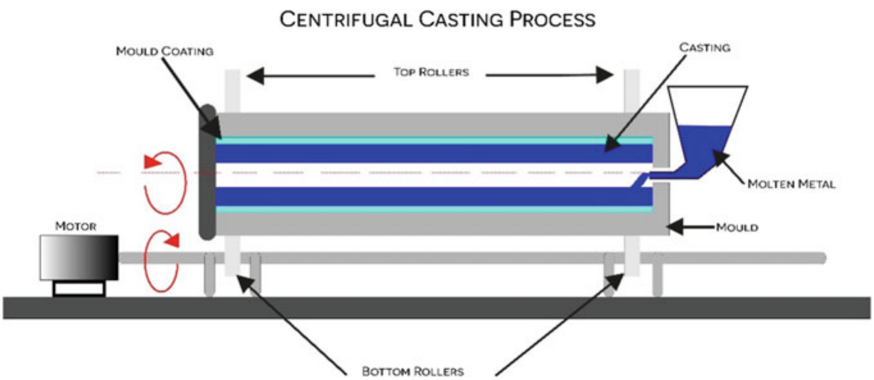

Fig. 5 Centrifugal casting process

Jamaludin et al. (2019) fabricated cylindrical tubes of functionally graded natural fibre-reinforced polymer (FGNF/epoxy) composite using horizontal centrifugal casting method. In this work, a coconut coir was mixed with epoxy; obtaining four different compositions 0%, 5%, 10%, and 15%. The fibres were chemically treated (NaOH solution) for 24 h and were mixed with epoxy using the centrifugal casting. When more natural fibre is added, decreases the material density from 1.1782 to 1.1656 (g/cm^3) at maximum load. The hardness and compression strength increased with natural fibres reinforcement from 66,25HD to 84HD 5% for hardness and 15 to 33,631 MPa, but it was slightly decreased with additional fibres load which was attributed to the porosity increase [147].

3.2.2 Pultrusion

Pultruded material has a constant cross section, manufactured through a mould (or mandrel). The matrix consists of mixing homogeneous resin and mineral fillers, whereas the reinforcement is a continuous filament (roving), as shown in Fig. 6 [141]. Fibre content varies between 50 and 80% by weight, depending on strength requirements; vinyl ester and epoxy resins provides up to 30% more strength than polyester resins; residual stresses and distortions can be minimized by specifying constant wall thicknesses, which cools more uniformly; approximately, 2–3% shrinkage can occur on the cross section when fully cured [143]. The range of the temperature for the pultruded composite profile is generally between 100 and 200 °C. The temperature setting for the pultruded fibre composites has to be carefully chosen to prevent the loss in their properties [148]. The main advantages of using pultrusion can be related to its high stiffness-to-weight ratio; ability to make tubes and sheets with precision

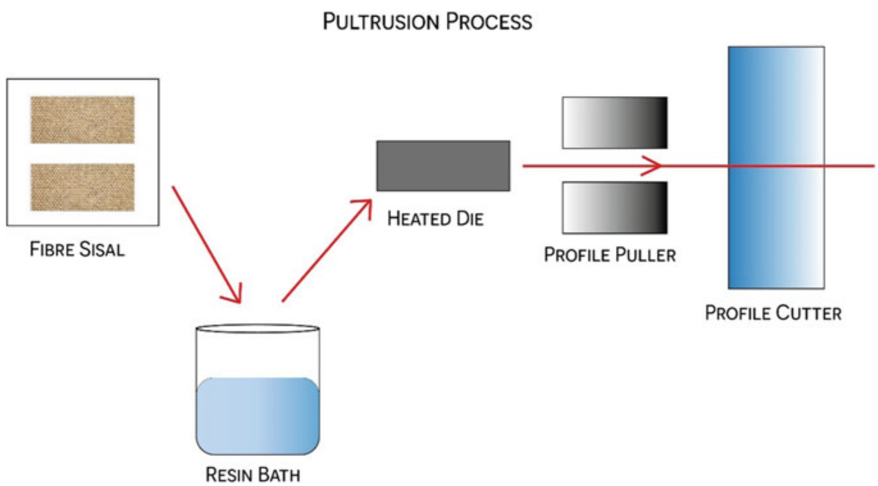

Fig. 6 Pultrusion process

wall thickness; high strength-to-weight ratio; low cost; high efficiency, durability, and noncorrosive character traits [141]. However, limited work has been reported on the processing parameters of pultruded natural fibre composites.

Chang et al. (2019) investigated the mechanical and wear properties of heat-treated pultruded kenaf fibre-reinforced polyester composites (PKFPCs) using pultrusion technique. The results showed that PKFPC with a 140 °C heat treatment exhibited better wear performance than untreated PKFPC and PKFPC using 120 and 170 °C heat treatments. The temperature of 120 °C had the best results regarding its flexural strength and modulus of PKFPC compared with untreated samples with an increase of 37% and 16%, respectively. The kenaf fibre in PKFPC was damaged when the heating temperature reached about 170 °C and led to the reduction in flexural properties. Physical modification by heat treatment, on the natural fibre, can be an effective way to improve the mechanical and wear properties of natural fibre-reinforced polymer composites [149].

Fairuz et al. (2016) studied the effect of filler loading on mechanical properties of pultruded kenaf fibre-reinforced vinyl ester composites. The tensile strength and tensile modulus showed an increase in the filler loading, improved the mechanical properties of the composites. The tensile strength had an increase of 20% when the percentage of the filler loading was increased from 20 to 50%. The tensile modulus increases 25% from 20 to 50% of filler loading, as in the case of tensile strength [150].

3.2.3 Compression Moulding

A measured quantity of raw, unpolymerized plastic material is introduced into a heated mould, which is subsequently closed under pressure, forcing the material into all areas of the cavity as it melts, this is shown in Fig. 7. Variation in raw material charge weight results in variation of part thickness and scrap; air entrapment is possible; internal stresses are minimal; dimensions in the direction of the mould opening and the product density will tend to vary more than those perpendicular to the mould opening. Surface detail is good and the surface roughness is a function of the die condition, in which typically, 0.8 μm is obtained [143].

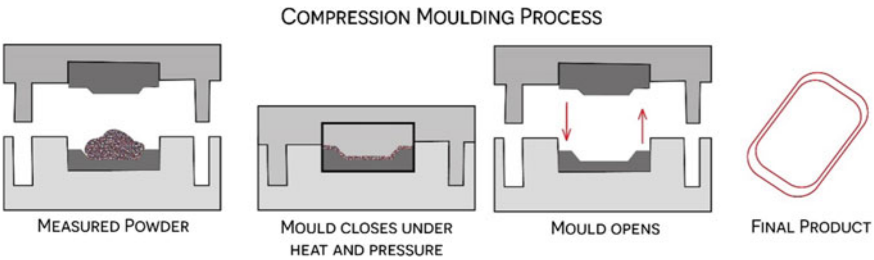

Fig. 7 Compression moulding process

Halim et al. (2019) studied the fabrication of unidirectional coir fibre as reinforcement for nonwoven melt-blown glass fabric using compression moulding. In this work, the fibres were chemically treated with NaOH and silane coupling agent, and the fibres were immersed in the materials which were oriented to achieve unidirectional preforms. A sandwich of preform with glass sheet in the middle was compacted in 5 MPa for 8 min at 170 °C. The tensile strengths were improved in samples with chemical modification, in addition to their storage modulus. The higher value indicates the higher stiffness and load bearing capacity of coir fibres in the reinforcement system. The chemical treatment of coir fibres acting as reinforcement to the nonwoven glass fabric increased the thermal resistance of the composite [151].

Yang et al. (2019) studied thermal and mechanical performance of unidirectional composites from Bamboo fibres reinforced with epoxy resin by the hand lay-up technique followed by compression moulding using various fibre volume fractions (0%-70%). Bamboo fibre-epoxy-based composites have a better thermal stability when compared with neat resin. The resin storage modulus is 2.5 GPa but when reinforced it increases from up to 9.7 GPa at the maximum filler addition. This increase in storage modulus with an increase in fibre content indicated that the composite stiffness is improved. The loss modulus of composites is much higher than that of neat resin, and the peak value of loss modulus increases with the increase of fibre content. In addition, the tensile strength increases from 21.0 to 134.3 MPa when increasing the fibre content from 0 and 70%. The improvement of mechanical performance makes it possible for epoxy-based composites to be widely used in certain practical applications [137].

3.2.4 Vacuum Bag Moulding

The vacuum bag moulding is one of the most versatile processes used for manufacture composite parts. This process combines a manual method using hand lay-up, or spray-up, on an open mould to produce a laminated component with a vacuum process and covering using a polymeric sheet. A vacuum is applied between the mould and the bag to squeeze the resin/reinforcement together, removing any trapped air, as shown in Fig. 8. Curing is normally performed in an oven [3, 152]. Some advantages of vacuum bag moulding are higher fibre content, laminates are easily produced with this technique; lower void contents and resin flow throughout structural fibres, with excess into bagging materials; the vacuum bag reduces the amount of volatiles emitted during cure, in addition to high-quality moulds, with complete elimination of voids and air bubbles.

Manjunath and Krishnamurthy (2019) studied the mechanical properties of hybrid composites using jute and e-glass by the hand lay-up and vacuum bagging technique. Tensile test results for hand lay-up technique resulted in 60.40 MPa and Young's modulus of 4306.6 MPa with flexural and hardness test of 159.47Mpa and 92 MPa, respectively. When the vacuum bagging technique was performed tensile strength and Young's modulus were 116.04 MPa and 8721.67 Mpa with flexural and hardness test of 450.50 MPa and 96 MPa, respectively. Mechanical and physical properties are

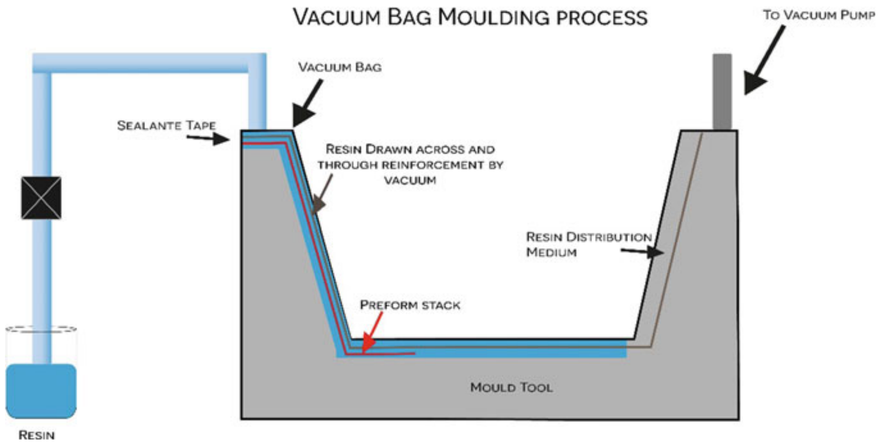

Fig. 8 Vacuum bag moulding process

greatly affected by fibre type and orientation. Vacuum bagging technique was found to be more suitable as compared to hand lay-up technique since vacuum bagging has yielded considerable better results [153].

Fajrin (2016) studied mechanical properties of natural fibre composite made of Indonesian grown sisal by vacuum bag process. In this work, Fajrin compared sisal fibre prepared using randomly orientation (RSO) and unidirectional oriented fibre (UOS). For UOS, the values of tensile, flexural, shear, and compressive stress were 40.25 MPa, 62.16 MPa, 23.26 MPa, and 60.88 MPa, respectively. Regarding the RSO, the values of tensile, flexural, shear, and compressive stress were 22.52 MPa, 51.5 MPa, 22.34 MPa and 49.12 MPa, respectively. This study shows that the orientation of sisal fibre alters the mechanical properties and unidirectional oriented provides laminates with higher mechanical properties [154].

Sanjay et al. (2018) studied the impact and inter-laminar strength of e-glass with jute/kenaf woven fabric epoxy composites, with the aim of evaluating the hybridization effects on different laminate stacking sequences made by these materials by the vacuum bagging method. The hybrid composite laminates with kenaf and jute containing e-glass fabrics demonstrated better results than composites laminates without the e-glass fibres. Laminate composites containing only natural fibre (jute, kenaf, or jute + kenaf) had impact strength ranging from 122.5 to 171.5 J/m. Nonetheless, the hybrids composites laminate exhibited values between 792.4 to 1078.4 J/m on impact strength. Therefore, the impact strength of composites depends on the inter-laminar and interfacial adhesion between the fibre and the matrix and also depends on the properties of individual fibres—fibre length, fibre loading and fibre orientation, for example, jute with e-glass composite the impact strength was 792.4 and kenaf with e-glass had 897.4 J/m [155].

3.2.5 Vacuum Infusion

Vacuum infusion is also known as the resin film infusion process. This technique consists of absorption of the matrix through a vacuum inside of a mould with the reinforcements, natural fibres, already arranged, and pre-oriented as shown in Fig. 9. The vacuum infusion process is widely used to manufacture large pieces [3, 143, 152] since it promotes better interfacial bonding between fibres and matrix phases that, consequently, produces a composite material with outstanding mechanical properties.

Bosquetti et al. (2019) studied the evaluation of the mechanical strength of sisal fibre reinforced with polyurethane composites panels using the vacuum infusion processing method. The tensile strength of the panels resulted in values of 146.34 MPa, 9.19 MPa, and 15.87 MPa for aligned, one-layer, and three-layered panels, respectively. When fibres were aligned to the load, they were responsible for bearing the applied load resulting in an improved resistance. When fibres were placed opposite direction to the load direction, the composite panel loses the resistance capability lowering its ultimate strength. Composites with one-layer of fabric and three-layers presented tensile strength of 9.19 MPa to one-layer and 15.87 for three-layered [156].

Yusuff and Ahmad (2019) studied the mechanical performances of a hybrid composites from kenaf/carbon with epoxy resin, which were fabricated via vacuum infusion technique in order to investigate the effect of various load of these natural fibres into the matrix. The kenaf fibre addition to the composite material improves the elongation at break, presenting highest elongation at break at 25 vol.% of kenaf fibre contents. The kenaf fibre exhibits good stiffness, which affects the elongation at break compared to carbon fibre [157].

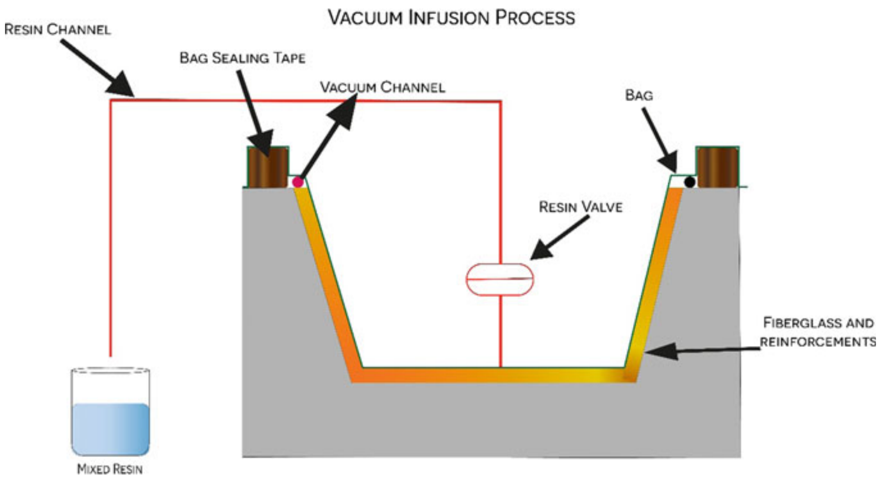

Fig. 9 Vacuum infusion process

Aisyah et al. (2019) studied the effects of carbon fibre hybridization on the thermal properties of woven kenaf-reinforced epoxy composites using vacuum infusion technique. In this work, a hybrid composite with higher kenaf fibre content had better thermal stability by presenting higher decomposition temperature, which was presented by the DSC, leading to a higher thermal stability was in pure carbon fibre composite [158].

3.2.6 Resin Transfer Moulding (RTM)

The resin transfer moulding (RTM) injects resin under pressure using an injection equipment into the mould cavity, in which the dry reinforcement materials are already arranged and pre-oriented. RTM is performed at room temperature with fast cycle times. A characteristic of the RTM is its low injection pressure. [3, 143, 152]. Some benefits of RTM are the possibility of producing large pieces; good dimensional tolerance; low cost of equipment for production; ability to produce parts with inserts; short production time cycles; possibility of automating the process; can operate with different types of resin; ability to vary the volumetric fraction of the composites; low solvent emission (operates within a closed mould), causing low environmental impact. Figure 10 shows a schematic of the RTM process.

Pinto et al. (2019) evaluated the impact resistance of hybrid jute-cotton fabric composites by the RTM process. A comparative study of the mechanical properties on impact of composites with four and six layers of jute/cotton fabric was carried out. Non-ageing specimens proved to be more resistant to impact than aged specimens. This behaviour was already expected, as the absorption of water makes it difficult for the matrix to adhere due to the fact that water lodges between the fibre/matrix interface and degrades both materials. The impact strength increased with the amount of the fabric layers that reinforces the composite.

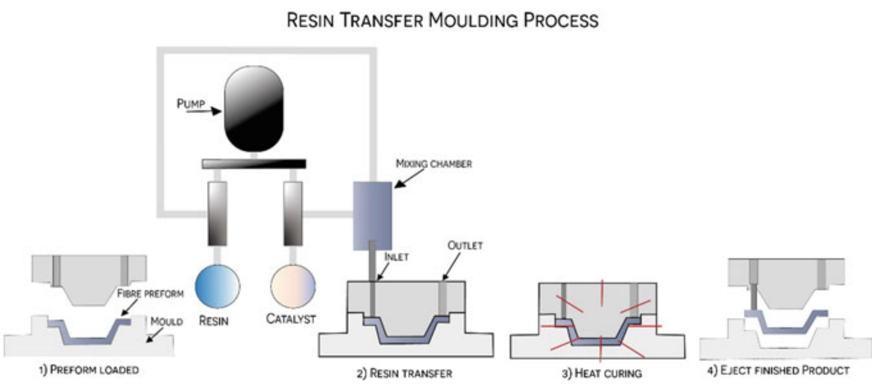

Fig. 10 Resin transfer moulding process

Mbakop et al. (2019) studied the effect of compacting parameters on preform permeability and mechanical properties of unidirectional flax fibre composites by RTM. In these reinforcements, the unidirectional fibre yarns were held together by a thin mat layer of short flax fibres. Reinforcements were compacted in dry or wet conditions at ambient or high temperature prior to permeability testing. Hot and wet compaction does not alter the permeability of the UD flax/mat reinforcement. The samples non-compacted and compacted followed by drying in ambient temperature exhibited the lowest value of tensile strength in 280 MPa and 290 MPa, respectively. Samples compacted without humidity using temperature of 100 °C and wet compacted at 23 °C and 100 °C exhibited the highest values of tensile strength as 340, 350, and 360 MPa, respectively [159].

3.2.7 Injection Moulding

This process can be performed using thermoplastic and thermosetting polymers. Composites are fed into a heated barrel, mixed, and forced into a mould cavity, where it cools and hardens to the configuration of the mould cavity, Fig. 11 [143, 152].

The main advantages of injection moulding are high production rates; repeatable high tolerances; ability to use a wide range of materials; excellent surface detail; *l*ow labour cost, minimal scrap losses, and little need to finish parts after moulding.

Correa-Aguirre et al. (2020) explored the reprocessing behaviour of polypropylene-sugar cane bagasse biocomposites, using neat and chemically treated cane bagasse fibres. These biocomposites were reprocessed five times using the extrusion process, followed by injection moulding after each reprocessing cycle. The mechanical properties indicate that microfibers bagasse fibres addition and chemical treatments generate improvements in the mechanical properties. The first processing

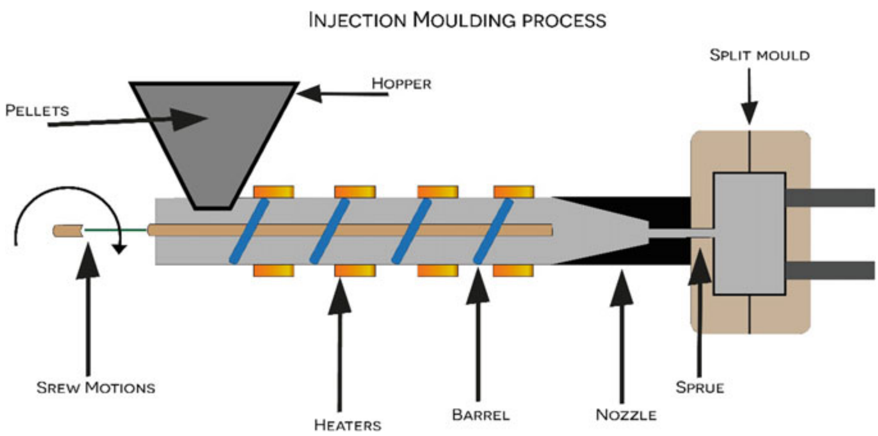

Fig. 11 Injection moulding process

cycle presented a flexural modulus of biocomposites PP-Bagasse (2069 MPa) and PP-Bagasse + alkali (1847 MPa), increased by 60% and 42% compared to neat PP (1296 MPa). Additionally, the flexural strength values increased for PP-Bagasse (48 MPa) and PP-Bagasse + alkali (43.3 MPa), increased by 20% and 8% compared to neat PP (40 MPa). For the third processing cycle, all flexural modulus and flexural strength values of the biocomposites presented significant differences compared to the flexural modulus value of the pure PP matrix. These increments were 57% and 48% for PP-Bag., PP-Bag. + alkali, respectively; also, flexural strength values increased by 11% and 7%, respectively. The last cycle had no significant differences were detected among the biocomposites in regard to flexural modulus and flexural strength. This could be related to the higher thermal stability of chemically modified fibres and a better interaction fibre–matrix, generated by the reprocessing cycles. Reprocessing and chemical modifications induced a better adhesion on the interface between bagasse fibres and PP matrix, while also increased the PP capacity to absorb energy perceived by the DMA.

3.2.8 Extrusion

Extrusion as a single- or twin-screw is the main industrial process to incorporate lignocellulose fibres into polymers [160]. In this process, plastics are continuously melted and pressed as a viscous mass from a pressure chamber through a shaping die. The moulding compound is granulated or powder, which is plasticized and compacted. The finished compressed part is cooled in the next step by water or air so that hardens, as shown in Fig. 12 [161]. Low cost per part; flexibility of

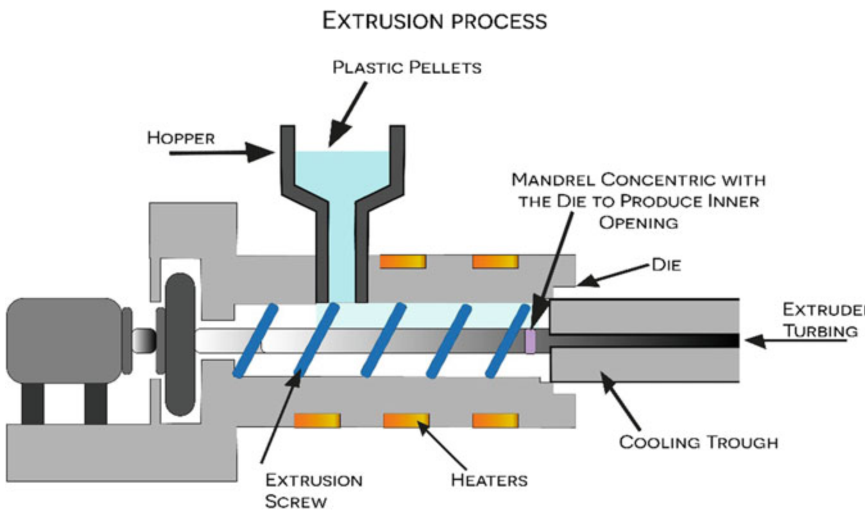

Fig. 12 Extrusion process

operation; in hot extrusion, post-execution alterations are easy because the product is still heated, continuous operation; high production volumes; many types of raw materials can be used; good mixing (compounding); surface finish obtained is good and good mechanical properties obtained in cold extrusion are some benefits of extrusion process.

Munde et al. (2019) studied the effect of sisal fibre loading on mechanical, morphological, and thermal properties of extruded polypropylene composites. The tensile modulus increases from 760.5 MPa to 1009 MPa for 10 wt% fibre loading, compared with neat PP, showing 33% improvement. Further addition of fibre at 20 wt% and 30 wt%, tensile modulus increases by 105% and 153%, respectively, due to the stronger interfacial interaction between the fibre and polymer. Thermal analysis shows a considerable improvement in thermal stability of composite compared to pure PP. The maximum 15.38% improvement in decomposition temperature is observed for 20% weight fraction of sisal fibre [162].

Miyahara et al. (2018) prepared and characterized composite materials using plastic waste from hydrapulper (PWH) obtained from paper industries and extruded with sugar cane fibre (SCF) residues from ethanol industries. Higher fibre proportion in the composite presented positive effects, mainly in the compression and impact tests. Thermal analysis showed that between 250 °C and 450 °C the composite with 40% fibre loses lower mass and degrades more slowly than the sample with 30% fibre, this is because natural fibre compounds have higher heat resistance and consequently high resistance to decomposition [163].

Teixeira et al. (2019) studied the impact of content and length of curauá fibres on mechanical behaviour of extruded cementations composites. The fibre content directly influenced the mechanical performance and fibres with greater lengths which presented better mechanical results for the modulus of rupture and fracture energy. These results demonstrated that curauá fibres after 200 accelerated ageing cycles were better in comparison with composites at 7 days, because of the cement hydration, which filled the pores, densified its structure, and improved the transition zone fibre matrix [164].

4 Biomaterials

Biomaterials are defined as devices that works within biological systems (including biological fluids) and may consist of compounds of synthetic or natural origin, as well as chemically modified natural materials. They can also consist as solids, gels, pastes, or even liquids, not necessarily being manufactured, such as pig heart valves and human skin flaps treated for use as implants [18, 165, 166]. These biomaterials comprise all, or part, of a living structure or biomedical device that performs, augments, or replaces a natural function to improve patients' quality of life. The scope of biomaterials includes simple implants like intraocular lenses, sutures, wound dressings, cell matrices, bone plates, joint replacements to more complex materials like biosensors, catheters, pacemakers, blood vessels, and artificial hearts. Biocompatibility means that the biomaterials must not form thrombi in the blood system, resulting in tumours in the surrounding tissues, or be immediately attacked, encapsulated, or rejected by the body [167]. The term biocompatibility was redefined in 1987 by Williams as the ability of a material to perform with an appropriate tissue response in a specific application. There are some factors that impact negatively in biocompatibility such as toxicology; reactions related to products from extrinsic microbiologic organisms colonizing the biomaterial; mechanical effects (rubbing, irritation, compression, and modulus mismatch) and also a broad range of interactions with surrounding proteins, and cells, inducing cell–biomaterials interactions (and tissue–biomaterials interactions) that might direct longer-term in vivo bioreaction.

The properties of biomaterials are classified into chemical, physical, mechanical, and biological in relation to the bulk and surface of the material. Figure 13 shows the different factors involved in biomaterials properties [168].

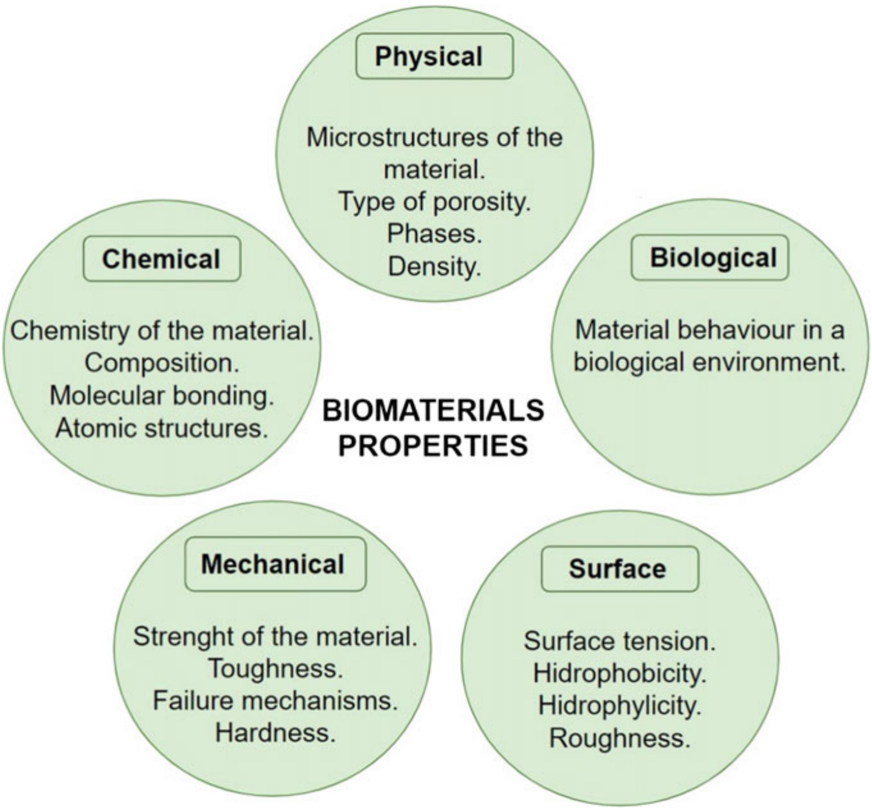

Fig. 13 Biomaterials properties

The properties and biocompatibility are important factors when choosing biomaterials; related with performance and success of implants and medical device to accomplish specific functions in the human body. Besides, these properties have been shown to have an important influence in their dynamic interactions with the biological surroundings when used as medical implants and organ or tissue replacements. Biomaterials are used in diagnostics (gene arrays and biosensors), medical supplies (blood bags and surgical tools), therapeutic treatments (medical implants and devices), and emerging regenerative medicine (tissue-engineered skin and cartilage) [18, 168].

A large number of polymers are used in multiple applications. Polymers are available in the most varied compositions, properties, and forms (solids, fibres, films, and gel), and they can be produced in diverse shapes and structures.

In addition, the success of the biomaterial in the body depends on several factors; Table 3 summarizes these in the selection of materials for biomedical applications.

The detailed analysis of the categories for chemical compounds used in the constitution of biomaterials, properties, advantages, limitations, and applicability are of great importance. Many materials can be used in biomedical application and they may be grouped into metals, ceramics, polymers, and composites. In this section, we will focus on polymers and composites.

Polymers are well suited for biomedical applications because of their diverse properties. The main advantages of polymeric biomaterials compared to ceramic or metallic materials includes the ease of manufacture to produce, varied shapes (particles, films, wires, among others), secondary processing, reasonable cost, and availability in finding materials with mechanical and physical properties desired for specific applications [170, 171]. Polymers can be obtained from polymerization reactions or by means of living organisms, thus, being classified, respectively, as synthetic and natural, which can also be chemically modified.

Natural polymers can include proteins (such as collagen, elastin, and silk fibroin) and polysaccharides (such as chitosan, alginate, xanthan gum, hyaluronic acid, and pectin.

Synthetic polymers can include polyamides, polyethylene, polypropylene, polyacrylates, fluorocarbons, polyesters, polyethers, polyurethanes, and among others. Table 4 can be observed some natural polymers and synthetic polymers and their application.

Natural Materials:

Composite materials consist of two or more distinct parts. The formation of composite biomaterials can occur by various methodologies, and the main associations being of the polymer-ceramic and metal-ceramic type [29, 170].

Table 3 Various factors of importance in material selection for biomedical applications [169]

Factors	Description		
First-level material properties	Chemical/biological characteristics Chemical composition (bulk and surface)	Physical characteristics Density	Mechanical/structural characteristics Elastic modulus Poisson's ratio Yield strength Tensile strength Compressive strength
Second-level material properties	Adhesion	Surface topology (texture and roughness)	Hardness Shear modulus Shear strength Flexural modulus Flexural strength
Specific functional requirements (based on application)	Biofunctionality (non-thrombogenic, etc.) Bioinert (non-toxic, non-irritant, non-allergic, non-carcinogenic, etc.) Bioactive Biostability (resistant to corrosion, hydrolysis, oxidation, etc.) Biodegradation	Form (solid, porous, coating, film, fibre, mesh, powder) Geometry Coefficient of thermal expansion Electrical conductivity Colour, aesthetics Refractive index Opacity or translucency	Stiffness or rigidity Fracture toughness Fatigue strength Creep resistance Friction and wear resistance Adhesion strength Impact strength Proof stress Abrasion resistance
Processing and fabrication	Reproducibility, quality, sterilizability, packaging, secondary processability		
Characteristics of host: tissue, organ, species, age, sex, race, health condition, activity, systemic response			
Medical/surgical procedure, period of application/usage			
Cost			

4.1 Biomedical Applications of Natural Fibres

Several studies in the literature incorporating natural fibres such as aloe vera, hemp, jute, banana, kenaf, ramie, sisal, coir, bamboo, flax, and abaca have been reported for biomedical applications. This section will describe recent works that have been published using natural fibres for biomedical applications.

Rodrigues et al. (2019) used the acemannan (ACE), which is a phytocompounds from aloe vera, which is reported to have beneficial biomedical properties such as cytocompatibility, wound healing inducing capability, as it promotes the release of several growth factors, antibacterial and immunomodulatory activities. In this work, ACE-based films were prepared through the combination of ACE with chitosan

Table 4 Polymeric biomaterials and application [18, 165, 170–172]

Natural polymers	Application
Proteins: Collagen, gelatine, elastin, silk, albumin, fibrin, keratin	Tissue regeneration and engineering, scaffold materials, drug delivery vehicles, and wound healing
Polysaccharides: Chitosan, alginic acid, hyaluronic acid, cellulose, chondroitin sulphate	Wound dressing, regenerative medicine (sponges, hydrogels, fibres, and membranes), tissue engineering, regeneration of bone, adipose-derived stem cell treatment, cartilage regeneration, surgical tools, dialysis membranes, biosensors, and drug delivery. Postoperative adhesion prevention, ophthalmic and orthopaedic lubricant, and cell scaffold
Synthetic polymers	
Poly(α-Esters): Poly (glycolic acid), polylactic acid, poly(lactide-co-glycolide), bacterial polyesters, polydioxanone and polycaprolactone	Load bearing, scaffolds, biocomposite materials, prostheses, tissue engineering, orthopaedic, bone pins and plates, suture, a drug/vaccine carrier, and a long-term contraceptive with zero-order drug release
Polyurethanes	Use in long-term implants such as cardiac pacemakers, vascular grafts, and tissue replacements
Polyphosphazenes	Scaffolds for tissue engineering, microencapsulating agents, biodegradable materials, biocompatible coatings, and carriers for gene delivery
Polyanhydrides	Drug delivery, eye disorder, local anaesthetics, and anticoagulants and used for treating brain tumours
Poly(propylene fumarate)	Bone tissue, ocular drugs, and estrogenic tissue engineering
Poly(ethylene glycol)	Hip and knee implants, artificial tendons and ligaments, synthetic vascular grafts, dentures, and facial implants
Poly(ortho esters)	Drug delivery, ocular delivery, periodontal disease treatment, and applications in veterinary medicine
Poly(ester amide)s	Tissue engineering, controlled drug release systems, hydrogels, adhesives and smart materials

or alginate at various concentrations. Blended films had a good homogeneity and mechanical stability. Films containing alginate and ACE had large storage modulus which indicates higher resistance to deformation. Films containing chitosan had lower values when ACE was incorporated; a significant weakening of the strength of the formed networks with the increase of ACE content was seen. The films produced by the authors revealed a very promising alternative to further biomedical applications; especially considering the polymeric blends of alginate containing higher ratios of acemannan, showing an improved resistance and stable structure[173].

Atmakuri et al. (2019) investigated the mechanical properties of hemp and flax fibres within an epoxy resin hardener. From the contact angle measurements, pure flax shows the maximum contact angle of 65.98° and a mixture of 20% hemp and flax show a decrease in the contact angle of 10 degrees. The materials presented a maximum flexural strength at 84.80 MPa to hemp/flax composites with a weight fraction of 25/15 and 3.30 GPa of flexural modulus. Because of its low density, high strength, and availability, they can be used in biomedical applications[174].

Furlan et al. (2019) used sisal cellulose and magnetite nanoparticles in order to obtain a magnetic hybrid film. A sisal cellulose film was prepared by a solvent casting evaporation method. The SEM images of the hybrid films exhibited a fibrous structure. The films presented higher tensile strengths (14.3 MPa and 12.1 MPa, respectively) than the neat cellulose film (9.9 MPa). The elastic modulus of cellulose film (1860 MPa) is higher than of hybrid films 1500–1650 MPa and 780 for a higher dosage, indicating that the incorporation of nanoparticles in the cellulosic matrix decreased the films' stiffness[175].

Tejero et al. (2019) investigated suitable agronomical approaches for industrial hemp (*Cannabis sativa* L.) cultivation for biomedical applications. This work evaluated the agronomical response of two industrial hemp cultivars (Carma and Ermes) subjected to different management practices [176].

5 Conclusion

Natural fibre-reinforced polymer composites show a great deal of promise as biomaterials in medical applications. They can be used for high-tech applications, and in comparison, to certain fibre-reinforced composites, have particular advantages such as low density and improved thermal insulation. Composites reinforced with natural fibres are an area attractive to researchers and industries in creating effective and low-cost alternative materials that are environmentally friendly. These aspects are becoming more pertinent with the present ecological crisis that is occurring throughout the world.

Several manufacturing techniques are used in the manufacture of composites. Processing mediums are chosen according to the fibre used and according to the desired final product. These product characteristics will define the type of resin used and the most suitable technique for processing. The polymeric matrix is responsible for distributing the stress applied to the composite; however, the choice of polymer is limited mainly by the temperature required for processing, so, it is necessary to choose a polymeric matrix and a type of natural fibre that does not degrade in its processing phase. *Aloe vera*, hemp, jute, banana, kenaf, ramie, sisal, coir, bamboo, flax, and abaca are some of the possible materials for use in biomaterial applications.

The use of biomaterials is already well established in the most diverse applications. However, the development of innovative composite materials has potential in the area of tissue engineering and stem cell cultures and other novel biomedical applications. In addition, there are few reports in the literature that have focused on the application of natural fibres in the production of composites for biomedical applications. There is extensive work necessary in the future to fully realize the potential of natural fibre-reinforced composites for biomedical applications, in particular, studies on the processing, characteristics, and end-of-life implications. However, there is a renewed emphasis on natural alternatives and as such the future looks promising.

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Polymeric Biocomposites from Renewable and Sustainable Natural Resources

Daniela M. Fidalgo, Mario D. Contin, Adriana A. Kolender, and Norma D'Accorso

Abstract The use of polymeric composite materials from renewable biomass has acquired great importance in different and varied fields. Moreover, its application in the biomedical applications has found a fast development in recent years. In this context, this chapter is focused on the use of biocomposites in tissue engineering and analytical applications. The studied materials include polysaccharides such as chitosan, cellulose, and alginate, as well as polyhydroxyalcanoates as matrixes, and fillers like nanoparticles, carbon nanotubes or polymers, among other combinations.

Keywords Chitosan · Nanocellulose · Gelatin · Polyhydroxyalcanoates · Analytical applications · Tissue engineering

D. M. Fidalgo · A. A. Kolender (✉) · N. D'Accorso (✉)
Facultad de Ciencias Exactas y Naturales, Departamento de Química Orgánica, Universidad de Buenos Aires, Buenos Aires, Argentina
e-mail: adrianak@qo.fcen.uba.ar

N. D'Accorso
e-mail: norma@qo.fcen.uba.ar

D. M. Fidalgo
CONICET- Centro de Investigaciones en Bionanociencias (CIBION), Buenos Aires, Argentina

M. D. Contin
Facultad de Farmacia y Bioquímica, Departamento de Química Analítica, Universidad de Buenos Aires, Buenos Aires, Argentina

Consejo Nacional de Investigaciones Científicas y Tecnológicas, CONICET, Buenos Aires, Argentina

A. A. Kolender · N. D'Accorso
CONICET- Universidad de Buenos Aires. Centro de Investigaciones en Hidratos de Carbono (CIHIDECAR), Buenos Aires, Argentina

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Abreviations and Acronims

ALP	Alkaline phospatase
BTE	Bone tissue engineering
CCNWs	Carboxymethylcelullose nanowhiskers
CPAs	Chlorophenoxy acids
CNCs	Cellulose nanocrystals
CQ	*Cissus quadrangularis*
CS	Chitosan
DSPE	Dispersive solid phase extraction
EDS	Energy –dispersive X-ray spectroscopy
ECHNN	Electrospun cellulose/nano-HA nanocomposite nanofibers
GC-MS	Gas chromatography–mass spectrometry
Gel	Gelatin
GO	Grapheme oxide
HAp	Hydroxyapatite
hASCs	Human adipose derived stem cells
HECA	Hydroxyethyl cellulose acetate
IgG	Immunoglobulin
MCNPs	Magnetic cellulose nanoparticles
MNPs	Magnetic nanoparticles
MOFs	Magnetic organic frameworks
mcl-PHAs	Medium chain length PHAs
MNPs@X	Modified magnetic nanoparticles
MSPE	Magnetic solid phase extraction
MWCNTs	Multi-walled carbon nanotubes
NBGC	Bioactive glass ceramic nanoparticles
NC	Nanocellulose
NHAp	Nanohydroxyapatite
PAHs	Polycyclic aromatic hydrocarbons
PAEs	Phthalate esters
PCBs	Polychlorinated biphenyls
PCL	Poly(ε-caprolactone)
PDA	Polydopamine
PEG	Poly(ethylene glycol)
PHAs	Polyhydroxyalkanoates
P3HB	Poly(3-hydroxybutyrate)
P4HO	Poly(4-hydroxyoctanoate)
P3HHx	Poly(3-hydroxyhexanoate)
P(LcG)	Poly(lactic-*co*-glycolic acid)
PPD	poly(*m*-phenylenediamine)
PP	Polypropylene
PVA	Poly(vinyl alcohol)
SBF	Simulated body fluid

SWCNT	Single-walled carbon nanotubes
SBSDME	Stir bar-sorptive dispersive microextraction
SPE	Solid phase extraction
TE	Tissue engineering
XRD	X-ray diffraction

1 Introduction

A composite can be defined as the arrangement of two or more different compounds, one of them plays the role of filler and the other one is the matrix in which the filler is dispersed. This combination leads to a new material with improved performance over the individual constituent materials [1]. The nature of both components may differ depending on the proposed application, thus composites can be classified into different categories.

In the last years, it was observed a renaissance of renewable polymers and development of bio-based macromolecular materials. There are several reasons that promote a gradual shift from oil-based materials to bio-based materials, from economical to environmental awareness. The last one is due to global demand for sustainable and "green" products, as well as new environmental legislation and regulations that encourage the development of environmentally friendly products with low carbon footprint [2].

In the case of biocomposites, the matrix is a polymer where the monomers are entirely or partially derived from biomass, and thus, the matrix can be classified as natural polymers or biopolymers. Natural polymers include only materials synthesized as such in nature like cellulose or starch, in opposition to biopolymers. Thus, all natural polymers can be considered as biopolymers, but not all biopolymers are natural polymers [3]. It is worth mentioning, the term "biodegradable biocomposites" where the matrix can be obtained either from biomass or petroleum-based, but it is degraded by anaerobic or aerobic biological processes, leading to the formation of carbon dioxide, water, methane, biomass, and mineral salts.

The other component of a composite is the filler, which is added to a polymeric matrix in order to reduce cost, improve processing, or modify some property. In the case of biocomposites, the nature of the fillers may differ from silicates, carbonates, magnetite, active pharmaceutical compounds, or other polymers [4].

Regarding the "green" nature of biocomposites, it may be expected that some of them would be biocompatible and useful for biomedical proposes. For example, several commercially natural polymer-ceramic composites are currently available for bone reconstruction [5]. However, there are many other fields where biocomposites can be useful as it is further described in this chapter.

The aim of this chapter is to summarize the usefulness of biocomposites in two well-established fields like tissue engineering and stationary phases for analytical applications, as well as other miscellaneous and not widely used applications.

2 Application of Polymeric Biocomposites in Analytical Chemistry

Polymeric biocomposites have been extensively used as sorbent for contaminant removal in waste eluents or in downstream procedures to recover valuable products [6]. However, only recently sorbents based on polymeric biocomposites have been used for analytical proposes.

A classical analytical procedure can be divided into three mayor steps: sample preparation, measurement, and data analysis. Despite there is not always a clear limit, sample preparation concerns the procedures that take place after sampling and before the introduction in the measurement system. The aim of sample preparation may differ depending on the type of compounds to be analyzed, the nature of the sample, and the purpose of the analysis. However, usual goals of sample preparation are to isolate the analytes from a complex matrix, to remove interferences from the sample and to concentrate the analytes in order to reach proper limits for detection [7, 8].

In the wide field of sample preparation, polymeric biocomposites have been used as sorbents in solid phase extraction (SPE). In traditional and miniaturized SPE devices, the solid phase (sorbent) is held on different supports which lead to different types of SPE techniques. Abundant examples and description of SPE techniques can be found in more specific literature [7]. However, a brief summary of the most relevant SPE techniques is given herein.

Conventional solid phase extraction is largely known as SPE, where basically a sample solution is forced to pass through a column filled with a sorbent. Then, the retained analytes are subsequently eluted with a proper solvent. In order to overcome some disadvantages of conventional SPE, different miniaturized solid phase extraction techniques were developed. The starting point was the development of solid phase microextraction (SPME) in 1990, by using a solid inert fiber covered with an active phase layer where actual extraction takes place. On the other hand in dispersive solid phase extraction (DSPE), the sorbent is dispersed through a sample solution and recovered by centrifugation together with the adsorbed analytes. Similarly, dispersive micro-solid phase extraction (DMSPE) takes advantage of nanomaterials as sorbent material. Magnetic solid phase extraction (MSPE) is a variation of DSPE; but in this case, an external magnetic field is used to recover the sorbent with magnetic properties. In stir bar-sorptive extraction (SBSE), the sorbent covers a magnetic stirrer. While stirring, the bar adsorbs the analytes to be extracted. Stir bar-sorptive dispersive microextraction (SBSDME) combine SBSE and DSPE by using a magnetic stir bar without any sorption property and magnetic nanoparticles (MNPs) that act as sorbent; while the stir bar is moving, the MNPs are dispersed but when it stops the MNPs and the adsorbed analytes come back to the stir bar surface.

In general, biocomposites meant to serve as solid phases are designed with a native or grafted natural polymer and a filler. The main natural polymers in this field are alginate, chitosan, and cellulose, among others. Fillers may be metal organic frameworks (MOFs) [9], grapheme oxide (GO) [10], MNPs, or modified magnetic nanoparticles (MNPs@X) [11].

Barium alginate was employed by Zhang et al. to overcome the low dispersion capacity of hydrophobic octadecyl (C-18) functionalized MNPs in aqueous solution. Basically, Fe_3O_4@C-18 was dispersed in a sodium alginate solution under sonication and vigorous stirring, while a barium chloride solution was dropped. The morphological analysis revealed a core shell structure based on Ba^{2+}-alginate vesicles as a light area surrounding a dark core of Fe_3O_4@C-18. Normally, that kind of hydrogel displays porous surfaces, and the swelling properties allow the free movement of water and solutes through the inner cavities [11]. The developed MSPE was successfully employed for the preconcentration of polycyclic aromatic hydrocarbons (PAHs) and phthalate esters (PAEs) from environmental water samples.

Usually MNPs, especially Fe_3O_4, are added to natural polymers**.** In some cases, MNPs do not play a primordial role as sorptive material but allow a simple material removal procedure by using an external magnetic field due to its magnetism.

Bunkoed et al. incorporated MNPs (Fe_3O_4) into alginate beads which were subsequently decorated with a layer of polypyrrole as sorbent material (Fig. 1) The authors evaluated the extraction efficiency of estriol, bisphenol A, β-estradiol employing alginate, alginate/Fe_3O_4, and alginate/Fe_3O_4/polypirrole as sorbent materials. Alginate/Fe_3O_4/polypirrole was the optimal sorbent due to the hydrophobic and π-π interaction established between the analytes and polypyrrole [12].

Graphene oxide (GO) is another interesting sorbent material due to its large surface area and functional groups. It is composed of carbon atoms in a sp^2 single-atom layer of a hybrid connection [13]. The presence of functional groups such as epoxy, hydroxyl, and carboxyl groups in GO promotes its dispersion in water, although that could obstruct its separation from aqueous media. Thus, the incorporation of MNPs onto the GO sheets makes the separation easier with an external magnet. Tasmia et al. took advantage of magnetic graphene oxide and included it into an alginate-Fe matrix to extract endocrine disrupting compounds from water compounds [10]. In

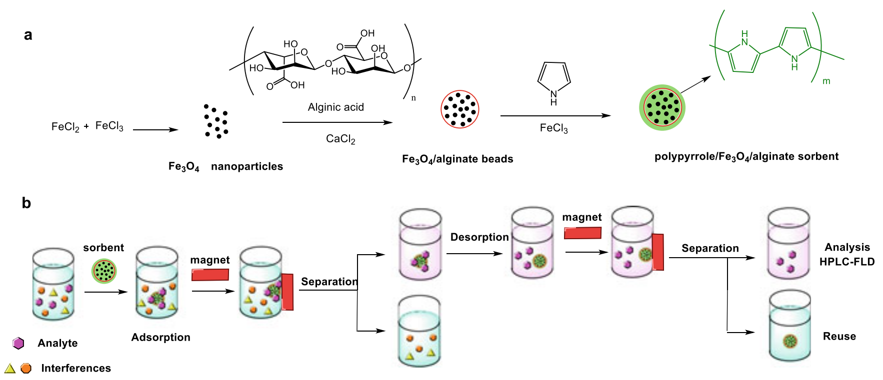

Fig. 1 **a** Synthesis of polypyrrole/Fe_3O_4/alginate sorbent. **b** Schematic illustration of SPE magnetic procedure. Adapted from Bunkoed et al. [12]

this case, the addition of alginate in the system reduced the magnetic-GO toxicity and enhanced the material biocompatibility.

Beyond magnetic materials, many other materials have been incorporated in natural polymers composites. Zirconia nanoparticles have been used as stationary phases due to its behavior as Lewis acid that can interact with Lewis-based functional groups, like $R\text{-}PO_3^-$, present in organophosphorus pesticides (OPPs). Zirconia nanoparticle/calcium alginate hydrogel fiber was employed by Zare et al. [14] for OPPs extraction from water and juice samples. An interesting point of this development was to perform the extraction without the need of centrifugation, filtration, or employing an external magnetic field as with usual magnetic adsorbents, because the obtained composite was a floating, fibrous solid phase that can be removed from the solution using forceps.

Metal organic frameworks (MOFs) also filled alginate hydrogels mutually benefiting for solid phase extraction. MOFs were found to be exceptionally stable and highly porous frameworks, first reported in 1999 [15], constructed from inorganic metal ions (node) and organic linkers. MOFs are characterized by a high porosity, crystallinity, large internal surface area, thermal stability, discrete order, and low density that render them interesting for sorptive material [16]. Tan et al. combined a hydrophobic MOF like MIL-101(Cr)-NH_2, (a supertetrahedral building unit constructed with terephthalate ligands and trimeric chromium octahedral clusters) with magnetite nanoparticles and alginate to yield an effective material for chlorophenoxy acids (CPAs) extraction from aqueous media [9]. The interaction experiment revealed that alginate polymer provided a polar hydrophilic surface that helped to draw the analytes closer toward MIL-101(Cr)-NH_2. On the other hand, magnetite provided magnetism for an easy separation. According to the performed extraction assays, neither magnetite nor alginate had a major impact over direct CPAs interaction, remarking the importance of MIL-101(Cr)-NH_2 as sorptive material.

It is worth to mention that alginates can directly interact with different analytes and work directly as a sorbent material. A novel alginate/zein hydrogel supported in a polypropylene (PP) fiber was developed by Castilhos et al. (Fig. 2) [17] to be used in a SPME procedure for hormones, pharmaceutics, and detergents of different polarities. Zein is a four fraction protein (α, β, γ, and δ) obtained from maize, with a particularly important fraction α due to its hydrophobic characteristic. The authors studied the effect of the zein addition over the hydrogel structure by performing loss of water, swelling, and methylene blue migration assays as well as the extraction performance. The results indicated that a more compact network with smaller pores and more plastic character is obtained when zein is incorporated to the alginate hydrogel, possibly due to the effect of the zein as an additional cross-linking agent, beside Ca^{2+} ions. In terms of analyte extraction, less polar analytes were more efficiently extracted when alginate/zein was employed compared to simple alginate hydrogel as sorption material. Conversely, more polar analytes were preferably extracted with alginate hydrogels. Zein modification produced a hydrophobic character in the sorption phase, decreasing the initial hydrogel water uptake and allowing for a better interaction between the analytes and the sorption phase through the functional groups of the alginate and zein. In conclusion, the solid phase based on alginate and zein was able

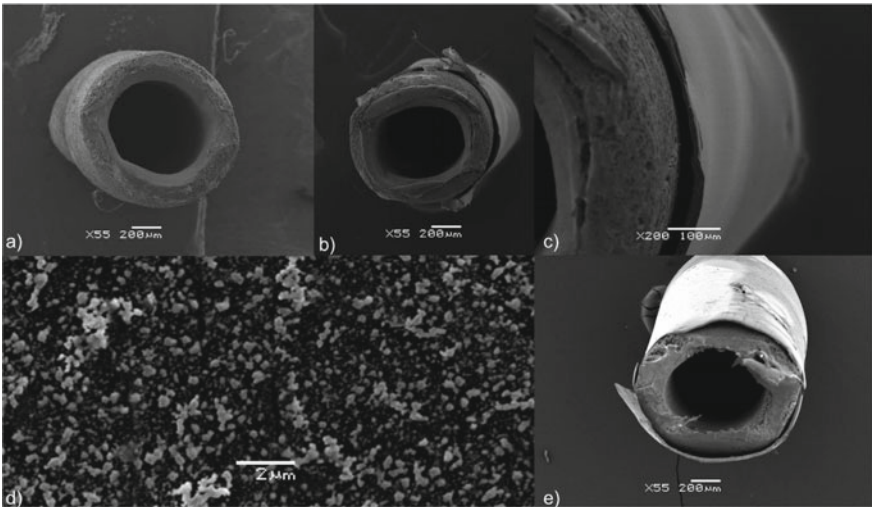

Fig. 2 Scanning electron microscopy of: **a** bare PP fiber, **b** PP fiber with alginate/zein hydrogel, **c** higher magnification of b, **d** alginate/zein film, and **e** PP fiber with alginate/zein hydrogel after extraction procedure. Reprinted from Castilhos et al. [17] Copyrigth 2017, with permission of Elsevier

to extract both polar and nonpolar compounds in water samples, due to its amphiphilic sorption behavior.

Nanocellulose (NC) is another biopolymer that has been studied as a sorbent material for analytical proposes. An excellent review from Ruiz-Palomero regarding NC as an analyte and analytical tool is highly recommended [18]. NC is characterized for a large superficial area, chemical reactivity, stiffness, and lightness that have been exploited for analytical goals.

A wide number of analytical applications is described in literature, but only cellulose in composites will be considered in this chapter.

Chemical modification of cellulose by oxolane, sulfonate, and amine groups allows the solid phase extraction of different metal ions, dyes, and silver nanoparticles [18]. β-Cyclodextrin moieties have also been incorporated into NC surface promoting the complexation with danofloxacin [19] with high recoveries.

Anirudhan et al. [20] developed a poly(methacrylic acid-co-vinyl sulfonic acid)-grafted-magnetite/nanocellulose composite for extraction of immunoglobulin (IgG). The authors extracted NC from saw dust and mixed it with iron salts producing magnetite nanoparticles in situ by co-precipitation. Finally, vinyl monomers methacrylic acid and vinyl sulfonic acid and ethyleneglycoldimethacrylate as cross-linker were added with the radical initiator ammonium peroxodisulfate to induce polymerization over the magnetic NC (Fig. 3). The composite material was physically characterized with special attention on swelling properties and pH dependence. The swelling percentage was higher than 450%. The material was applied to fully recover IgG from a protein mixture through five adsorption–desorption cycles.

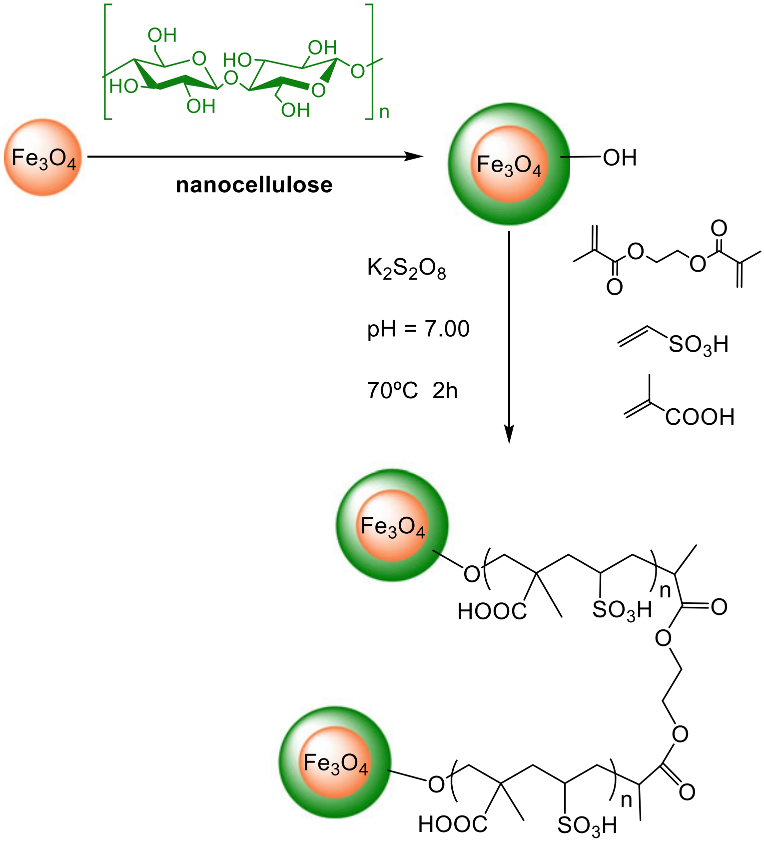

Fig. 3 Synthesis of poly(methacrylic acid-co-vinyl sulfonic acid)-grafted-magnetite/nanocellulose. Adapted from Anirudhan et al. [20]

Magnetic cellulose nanoparticles (MCNPs), without any further modification, were also employed as sorbent, although this is a vacant area. Abujaber et al., proposed a novel approach for MCNPs solid phase extraction based on SBSDME [21]. The methodology was employed for the determination of nine polychlorinated biphenyls (PCBs) in fruit juice samples by gas chromatography–mass spectrometry (GC–MS). MCNPs were able to be reused at least five times without significant loss in recovery.

Nurek et al. employed cellulose acetate as scaffold due to its porosity and high surface area, and coated cellulose acetate fibers (CFs) with calixarene-functionalized GO and polydopamine (PDA) [22]. Polydopamine had multifunctional groups, and GO was satisfactory proven as sorbent material, meanwhile calixarene provided π-π interactions due to the phenol units linked via methylene bridges. Polydopamine was prepared by auto-polimerization adding cellulose acetate fibers to a dopamine solution at pH 8.00. On the other hand, GO and 4-*t*-butylcalixarene were treated with hydrazine and ammonia solution, and the combined material was physically adsorbed and entrapped in the as-prepared porous PDA-CFs adsorbent. The final material was placed into a SPE polypropylene tube to yield a SPE device for the extraction of aflatoxins from corn samples.

Chitosan (CS) due to its biocompatibility, no toxicity, biodegradability, easy modification, and free amino groups is another biopolymer that has been widely studied as sorbent material. Chitosan as a sorbent material for analytical proposes has promising prospects as showed by Xu et al. [23] by developing a DMSPE for flavonol extraction using ultramicro chitosan. Definitely, composites based on CS can enhance or modify its own properties.

Guo et al. trapped MNPs into a chitosan matrix and modified its amino surface groups with rhein acid (Fe_3O_4@CS-rhenic acid), a dihydroxyanthraquinone with polar functional groups such as hydroxyl and carboxyl groups and a plane structure with a great conjugated system (Fig. 4) [24]. Thus, rhenic acid can form hydrogen bonds and $\pi-\pi$ or n-π transfer interaction with aromatic polar compounds like isoflavones. The authors also developed magnetite NPs with surface amine groups (Fe_3O_4@APTES) later modified with rhenic acid following the same procedure to yield Fe_3O_4@APTES-rhenic acid. Both materials were evaluated in the MSPE of isoflavones on soymilk. It is interesting to point out that the adsorption of polymeric microparticles coated by chitosan was higher than those ones prepared by APTES, because the surface of chitosan contains a large number of amino groups, so more

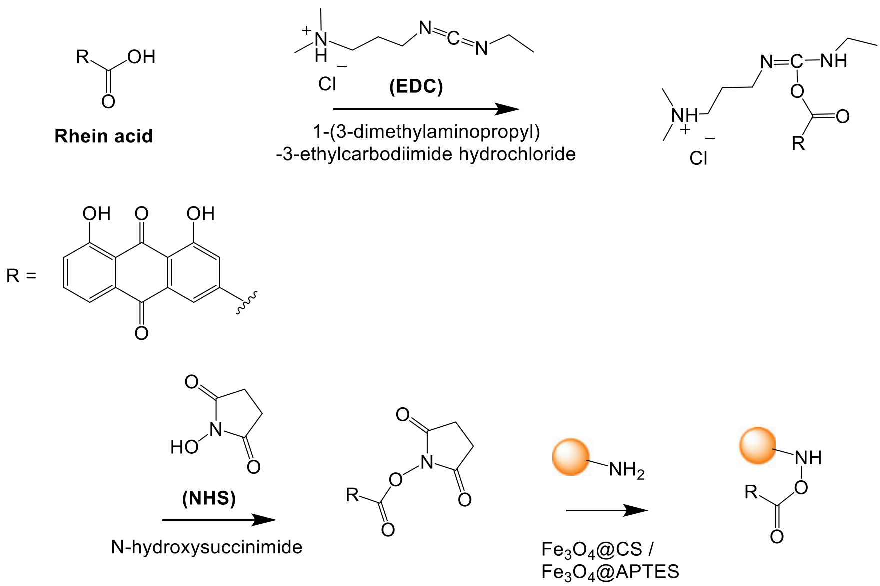

Fig. 4 Synthetic route of Fe_3O_4@CS-rhenic acid or Fe_3O_4@APTES-rhenic acid. Adapted from Guo et al. [24]

rhein acid can be connected via the amide formation to the magnetic particle. Thus, the importance of chitosan for this material was demonstrated.

Following a standard procedure to yield Fe_3O_4@CTS, Liao et al. covered this magnetic material with poly (*m*-phenylenediamine) (PPD) following a self-assembly approach. According to the authors, Fe_3O_4@CTS has hydrophobic and hydrophilic moieties that could facilitate the dissolution of *m*-phenylenediamine monomers before its polymerization by adding ammonium peroxydisulfate as initiator. The final material, Fe_3O_4@CTS-PPD, was employed for MSPE of polychlorinated biphenyls from water samples [25].

Chitosan-grafted polyaniline as sorbent material was developed in order to improve chitosan sorption properties. Razavi et al. applied a chitosan-grafted polyaniline for the preconcentration of phthalate esters in DSPE coupled to HPLC–UV detection. Basically, chitosan was dissolved in aqueous acetic acid with aniline as monomer and ammonium persulfate as initiator to yield the final composite after aniline polymerization [26].

3 Application of Polymeric Biocomposites in Tissue Engineering

It is important to remark the role of carbohydrates in many different and relevant applications. They are products obtained from renewable biomass have high functionality, low cost, and offer interesting chemical possibilities of functionalization. For example, cotton fibers modified with citric acid, sodium lignin sulfonate, and boric acid gave fabrics with flame retardancy, antioxidant activity, antibacterial activity, and UV protection properties [27]. Marine carbohydrates had found applications in pharmaceutical and cosmetic [28]; however, more recently, they have been used in the biomedical field.

On the other hand, Aparecida de Oliveira et al. [29] discussed the environmental impact derived from the exclusive use of thermoplastics, such as polylactic acid and their composites, in comparison with the use of products obtained from cotton fibers. The authors concluded that the products derived from natural polymers have less impact on the environment, despite its lower efficiency related to their mechanical properties. Therefore, it is important to emphasize the importance of the design of new materials that provide solutions and respect the environment simultaneously.

A scaffold for tissue engineering should fulfill some requirements including biocompatibility, biodegradability, mechanical durability, and porosity [30]. It is often necessary to make a combination of suitable biomaterials to achieve the necessary properties [31]. A 3D matrix-based scaffold system able to simulate the structure and function of a proposed tissue has become a main goal of biomaterials research [32].

4 Chitosan

Tissue engineering (TE) is an interdisciplinary field that aims to improve or replace biological tissues and involves the architecture of artificial cellular scaffolds which mimic extracellular matrix. Chitosan (CS) has been extensively used in the design of a variety of 3D scaffolds for its application in TE. Its physicochemical and biological properties make it an interesting material to apply as a base in the fabrication of scaffolds. CS is biocompatible, biodegradable, bioactive, and antibacterial; it is also endowed with a hydrophilic surface useful for wound healing properties. Besides, it has been reported that CS has an important role in enhanced cell adhesion and proliferation [25, 33–38] However, CS scaffolds present low mechanical properties and fast degradation, undesired properties for materials meant to be applied in TE. Looking forward to achieving an optimal material for a specific TE application, the properties of CS scaffolds can be tuned by the development of CS-based biocomposite by addition of other polymers, ceramics, and/or nanoparticles [31, 39–41].

In bone tissue engineering (BTE), the scaffolds should satisfy various properties such as adequate porosity, biocompatibility, water retention, protein adsorption, biomineralization, biodegradability, and mechanical properties [42]. Additionally, scaffolds must possess important properties like osteointegration and osteoconduction [43]. Several scaffolds have been developed for bone tissue engineering with compositions that mimic the extracellular matrix of native bone. Human bone composition includes 30% organic matter, mainly type I collagen (Col)1 and 70% of hydroxyapatite (HAp, $Ca_{10}(PO_4)_6(OH)_2$) depositing on the extracellular protein matrix. Collagen, a natural component of extracellular matrix protein, has been used as in scaffolds for a variety if tissues, including bone [44], skin [45], and cornea [46]. It has excellent biocompatibility, cell adhesion, and consequently enhanced tissue regeneration [47]. In addition, HAp has been applied as a reinforcement for scaffolds in bone tissue engineering, due to inherent biocompatibility and osteoconductivity properties combined with mechanical properties [48, 49].

The development of composites by combination of natural polymers with ceramics is one of the most used strategies to mimic the chemical composition of the tissue. Recently, a variety of CS/nanoceramic composite scaffolds were designed to obtain materials with enhanced biocompatibility, biodegradability, mechanical properties, and biological activity as a result of the combination of the individual advantages of each component [31]. The use of nanoceramics allows taking advantage of their known benefits compared to the bulk material. Moreover, they are structurally very similar to the inorganic component of the bone.

Nanoceramics could be incorporated into CS scaffolds by various methods. Depending on which one is used, different interactions will be observed between the polymer matrix and the ceramics. Considering what properties are sought for the material, the type and conditions of the method can be chosen. CS has been combined with nanohydroxyapatite (NHAp), bioactive glass ceramic nanoparticles (NBGC), $nSiO_2$, $nTiO_2$, and $nZrO_2$ bioceramics to obtain a variety of nanocomposites with potential application in the TE field [31]. Among them, NHAp is one of the most utilized biomaterials, especially in BTE. In addition to its relationship with the natural bond composition, NHAp is biocompatible and stimulates osteoconduction. Nevertheless, its crystalline nature confers poor mechanical properties, so it needs to be combined with polymers to be applied in TE. The structural characteristics of CS have been exploited in a variety of CS-NHAp or CS-HAp composites that were produced by different methods [50].

Yamaguchi et al. [51] prepared a CS/HAp composites by one of the most used methods, the co-precipitation. A CS/ H_3PO_4 solution was gradually dropped into a $Ca(OH)_2$ suspension. Then, the pH was slowly changed up to 9 until CS became insoluble and precipitated with small HAp crystals. The radio of CS/H_3PO_4 was adjusted to obtain composites with different weight ratio content of CS/HAp. The formation of the nanocomposites was a result of the interaction between the amino groups in CS and the Ca^{2+} ions of the HAp. It was observed that the CS content affected the ζ-potencial of the solutions, and it had a consequence in the size of the formed particles. The maximum size observed by TEM (17 μm) was using a CS content of 25 wt.%, where the ζ-potencial was closed to zero. All these results suggested that the size of the precipitate was affected by the surface charge, with an electrostatic repulsive force being minimal around the isoelectric point. Furthermore, a small amount of citric acid was added to a CS/HAp suspension after the formation of the composite. Citric acid is a multivalent negative ion that can participate in ionic interactions with positively charged ions and eliminate surface hydration water of particles. This could allow the formation of lower surface charged layers or shorter distances between charged particles. As it was expected, the addition of citric acid enlarged the precipitate size of the nanocomposites, probably due the formation of an ionic complex between the carboxyl groups of the acid and the NH_3^+ of the CS. Additionally, the incorporation of citric acid influenced the mechanical properties, since the compressive strength and Young's modulus were increased. This suggested that the bonding strength between particles was enhanced by the citric acid.

CS/HAp composites can also be obtained by the mineralization biomimetic method. When CS comes into contact with a simulated body fluid (SBF) solution, formation and precipitation of HAp occurs slowly. This process can occur directly on the CS surface or the surface can be modified someway. Fraga et al. [52] synthesized CS/HAp composites by mineralization of HAp over CS membranes with and without previous treatment with a sodium silicate (SS) solution, where SS acted as a nucleation agent. The free primary hydroxyl group of C-6 in CS can interact with silanol groups of the SS solution promoting the activation of the membrane surface. This way, the silicate ions were observed on the CS membrane until the nucleation of HAp began over the silicate ions. Finally, the HA crystals grow because of the saturated SBF solution to develop a HAp layer over the CS surface (Fig. 5). The characterization of the coated membranes by SEM, XRD, and FTIR demonstrated that the use of SS in the mineralization method and the exposure time to SBF affected the phase formation of HAp. The HAp layer homogeneously covered the entire surface of CS, and it had a semi-crystalline structure similar to the mineral phase of human bone.

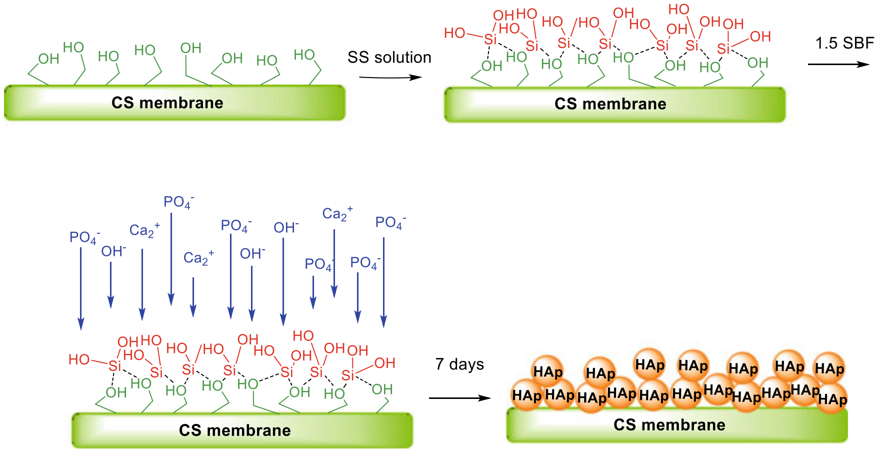

Fig. 5 Schematic illustration of the proposed coating mechanisms of CS membranes with HAp using the mineralization biomimetic method. Adapted from Fraga et al. [52]

CS can be chemically modified to enhance its physicochemical properties and cellular response. D. Depan et al. [53] created an organic/inorganic hybrid network structure nanocomposite based on grafted CS. First, CS was modified by reaction of the amino groups with propylene oxide to obtain hydroxypropylated CS which was subsequently linked with ethylene glycol functionalized NHAp (Fig. 6). Finally, the 3D-network of the nanocomposite (CS-g-NHAp) was prepared by lyophilization. Thus, NHAp was included as part of the network instead of being a filler. The SEM

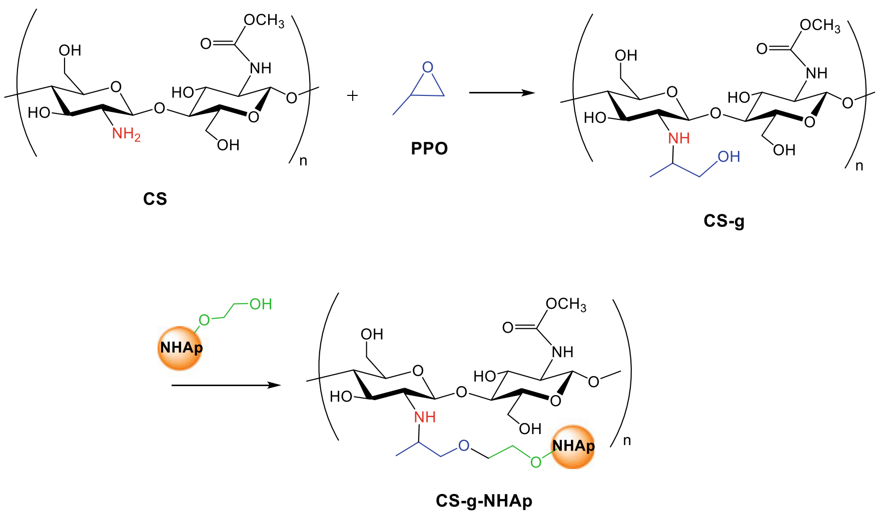

Fig. 6 Synthesis of CS-g-NHAp. Adapted from Depan et al. [53]

micrographs analysis showed that CS-g-NHAp was highly porous. The pore size was slightly larger than that of the unmodified CS scaffold (CS-NHAp), probably due to the grafting of propylene oxide onto CS. The modification of CS led to a more cross-linked matrix with more homogeneously distributed and interconnected pores than the pure CS. Consequently, CS-g-NHAp exhibited greater Young´s modulus, controlled degradation rate, and higher affinity toward pre-osteoblast compared to CS-NHAp.

Another strategy to improve the properties of CS scaffolds was the use of cross-linking reagents. Aiming to modulate the hydrophobic/hydrophilic balance and mechanicals properties of CS films, Li et al. [54] used genipin as a water-soluble bifunctional cross-linking reagent to develop a NHAp/chitosan cross-linking composite film (Fig. 7a). The NHAp was dispersed into a CS–genipin solution by ultrasound, and then, the suspension was dried at 37 °C for 2 days. The formation of a cross-linked matrix made the entrapment of NHAp more efficient. FTIR and TGA analysis demonstrated that part of the amino groups in CS had reacted with genipin and evidenced a hydrogen bond and electronic interaction between the positively charged amino groups of CS and PO_4^{3-}. The cross-linked composite films showed well cytocompatibility, water adsorption, and appropriate tensile strength.

In order to increase the cell attachment onto a scaffold, Atack et al. [55] used glutaraldehyde as a cross-linking agent (Fig. 7b) and modified NHAp nanoparticles to generate a CS-NHAp-NH_2 biocomposite for BTE. The NHAp nanoparticles were modified by reaction with 3-aminopropyltriethoxysilane (APTES) to incorporate NH_2 groups with cross-linking ability in their surface. CS was mixed with the NHAp-NH_2 nanoparticles, and then, glutaraldehyde was added to cross-link the CS matrix.

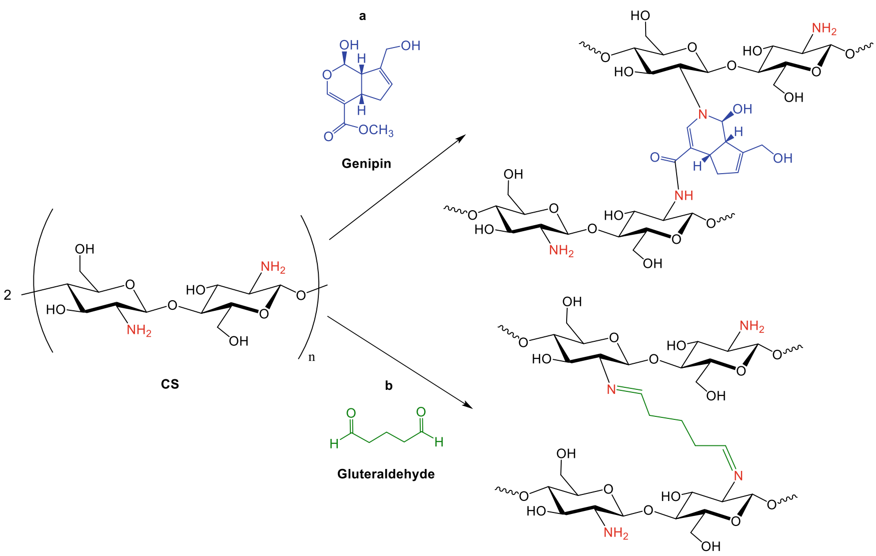

Fig. 7 Cross-linking reactions between CS and **a**. genipin or **b**. glutaraldehyde

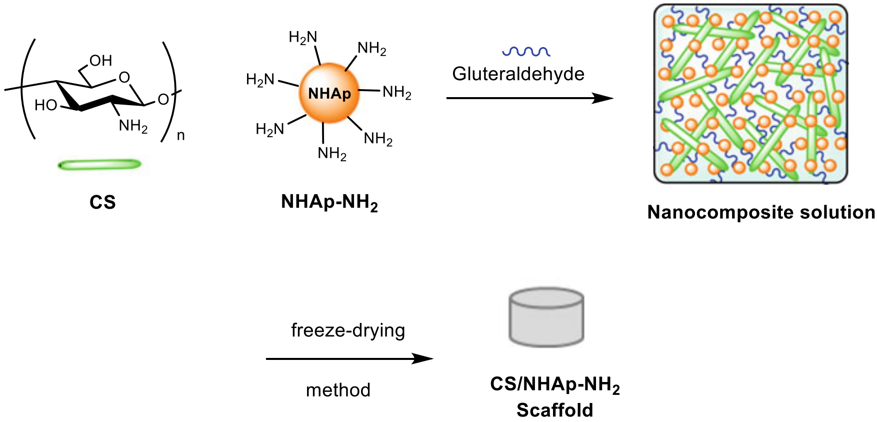

Fig. 8 Synthesis of the CS/NHAp-NH2 scaffold. Adapted from Atak et al. [55]

Finally, the scaffold was prepared via a freeze-drying method (Fig. 8). The FTIR analysis of the scaffold confirmed that NHAp-NH_2 nanoparticles were cross-linked in the polymer matrix, and the SEM images showed an increase of the distribution behavior of the nanoparticles in the surface of the biocomposite. Comparing with other scaffolds (CS and CS-NHAp), the CS-NHAp-NH_2 revealed a better degradation rate, cell attachment, survival, and proliferations of human bone mesenchymal stem cells and osteogenic differentiation.

Even though chitosan-NHAp composite biomaterials have demonstrated their importance as potential scaffolds, more complex composite systems can also be prepared. The incorporation of other biopolymers, synthetics polymers, or metals to the biocomposites can further enhance their properties. Because of their biocompatibility and potential biodegradability, other polysaccharides can be assembled with CS to develop biocomposites. Furthermore, the hydroxyl groups of their framework can interact with the amino groups of the chitosan to form cross-linked networks with enhanced mechanical properties. They can additionally incorporate more centers of interaction with the Ca^{2+} ions of HAp facilitating the crystallization process of NHAp. Taking this into account, tamarind seed polysaccharide (TSP, Fig. 9a) and starch (ST, Fig. 9b) were combined with CS to obtain NHAp/CS/TSP [56] and NHAp/CS/ST [57] nanocomposites by the co-precipitation method. A comparative assessment of their properties and NHAp/CS nanocomposite was done. In both cases, it was observed increased thermal stability, compressive strength, and Young´s modulus. This was probably due to the strong intermolecular interactions between the three components that were observed by FTIR analysis. Also, the SEM images demonstrated that the presence of the polysaccharides influenced the surface morphology of the scaffolds and that the NHAp nanoparticles were more homogeneously dispersed and could mimic natural bone apatite in terms of morphology and size. The biological properties were also improved suggesting that the nanocomposites are promising materials for applications in BTE.

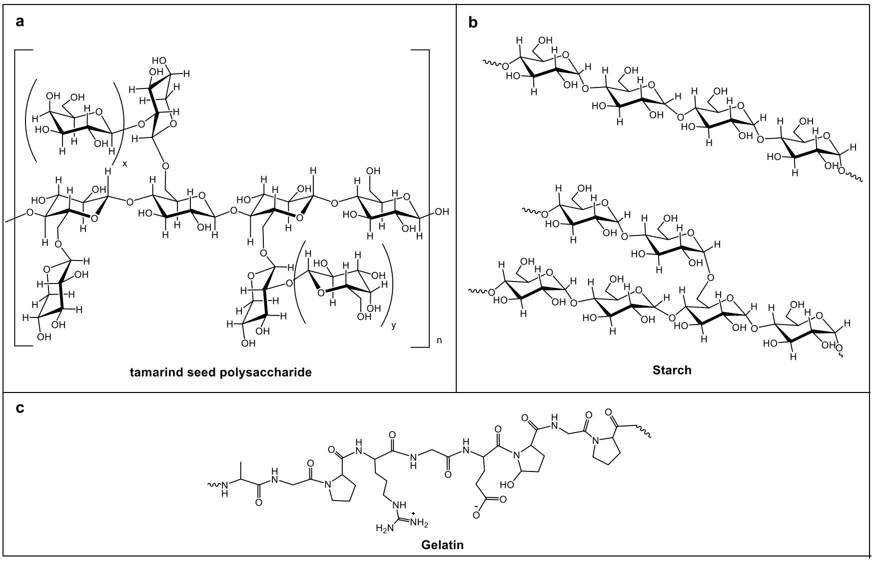

Fig. 9 Representative structures of **a**. tamarind seed polysaccharide; **b**. starch; and **c**.gelatin

Gelatin (Gel), a biocompatible and biodegradable biopolymer which can promote the adherence of cells because of the Arg-Gly-Asp sequences included in its structure (Fig. 9c), was also blended with CS to design more complex biocomposites [58, 59]. Neacsu et al. [60] mixed CS and Gel with a biomimetic HAp synthesized by microwave using an interwoven hierarchical structure of eggshell membrane (ESM). Also bone ash (BA), a natural source of both HAp and tricalcium phosphate (TCP), was added aiming to produce an even higher degree of biocompatibility [61, 62] and to obtain a scaffold more similar to natural bone, since its nonstoichiometric structure can contain other elements in addition to Ca and P (Na, Zn, Mg, K; Si, BA, F) [63, 64] After using glutaraldehyde as a cross-linking agent, the sample was subjected to the freeze-drying process to obtain a porous HAp/BA/CS/Gel composite material with uniform distribution of the inorganic powder. The in vitro studies proved that the scaffold was biocompatible and non-cytotoxic and allowed cellular proliferation, stem cells adhesion, and multiplication. The biological properties observed for the new composite suggested that it might accelerate the bone healing process.

Saravanan et al. [65] used silver nanoparticles (NAg) to prepare a CS/NHAp/NAg scaffold. The authors compared the swelling and biodegradation rate of the composites with and without the incorporation of NAg. The present of silver in the composite decreased the swelling percentage, which is important to ensure good mechanical strength and avoid loosening of the scaffold from the implanted site. Moreover, CS/NHAp/NAg biodegradation rate decreased compared to CS/NHAp scaffold suggesting that the material will be accessible for a longer time until bone tissue ingrowth. The NAg also increased the antibacterial activity of the new biocomposite. Although CS/NHAp scaffold exhibited antibacterial activity due to the presence of

CS which possesses this natural characteristic, the activity was considerably lower than that observed for CS/NHAp/nAg.

Another approach to keep the stiffness of chitosan would be to blend it with synthetic polymers with high mechanical properties. Poly(vinyl alcohol) (PVA) is useful for this purpose since it is a biodegradable, biocompatible, water-soluble polymer, and miscible with chitosan. Wu et al. [66] prepared films from PVA/CS blends and suggested that they were physically cross-linked by the formation of intermolecular hydrogen bonds. The use of PVA/CS blends could also be used to optimize the fabrication of nanofibers scaffolds by electrospinning, since the sufficient chain entanglement necessary for electrospinning was not achieved in the medium in which CS is soluble (aqueous acetic acid). This was probably due to the repulsive interaction between the chains generated by the amino groups of CS. The addition of PVA helped to reduce the repulsive interaction between the CS chains by forming hydrogen bonds with CS and thus facilitated the electrospinning process of the CS-PVA blend solution. Shen et al. [67] used CS/PVA blends to test the influence of different parameters (content of PVA, applied voltage, and flow rate) on the morphology of the nanofibers and determined the optimal conditions to produce a uniform and ultrafine nanofibrous CS bicomponent. Figure 10 shows that as the content of the PVA solution increased, the finer and more consistent nanofibers could

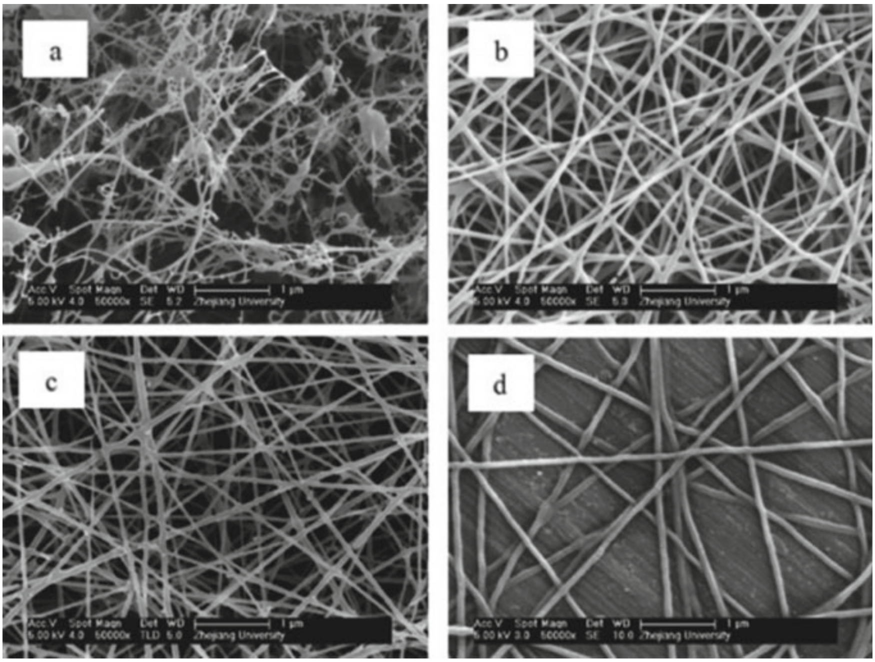

Fig. 10 SEM images of CS/PVA nanofibers electrospun under different volume radios **a** 90/10; **b** 80/20; **c** 70/30; **d** 50/50. Reprinted from Journal of Applied Polymer Science © 2019, with permission of John Wiley & Sons, Ltd. [67]

be electrospun. The CS/PVA bicomponent mats were filled with HAp nanoparticles to development new materials that have potential application in BTE.

As already mentioned, CS or its derivatives are fragile. Also, it has been proved that unplasticized CS cast films were very brittle when stored at low relative humidity [68]. Thus, the need of using a plasticizer, such as glycerol, became evident [69]. A plasticizer decreases the intermolecular interactions, increases the free volume, and enhances the molecular motions, but it decreases the barrier properties of the polymer films [70, 71]. Besides, the plasticizer may migrate to the polymer surface and give undesired effects on the performance of the polymer films [72, 73]. This issue may be overcome by the use a plasticizer with higher molecular weight with a hampered migration to the bulk of the matrix. Poly(ethylene glycol) (PEG) is a water soluble, biodegradable, and biocompatible polymer with a flexible backbone. It is available in a wide range of molecular weights and may be an efficient plasticizer for chitosan. A blend of CS with increased amounts of PEG can give higher swelling ratios and *in vitro* biodegradation [74]. In opposition, the addition of a plasticizer to CS decreases its mechanical strength and thermal stability [69]. Jiang et al. [75] dissolved CS in aqueous $AlCl_3.6H_2O$ and prepared PVA/chitosan blend films with glycerol as plasticizer. The combined use of glycerol and $AlCl_3{\bullet}6H_2O$ had a synergistic effect on the film properties. Chen et al. concluded that chitosan crystallinity can be decreased by the combination with PEG in blends [74]. Recently, PVA/CS blends, plasticized with PEG and glycerol, were prepared, and their physical and mechanical properties were investigated [76]. The plasticization efficiency and the compatibility of glycerol, PEG, and combinations with PVA/CS blends were evaluated using different characterization methods. A set of plasticizers including glycerol, PEG400, PEG1000 and the combination of glycerol and PEG1000 were used. The results from different analytical methods revealed that the optimum PEG: PVA ratio should be 1:9. Higher concentration of PEG would give phase separation and affect the performance of the film. For PVA/CS blends, PEG was also a compatibilizer, due to its ability to act as a hydrogen bond acceptor with the blend components. However, the high interaction affinity of glycerol with PVA and CS decreased the linkage through hydrogen bonds between PVA and the polysaccharide. On the other hand, glycerol enhanced the crystallinity of PVA/CS blends, whereas PEG reduced it (Fig. 11). Therefore, a combination of both plasticizers, glycerol, and PEG was employed and led to the highest level of compatibility. The antibacterial properties of the blends were investigated in order to analyze their potential for biomedical or food industry applications. The antibacterial activity of the blend plasticized with PEG/glycerol was notably decreased compared to the use of any single plasticizer. This was attributed a lower number of free amino groups in chitosan, as a consequence of the interactions between PVA and chitosan. In addition, the migration of PEG from the blend was much lower than that of glycerol, and the mixture of glycerol with PEG reduced the plasticizer migration.

Some biomolecules, growth factors, or stimulating agents have been added to the scaffolds to promote bone cell growth and to enhance the biological properties of the biocomposites. Considering that *Cissus quadrangularis* (CQ) extract was reported as beneficial for bone fracture healing [77–81], Tamburaci et al. [82] proposed a blend of

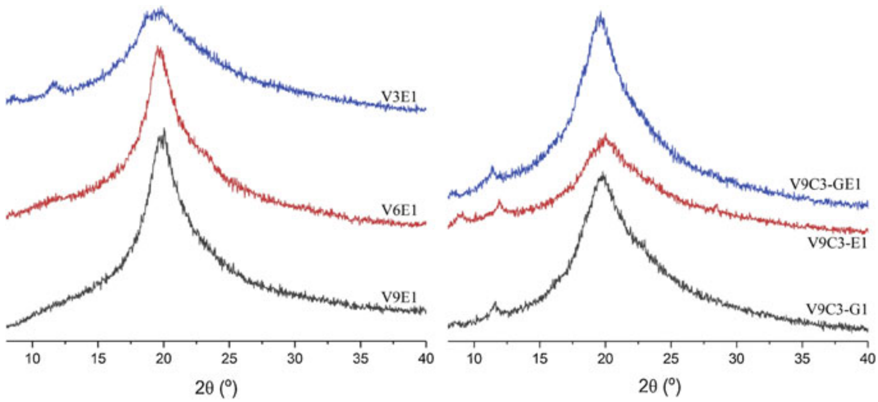

Fig. 11 XRD diffractions for PVA/PEG, PVA/chitosan/glycerol, PVA/chitosan/PEG, and PVA/chitosan/glycerol/PEG. To compare all the spectra, the y-axis was kept constant. The samples were coded as VxE1 in which V and E represent PVA and PEG; V9C3-G1 and V9C3-E1 where C and G account for chitosan and glycerol, respectively, sample V9C3-GE1was prepared using a combination of glycerol and PEG. Reprinted from Sofla et al. [76] © 2020, with permission from Elsevier

CQ extract with CS/Na-carboxymethyl cellulose, in scaffolds to enhance the alkaline phospatase (ALP) activity of Saos-2 osteosarcoma. Soumya et al. [83] included CQ extract in alginate/O-carboxymethyl CS scaffolds to promote osteoblast proliferation and ALP activity. The resulting biocomposite scaffolds had different porosity and swelling properties. Recently, Thongtham et al. [84] proposed a controlled release of the bioactive compounds from CQ extract, included in nanoparticles, in scaffolds made of Col, CS, and HAp. An ethanolic extract of CQ was encapsulated in polymer nanoparticles, instead of using a direct blend with the biopolymers. As a hydrophilic component, CQ was encapsulated by the double emulsion technique [85]. Poly(lactic-*co*-glycolic acid) (PLcG) and PEG are commonly used to form the first emulsion, while PVA and pluronic are utilized for the second emulsion formation to give nanoparticles (CQ-PCL NPs) with homogeneous size distribution [86, 87]. The CQ-PCL NPs were included in the CS/Col/HAp scaffolds. The scaffolds with and without CQ-PCL NPs were studied in terms of morphology, chemical composition, compressive modulus, water swelling, weight loss, and biocompatibility. The results showed that 20 mg/mL PCL and 0.5% (w/v) PVA were suitable for CQ-PCL NPs preparation, which were then included into the porous CS/Col/HAp scaffolds (Fig. 12). The surface of the nanoparticles was modified as a consequence of the blend, giving a slow release rate of CQ. The loaded CS/Col/HAp scaffolds showed similar properties to those of pristine CS/Col/HAp scaffolds. In addition, the CQ-PCL NPs-loaded CS/Col/HAp scaffolds were nontoxic to MC3T3-E1 murine osteoblasts (Fig. 13).

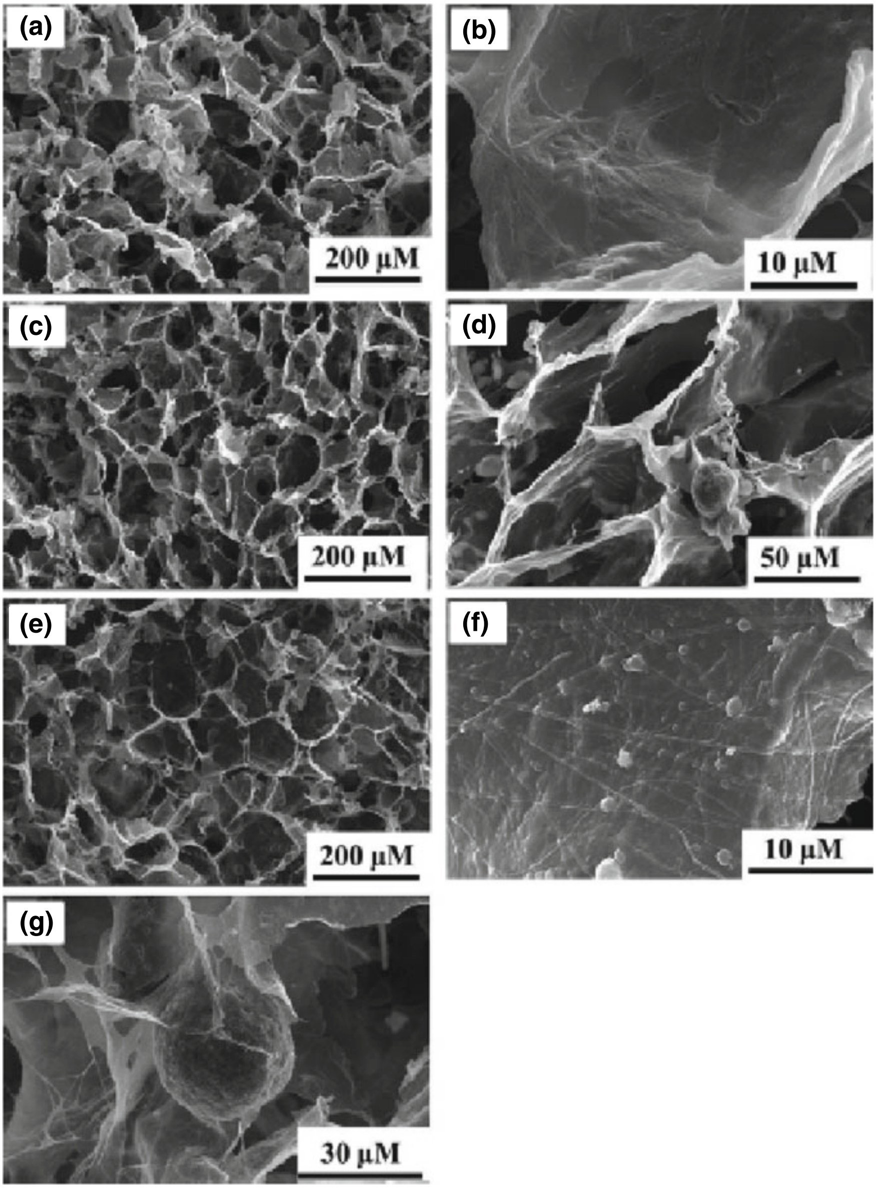

Fig. 12 SEM images of cross-sections of (**a**, **b**) CS/Col scaffolds, (**c**, **d**) CS/Col/HA scaffolds, (**e**, **f**, **g**) CQ-PLC-NPs-loaded CS/Col/HA scaffolds. Reprinted from Polymers for Advanced Technologies © 2019, with permission of John Wiley & Sons, Ltd. [84]

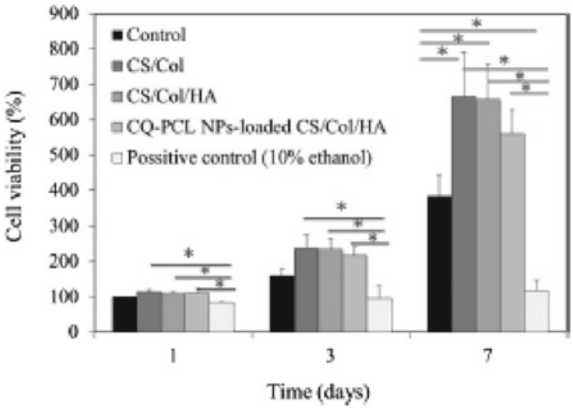

Fig. 13 Cell viability of MC3T3-E1 cells cultured with different scaffolds at 1, 3 and 7 days. $*p < 0.05$. Reprinted from Polymers for Advanced Technologies © 2019, with permission of John Wiley & Sons, Ltd. [84]

5 Cellulose

Cellulose is one of the most abundant natural renewable materials of the world. It is a diverse material with tunable chemical, physical, and mechanical properties [88]. Additionally, it has excellent biodegradability and biocompatibility. All these qualities make it an ideal candidate to be applied in the development of cellulose-base biomaterials for TE. In order to achieve materials with excellent performance in terms of their mechanical and biological properties [89, 90], cellulose could be modified, used in its nanoscale form, and combined with other polymers or ceramics to design composites. As in the case of CS, many research groups have reported recently its use in combination with HAp to enhance their mechanical and biological properties and produce scaffolds that could mimic bone tissue.

The chemical modification of cellulose is a widely used strategy to improve the solubility, the ability to fuse with the other materials that are part of the composite, and to modify the final biological and mechanical properties of the new material. Azzaoui et al. [91] synthetized hydroxyethyl cellulose acetate (HECA) and obtained an inorganic-organic film with potential application in BTE by evaporating the solvent of a solution of HECA and HAp in DMF. The structure and properties of the films were characterized with SEM, TGA, DTA, FTIR, and NMR. The cellulose modification allowed the improvement of the material workability and the formation of a specific interaction between the carbonyl groups in HECA and the HAp which probably contributes to the formation of uniform films. The HAp particles were well dispersed and immobilized throughout the formed films.

Nanocellulose (NC) properties (high surface area and crystallinity, low thermal expansion and cost, high specific strength and modulus, good biodegradability, and environmentally friendly) made it attractive to use in the design of composites. In the TE field, the electrospinning of biopolymers results an efficient procedure to obtain nanofibers. These kinds of polymers could easily form 2D or 3D nano-scaled systems with the ability of mimic the nanofibrillar components of the extracellular matrix (ECM) that surrounds cells within tissues. Thus, the use of biopolymers-based composites with a nanoscale matrix has been widely investigated for the application in TE. Ao et al. [92] fabricated electrospun cellulose/NHAp nanocomposite nanofibers (ECHNN) based on native cotton cellulose and different NHAp concentration. The authors studied the physical properties of ECHNN and their potential application in BTE. The morphological analysis by SEM demonstrated that the nanofibers in scaffolds had a diameter distribution comparable to the natural ECM fibers (50–500 nm) (Fig. 14). The mechanicals properties were enhanced with the addition on NHAp, especially when the NHAp loading was 5%. In this case, the SEM images proved that the nanoparticles were well dispersed. The 5% ECHNN showed distinctively higher values for tensile strength and Young's modulus (70.6 MPa and 3.12 GPa, respectively) than those of neat cellulose nanofiber mat (52.9 MPa, 1.64 GPa). The observed mechanical properties are promising for the ECHNN to be applied in BTE due to their similarity with those of natural bone.

NC can be blended with other polymers by the electrospun method to develop bio-nanocomposites. Turng et al. [93] incorporated cellulose nanocrystals (CNCs) functionalized with PEG (CNC-g-PEGs) into a PLA matrix by electrospinning. The PLA/CNC-g-PEG composite nanofibers were characterized in terms of morphology, thermal behavior, contact angle, wettability, and biocompatibility in human mesenchymal stem cells (hMSC). The use of CNC grafted with PEG enhanced the dispersion of cellulose nanocrystals in the PLA matrix. Consequently, it was demonstrated an enhanced mechanical strength and improved cell viability and proliferation. Therefore, they could be efficient apply in TE. Si et al. [94] designed an electrospum poly(ε-caprolactone) PCL/NC scaffold with HAp mineralized on the surface. In this study, the authors decided to incorporate NC to improve the hydrophilicity and mechanical properties of the scaffold and to take advantage of the hydroxyl groups in the NC to generate nucleation sites to form mineral crystals over the polymer surface. It is known that during mineralization, the hydroxyl

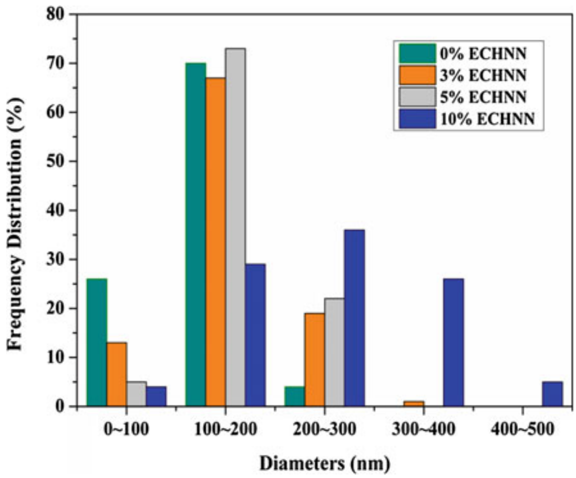

Fig. 14 Fiber diameter distributions of ECHNN. Reprinted from Ao et al. [92] © 2017, with permission from Elsevier

groups can form ionic bonds with Ca^{2+} and hydrogen bonds with PO_4^{3-} generating local supersaturation and then nucleation of the crystals [95]. The scaffold was prepared starting from a fibrous PCL/NC matrix produced by an electrospun methodology. Then, this matrix was mineralized by immersion in SBF solution, which contained different salts of sodium, potassium, magnesium, and calcium to simulate body corporal medium (Fig. 15). An interesting conclusion was the influence of the NC content in the nanofibers to induce the HAp nucleus deposition and its efficient subsequent growth. SEM, energy-dispersive X-ray spectroscopy (EDS), and X-ray diffraction (XRD) analysis revealed that HAp crystal layers were formed after few days and that this was only possible on the composite fiber surfaces that included NC. The higher content of NC not only favored formation of crystals on the surface of the electrospun fibers, but also increased the hydrophilicity and the mechanical properties of the fiber mats.

Another way to improve nucleation and locally control the growth of HAp crystals is to use chemically functionalized NC [96]. Fragal et al. [97, 98] introduced sulfate, phosphate, carboxylate, and amino groups on the surface of cellulose nanowhiskers (CNWs) obtained through acid hydrolysis of the cellulose extracted from sugarcane bagasse. The functionalization of the primary hydroxyl groups of the CNWs is important because it might enhance the reactivity of the nucleation sites to promote homogeneous growth of HAp crystals [99]. The crystallization of the HAp layer at

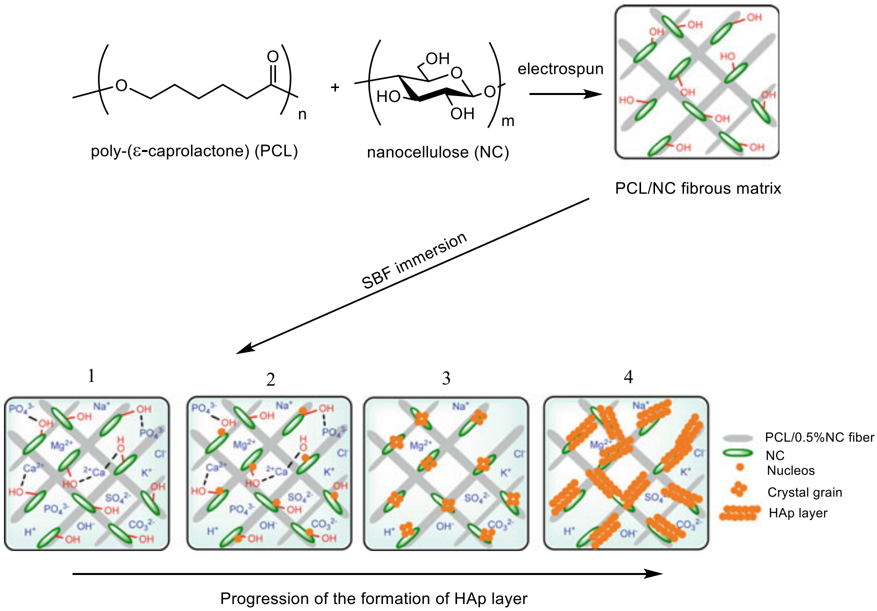

Fig. 15 Methodology used for the preparation of PCL/NC scaffold and schematic illustration showing the influence of NC content in the nanofibers to induce the HAp nucleus deposition and the progression of the HAp layer mineralization over its surface. Adapted from Si et al. [94]

the CNWs bearing carboxylate and amino groups was achieved by the interaction of these groups with Ca^{2+} using the biomimetic method under SBF for 14 and 28 days (Fig. 16). The stable nuclei, necessary to achieve the growth of the HAp layer over the CNWs surface, were formed through ionic and hydrogen interactions between the carboxylate, amino, and hydroxyl groups of the CNWs and Ca^{2+} and PO_4^{3-} ions. Moreover, it was revealed that the functionalized cellulose nanowhiskers could promote cell viability for the pre-osteoblasts (MC3T3-E1) comparable to HAp itself.

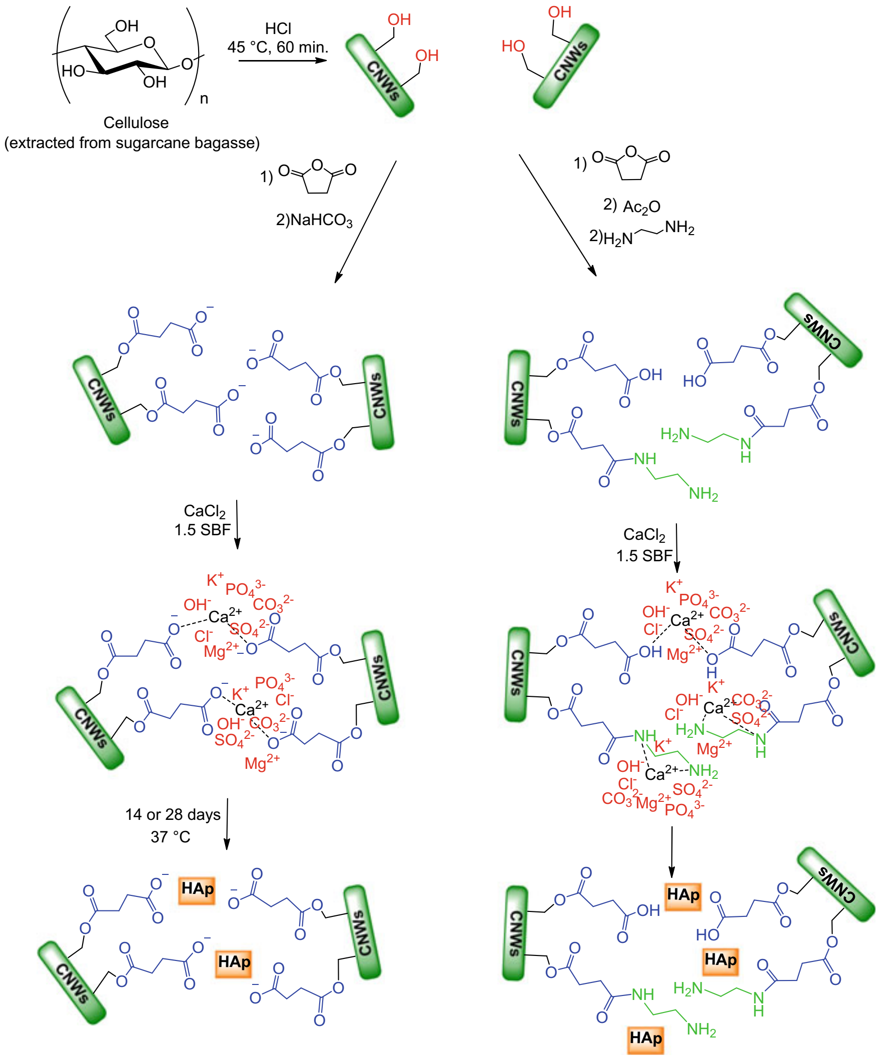

Fig. 16 Schematic illustration for the synthesis of modify CNWs and HAp crystal growth in the biomimetic method for 14 or 28 days. Adapted from Fragal et al. [98]

NC can also be used in the design of functional film bone scaffolds that are very interesting due to their tuneable surface structure, chemical and biological properties, and diverse functions. As already mentioned, there is a great interest in incorporating HAp to biocomposites due to the properties that it provides to the material [100]. However, it has been showed that combining HAp and thin films lead to development of materials with mechanical instability and susceptible to the surrounding environment [101]. Looking to solve this problem, Ragauskas group [102] described a methodology of layer-by-layer coating to produce a bone film scaffold. First, they prepared a CAN/HAp matrix by an in situ coating of HAp on the nanocrystal of cellulose (CNC). Then the CNC/HAp matrix was used as a template for building up the layer-by-layer assemblies with CS and hyaluronic acid (HA) (Fig. 17). The authors described that the HAp coating produced an increase in Young's modulus and matrix hardness. The incorporation of CS and HA layers increased the hydrophilicity of the material, identified by the decrease in the contact angle. Although the authors reported a decrease in the composite mechanical resistance with the layer-by-layer assembly, it was higher than CNCs, and therefore, it could be used as a bone scaffold.

6 Polyhydroxyalcanoates

In the field of biopolymers, the polyhydroxyalkanoates (PHAs) play an important role. The discovery of these biopolymers is due to the Maurice Lemoigne [103–107], who reported that their chemical structures depended of the bacterial species as well as the substrates present in the culture media. PHAs are biodegradable and biocompatible polyesters, synthesized by several microorganisms as intracellular carbon and energy storage compounds [108–110]. The repetitive unit of these polyesters consists of a 3-hydroxyacid with an alkyl substituent on C-3. A relevant representative of these biopolymers is the poly(3-hydroxybutyrate) (P3HB), which is a biodegradable thermoplastic derived from [*R*]-3-hydroxybutyric acid (Fig. 18). This chiral monomer may generate an optically active polymer. PHAs can be divided into two main groups depending on the number of carbon atoms in the monomeric units: short chain length PHAs (scl-PHAs), that contain between 3 and 5 carbon atoms (e.g., P3HB), poly(4-hydroxybutyrate) [P4HB], etc.) and medium chain length PHAs (mcl-PHAs) containing 6–14 carbon atoms (e.g., poly(3-hydroxyhexanoate) [P3HHx], poly(3-hydroxyoctanoate) [P3HO], etc.)

Lenz and Marchessault [111] reported an interesting work describing the biosynthesis and biotechnology of these polyesters, while Sudesh et al. [112] described their biochemistry and physicochemistry. Considering the biodegradability of these biopolymers, they have found different and varied applications. Since they are biocompatible as well, they are widely applied in the medicinal field in drug delivery, tissue engineering, scaffolds supporting regrowth of damaged tissues, and biodegradable implants biocontrol [113, 114]

Despite the interesting properties of PHAs, they are often combined with other polymers for several applications. The blending is prone to the generation of surface

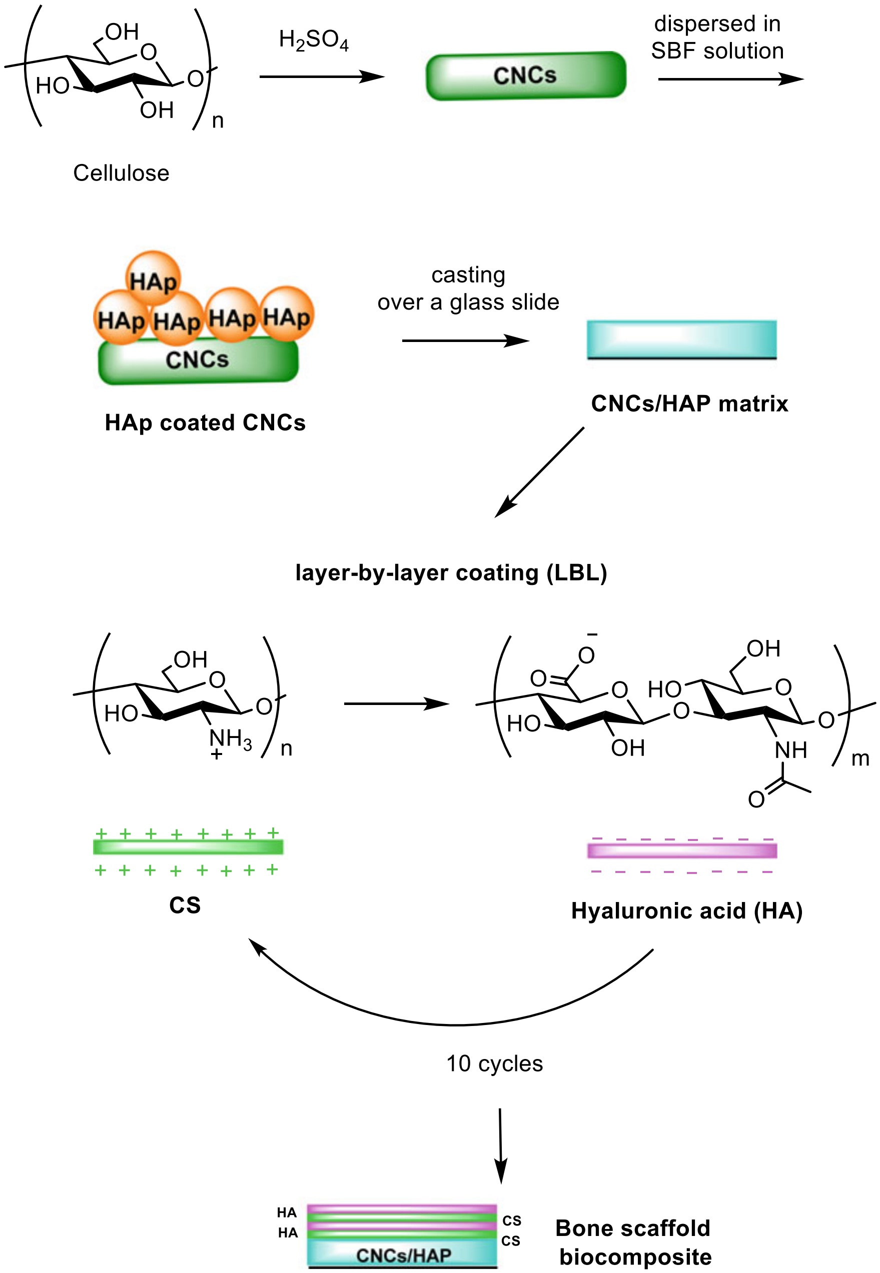

Fig. 17 Nanocomposite synthesis by layer-by-layer coating of CS/HA multilayers on a hard CNAs/HAp matrix

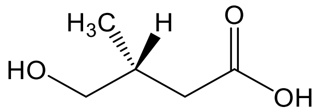

Fig. 18 Chemical structure of [*R*]-3-hydroxybutyric acid

separation, with the consequent fragility of the material. In this sense, Dufresne and Vincendon [115] studied polymeric blends with different thermomechanical properties, such as P3HB with P3HO using casting methodology and chloroform as solvent, and have determined the physical behavior and morphology of the biphasic mixtures.

A challenge for materials science is the development of biocompatible materials able to mimic neural tissue characteristics, maintain functionality in chronic devices, with minimal inflammation and neuronal cell loss. Soft electrically conducting polymers or elastomers have been proposed as alternatives to metallic implants [116–119] considering that biomaterial-tissue interface is a dynamic region where chronic inflammation may take place from incompatibilities between elastic modulus and relative micromotion of the implanted materials [120]. Usually, a threshold material stiffness must be kept to ensure the implant to penetrate the neural tissue to reach the final insertion point. However, the tissue would give a glial scar formation and electrode encapsulation, contributing to a chronic peri-electrode inflammatory response in vivo [120, 121] Elastomeric polymers may be helpful to decrease the mechanical gap between the brain tissue and soft polymers at the neuroelectrode interface [122] and to improve the integration of the electrode with the tissue [123]. Relative micromotion of the device may be solved by the use of flexible materials, improving the electrode change transfer capability [124].

Medium chain length PHAs (mcl-PHAs), containing 6–14 carbon atoms, are relevant due to their low crystallinity (T_m 45–60 °C), low T_g (in the range −25 to −50 °C), Young's modulus (3–70 MPa), and up to 500% elongation at break. Besides, they may be easily processed via traditional thermoplastic techniques, and their properties can be improved by their use in composite systems [125, 126].

On the other hand, nanocomposites incorporating multi-walled carbon nanotubes (MWCNTs) have been successfully employed in compliant electrode technologies to yield materials with interesting neuronal adhesion, survival, and support neurite elongation [27]. Carbon nanotube interfaces also stimulate spontaneous synaptic and can be applied to distribute electrical stimulation to neuronal pathways in vitro [128, 129].

MWCNTs were included in a mcl-PHA copolymer based on 3-hydroxyoctanoate and 3-hydroxyhexanoate to give a conductive elastomeric composite that could be interesting as a neuroelectrode material (Fig. 19) [123].

The thermoplastic elastomer behavior of the mcl-PHA was modified by the incorporation of MWCNTs, with the aim of applying the new material as a neurointerface-specific biomaterial. The stiffness of mcl-PHA gradually increased with the amount of MWCNTs from approximately 8 MPa (pristine mcl-PHA) to approximately 50 MPa (1 wt.% MWCNTs). The inclusion of the stiff nanofillers would promote the

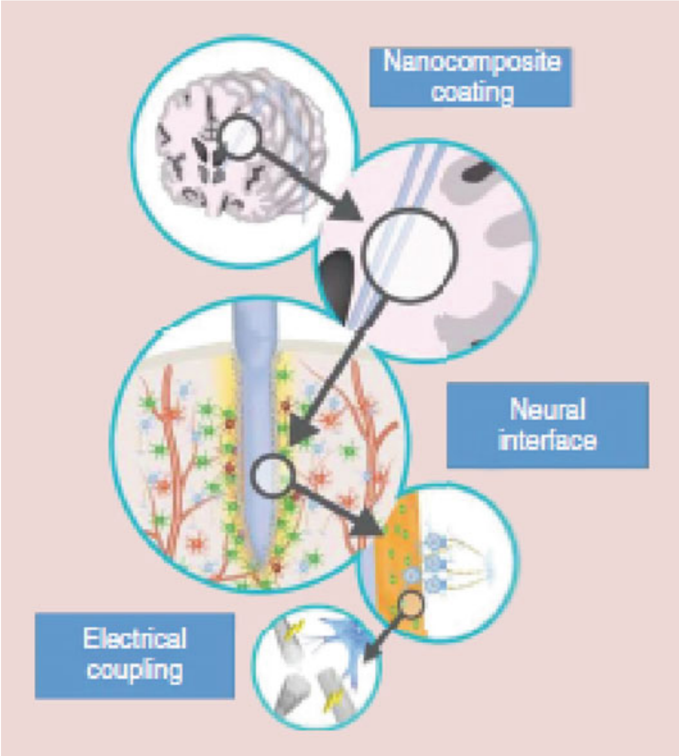

Fig. 19 Schematic overview of the challenges associated with neural interfaciong technologies republished with permission of Future Medicine Ltd., from Vallejo-Giraldo et al. [123]

entanglement of the polymeric chains, thus the material had an enhanced stiffness. Incorporation of MWCNTs also promoted the conductivity of the composite. Incorporation of 0.5 wt.% of MWCNTs changed the behavior of the samples from resistive to capacitors. Both mechanical and electrical properties would have impact on the biological response. The mcl-PHA/0.5 wt.% MWCNTs composite was particularly interesting since it promoted neuron maintenance without any detrimental modification to neurite length (with respect to platinum control). Furthermore, this material produced a characteristic neural physiological type response in electrically stimulated VM neuronal populations (Fig. 20).

Hore and Laradji [130] performed theoretical studies using dynamics simulations, and they have proposed the introduction of nanorods, as emulsifying agents. Russell et al. [131] reported on the blending of poly(3-hydroxybutyrate) (PHB) and poly(3-hydroxyoctanoate) (PHO) with different thermomechanical properties and the effect of single wall carbon nanotubes (SWCNT) on their miscibility, electrical conductivity, and thermomechanical performance. Miscibility of scl-/mcl-PHA solvent

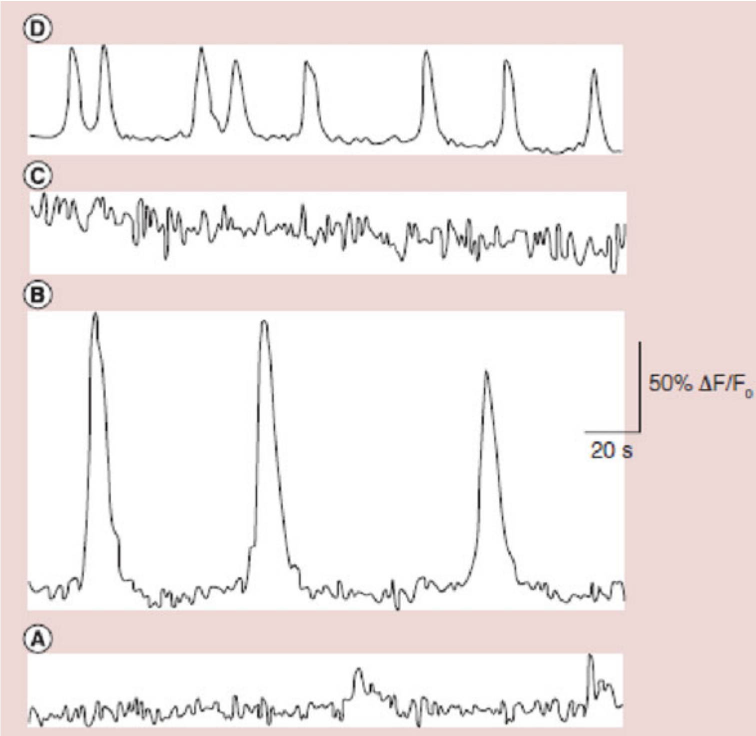

Fig. 20 Representative traces showing responses of selected regions of interest (single neurons) to stimulation induced changes in intracellular calcium. Stimulation was delivered to the films as biphasic voltage pulses (900 mV/cm^2, 0.003 s pulse, 0.2 Hz). Cells were grown on **a** pristine mcl-PHA, **b** mcl-PHA/0.5 wt.% MWCNTs, and **c** mcl-PHA/1.0 wt.% MWCNTs. **d** Control stimulation experiments were conducted on Pt coated glass. Republished with permission of Future Medicine Ltd., from Vallejo-Giraldo et al. [123]

cast blends had been probed by scanning electron microscopy (SEM) and thermomechanical analysis [115]. The chemical imaging of deuterium-labeled poly(3-hydroxyoctanoate) (D-PHO) using infrared microspectroscopy (IRM) revealed phase separation in films containing PHB and P3HO [115, 132]. The addition of SWCNT to these polymer blends was expected to enhance miscibility of the components and therefore yield a biomaterial with improved thermomechanical properties. Moreover, a good dispersion of the SWCNT would increase the electrical conductivity of the films that could be applied for electrical stimulation through the material. The results suggested improved phase miscibility due to nanoparticle addition. The electrical percolation threshold in nanocomposite films was observed at 0.5–1 wt% SWCNT. At these levels, the surface resistivity was eight orders of magnitude lower than the insulating polymer blend. In addition, a SWCNT content up to 2.5 wt % did not affect significantly on mechanical properties of films. A solvent cast bionanocomposite film with 1 wt% SWCNT gave a material with enhanced electrical conductivity compared to the SWCNT-free blend. This optimized blend held the growth of olfactory ensheathing cells and established a basis for developing biopolymer scaffolds able to conduct electrical stimulation. A biomaterial to support nerve repair should be stiff enough to allow implantation without tissue compression, but also flexible to tolerate movement, and give a stable electrode-tissue interface [133].

Many natural materials have been proposed for biomedical, biotechnological, and tissue engineering applications, and among them, protein-derived biomaterials are interesting potential candidates [134]. Keratin-based biomaterials have been considered due to their biocompatibility, biodegradability, mechanical properties, and natural abundance [135, 136]. In vitro biocompatibility studies of keratin-based scaffolds have been shown good swelling, attachment, and proliferation of mice fibroblast cells on the surface of the scaffolds [137]. Other cell lines have been tested, and the in vitro biocompatibility was validated for keratin biomaterials [138, 139]. Moreover, promising results were obtained for in vivo evaluation of keratin biomaterials in different animal models [140, 141].

Several research groups have described P(3HB)-based scaffolds with suitable structure and biocompatibility for tissue engineering applications [142–144]. Hydroxyapatite, bioglass, chitosan, silk, and carbon nanotubes have been blended with P(3HB) to promote biomedical applications [144]. Nanofibers can be easily obtained by electrospinning [145]; they allow cell migration and proliferation, may replace the extracellular matrix [146, 147], and find application as scaffolds in tissue engineering [148]. Zarei et al. [149] used chicken feather-derived keratin and P(3HB) to prepare porous structure scaffolds for tissue engineering. These scaffolds were able to mimic the extracellular matrix environment as they showed good uniformity, structural integrity, enhanced mechanical strength, and bioactivity. The results implied a high potential for these electrospun scaffolds in tissue regeneration or tissue repair applications. In addition, the simple and cost-effective methods used may lead to an easy scale-up. The approach of blending and spinning protein-based polymer with other biocompatible polymers may be extended to give a variety of biocomposites to mimic natural ECM for various tissue engineering applications.

7 Miscellaneous Applications

Most of the materials used in the construction industry derive from non-renewable resources or resources that require considerable time to be renewed [150]. Biopolymers and natural fibers (NF) may have interesting applications in this area [151, 152]. Some biocomposites have been considered in construction applications [153–155]. In particular, the application of NFs and biocomposites in construction was studied [156–161]. The effect of foam core density on the behavior of sandwich panels was studied by CoDyre et al. [159]. In this work, novel biocomposite unidirectional flax fiber-reinforced polymer skins were compared with panels of conventional glass-FRP skins. Structural sandwich panels were developed as replacement of conventional glass fiber-reinforced polymer (GFRP) skins [160]. This panels were made with bio-based skins that were prepared with unidirectional flax fibers and a resin blend of epoxidized pine oil.

The use of cellulose fibers as reinforcement in green composites has been recommended, as an alternative to petroleum-based plastics [162], petrochemicals, and minerals [163, 164]. Inherently, green or biocomposites made from renewable resources are biodegradable and degraded into substances harmless to the environment [165, 166]. In biocomposite formulations, NFs are stronger and stiffer than polymeric matrix [167, 168]; however, a fundamental role is the binding between fibers and matrix, which contributes to stress distribution. When biopolymers are used as matrices, they can protect the fibers, and the overall behavior of the biocomposite depends on: type of fiber, matrix, distribution pattern of the fibers in matrix, etc.

Biopolymers may be obtained from renewable resources, synthesized microbially, or synthesized from petroleum-based chemicals [169]. Polyhydroxybutyrate (PHB) has mechanical properties equal or even better than traditional thermoplastics [170]. Additionally, the replacement of synthetic fibers by biofibers has been considered [171, 172]. Bast fibers, as majority of NFs, have several advantages as replacements for classic glass fibers in composites [173], namely they may lead to materials with reduced weight, less abrasive than glass particles, thus inducing less damage to machinery and personnel during the manufacturing process [174]. Kenaf bast fiber (KBF) has high strength-to-weight/stiffness-to-weight ratio in comparison to other fibers. Additionally, it has a very high carbon dioxide absorption capacity, which is valuable in the prevention of global warming [175]. Hybrid composites composed by two or more types of fibers together with a matrix would overcome the deficiencies of one fiber with another one [176, 177]. Among the NFs, oil palm empty fruit bunches (EFB) is hard and tough and found to be a potential reinforcement in composite applications [178]. The main advantages of EFB hybrid composite are low density, non-abrasiveness, and biodegradability. Hybridization of EFB with jute fibers [179, 180], sisal [179, 180], and glass [181, 182] led to enhancement of physical and mechanical properties of the hybrid composites. Therefore, a hybrid composite of two fibers may include the beneficial properties of the constituents. Tensile strength (TS) is one interesting feature of the NFs, and it is defined as the greatest longitudinal stress that a material can bear without tearing apart. Kenaf fibers have high TS, and thus, they could be employed as a reinforcing component in structural composites for construction industry. In contrast, kenaf fibers have low toughness leading to low impact resistance of the composite. Therefore, kenaf fibers were combined EFB, due to its toughness. A KBFw/EFB hybrid reinforced with PHB biocomposite was developed for application in building material [183]. The KBFw/EFB hybrid reinforced PHB biocomposite was made from polymer films and kenaf together with EFB fabric by lamination and compression molding. PHB is stiff and brittle, so triethyl citrate (TEC) was chosen as a plasticizer [184] in order to improve the mechanical properties and handling of PHB films [185, 186]. Also, a silane coupling agent was chosen to promote interfacial adhesion between reinforcements and matrix of the biocomposite [187]. Figure 21 shows the sample arrangements of the KBFw/EFB hybrid

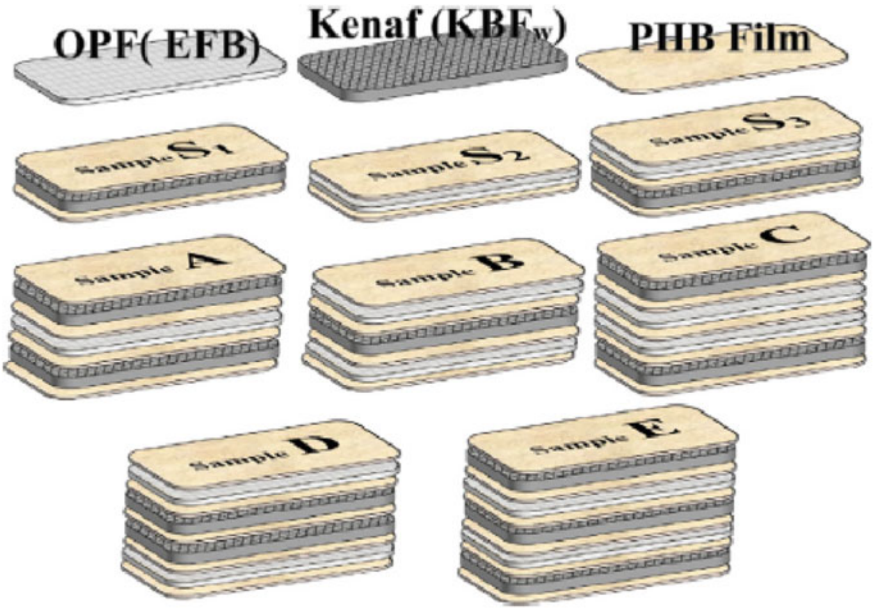

Fig. 21 Sample arrangement of KBFw/EFB hybrid reinforced PHB biocomposite. Reprinted from Construction and Building Materials © 2017, with permission from Elsevier [183]

reinforced PHB biocomposites. The results showed that the tensile and flexural properties would be increased when NFs with higher TS were used. The flexural stiffness of the biocomposite increased with a EFB reinforcement. The sample composed by 11 layers (sample E) might replace some wood and woody production in some applications.

Hasan et al. [188] described nano-biocomposite scaffolds with interesting antimicrobial activity as well as mechanical strength. The synthesis, described in Fig. 22, started with the acid hydrolysis of cellulose to produce cellulose nanowhiskers (CNWs), which were treated with TEMPO to produce the oxidation of primary hydroxyl groups yielding carboxymethylcelullose nanowhiskers (CCNWs). Then, silver nitrate ($AgNO_3$) was added to an aqueous dispersion of the nanowhiskers to give CCNWs/Ag^+. The reduction of silver cations was accomplished by addition of sodium borohydride, according to the methodology described by Liu et al. [189, 190]. Finally, the composites were lyophilized and kept at 4 °C.

Bacterial cellulose BC is synthesized by different species of bacteria, being *Gluconacetobacter xylinum* the most common. It presents chemical purity, high crystallinity, high porosity, high thermal stability, high hydrophilicity, high surface area, and Young's modulus. In addition, it forms well-organized 3D structures with different sizes and shapes. These unique properties allowed it to be used in the development of a variety of composites with possible applications in the TE field.

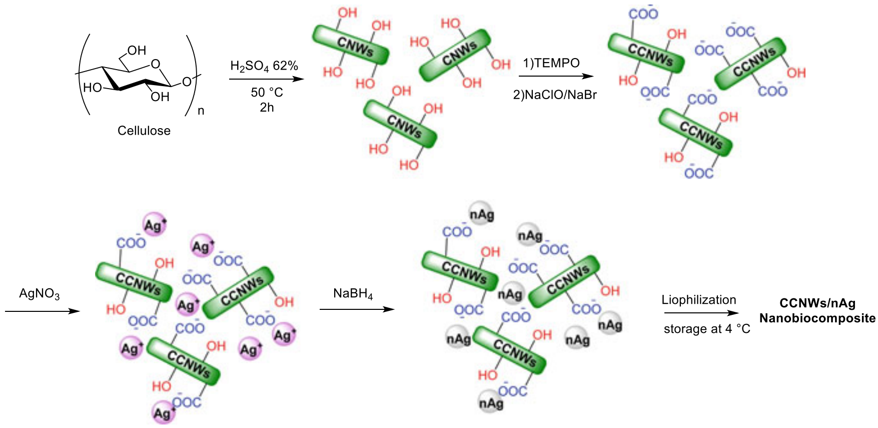

Fig. 22 Synthesis of CCNWs/nAg. Adapted from Hasan et al. [188]

The structural properties of the bacterial cellulose can be modified by election of the growth medium and the environmental conditions during the production of the cellulose. Costa et al. [191] demonstrated by SEM and AFM analysis that the surface morphology of BC biocomposites could be altered by changes in the fermentation medium incorporating some additives such as sugar cane, honey, and dates paste. Also, DSC and TGA analysis showed a change in the crystallinity and higher thermal properties of the new biocomposites. Another common way of interfering with the production rates and the overall properties of biocellulose is the incorporation of carboxymethylcellulose (CMC) in the BC culture medium. The changes in either the cross-linking density or overall network porosity of the biocomposite can be modulated using CMC with different degree of substitution (DS, the average number of carboxymethyl groups per monomeric unit). Thus, De Lima Fontes et al. [192] prepared BC/CMC biocomposites by in situ modification of a static culture medium using *Gluconacetobacter* bacteria and examined the effect of the incorporation of different DS-CMC (Fig. 23). The addition of the cellulose derivative did not generate chemical changes in the biocomposite structure, but a slight decrease in the crystallinity was observed. The FEG-SEM micrographs of the composites revealed that the CMC incorporation into BC nanofibers promoted the reduction of the material porosity. Also, the average elastic modulus decreased with the incorporation of CMC and the increase of the DS, probably due to low elastic modulus of the CMC phase and the decreasing inter/intra hydrogen bonds interaction between the chains of the CMC as a result of the substitution of hydroxyl groups in the glycopyranose molecule. Finally, the authors tested the in vitro release of methotrexate (MTX), a poorly water-soluble drug used in the treatment of cancer, inflammatory, and autoimmune diseases. It is well known that the highly porous structure of BC does not constitute a barrier against the drug molecules diffusion, but the addition CMC demonstrated to reduce the MTX release rate. These results suggested that this kind of biomaterials could be promising for the application through the cutaneous route in different pathologies.

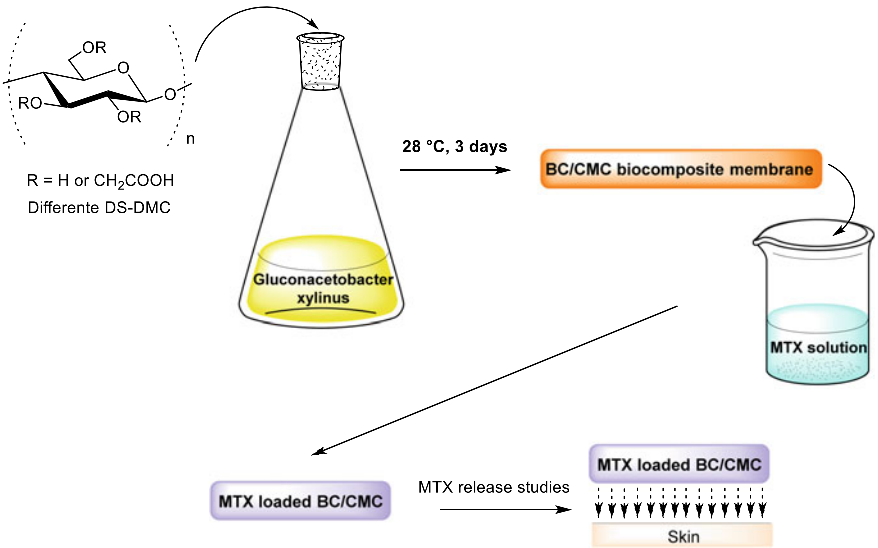

Fig. 23 Schematic representation of the synthesis of BC/CMC biocomposites, MTX loading, and release studies for topical applications. Adapted from Lima Fontes et al. [192]

According to the intended application of a biomaterial, its properties could be adapted by the incorporation of nanoparticles to a BC matrix. In order to give cellulose nanofibers antibacterials properties, Jalili Tabaii et al. [193] developed a silver nanoparticles (AgNPs)/BC composite to consider its application as infectious wound dressing. The AgNPs were successfully impregnated in the BC matrix through in situ reduction of silver nitrate in the presence of sodium tripolyphosphate (TPP) or sodium hydroxide (NaOH). High transparent nano sheets were obtained with the AgNPs firmly and uniformly attached to BC nanofibers. This is very important to ensure a control release of Ag ions which can minimize the potential cytotoxicity and prolonged the antibacterial activity. The (AgNPs)/BC composite exhibited high swelling ability, strong antibacterial activity against both Gram-negative and Gram-positive bacteria, biocompatibility, and no cytotoxicity. The showed characteristics of the biocomposited were a consequence of taking advantage of the individual properties of the materials that constituted it and gives it a promising potential application as antibacterial wound dressing. Galateanu et al. [194] fabricated bio-nanocomposites based on BC and magnetic nanoparticles (Fe_3O_4) for efficient chronic wounds healing. In this case, the composites were obtained directly during the biosynthesis process of BC by dispersing various amounts of magnetite nanoparticles (MNPs) in the culture medium. The biocompatibility of these innovative materials was studied in relation to human adipose derived stem cells (hASCs) in terms of cellular morphology, viability, and proliferation as well as scaffolds cytotoxic potential. The hASCs have an important role in stimulate recovery into injured or diseased tissue by promoting the recruitment of endogenous stem cells to the site of injury. All the BC-based nanocomposites promote hASCs proliferation but the one with highest percentage of MNPs (BC/MNPs 5%) exhibited the greater cell density, viability, and adhesion over it surface.

8 Conclusions

Biocomposites are a hot spot area in material science according to the novel advances and current developments. Despite bio or natural polymers have several useful properties, such as biocompatibility, biodegradability, and abundance in nature, the incorporation of different fillers into the polymeric matrix or a chemical modification can enhance those properties or even add new ones.

Tissue engineering seems to be one of the most promising as well as the best established area for biocomposites, mainly due to the high demand of safe and compatible biomedical devices. It is expected that in future, the use of biocomposites for bone replacement will replace the use of autografts (patients own tissue) which is the current standard treatment.

On the other hand, there are several developments involving biocomposites, from glass production for construction to analytical processes. Regarding this last issue, considering that one of the principles of green analytical chemistry stipule that: "reagents obtained from renewable source should be preferred" [195] biocomposites involved in any kind of analytical procedure will be more than welcomed and will be increasingly found in future analytical developments.

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Polymer/Carbon Nanocomposites for Biomedical Applications

Jyotendra Nath, Kashma Sharma, Shashikant Kumar, Vijay Kumar, and Rakesh Sehgal

Abstract The technological need for novel and intelligent materials as well as the drive for basic understanding has led to noteworthy progress in the field of polymer science. The current interest in polymer matrix-based nanocomposites (NCs) has materialized mainly due to research including exfoliated clay, carbon nanotubes (CNTs), carbon nanofillers, graphene, nanocrystalline metals, and a host of additional nanoscale inorganic fillers. This chapter presents a comprehensive survey of the existing and current literature on different aspects of CNTs, their NCs with polymeric materials and their biomedical applications. This chapter also highlights a variety of methods used to produce CNTs polymer nanocomposites, along with their characterization techniques. Polymer nanocomposites (PNCs) based on CNTs offer remarkably improved mechanical, electrical, and sensing properties. All this justifies the emergent interest in both academia and industrial development. Likewise, the present status and upcoming possibilities of CNT/PNCs are examined in general along with appropriate examples drawn from existing literature.

Keywords Carbon nanotubes · Polymers · Nanocomposites · Drug delivery · Tissue engineering · Biomedical

J. Nath · S. Kumar
Department of Chemical Engineering, National Institute of Technology Srinagar, Srinagar, Jammu and Kashmir 190006, India

K. Sharma
Department of Chemistry, DAV College, Sector-10, Chandigarh 160014, India

V. Kumar (✉)
Department of Physics, National Institute of Technology Srinagar, Srinagar, Jammu and Kashmir 190006, India

R. Sehgal (✉)
Department of Mechanical Engineering, National Institute of Technology Srinagar, Srinagar, Jammu and Kashmir 190006, India

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1 Introduction

As cutting-edge innovations keep on developing every day, there is a consistent need for novel materials such as polymers, ceramics, glasses, nanomaterials, hybrid materials, and colloidal materials with unusual properties and/or combinations of distinctive properties. In the pursuit of modified and improved properties of polymers, numerous endeavors have been made for the last few decades utilizing novel nanotechnology and nanoscience information to get novel materials with improved properties which in turn may be used in various applications including energy generation and storage [1], engineering and construction [2], electronics [3, 4], display technologies [5], food packaging [6, 7], and environmental and biomedical applications [8], etc. In the sector of nanoscience and nanotechnology, polymer matrix-based nanocomposites (NCs) have created noteworthy attention in recent years. Polymer nanocomposites (PNCs) have revolutionized the research attempt in the field of composites as they lead to the realization of synergistic properties from the organic and inorganic components of the system and have led to the growth of the spectrum of application of the commodity polymers to innovative end products [9, 10]. These NCs offer the prospect of significant enhancements in materials properties. Nanoscience and nanotechnology are widely seen as having vast prospects in various fields of science and technology (viz, chemical, physical, and biological, to medicine, engineering, and electronics) [11, 12]. The prefix "nano" is denoted to a Greek prefix meaning "dwarf" or something very small and is equal to one-billionth of a meter, 10–9 m [11, 12]. As a correlation, one must understand that a single human hair is 60,000 nm thickness and the DNA double helix has a radius of 1 nm (Fig. 1) [12].

Nanotechnology is the study and fine-tuning of materials at molecular, atomic, and macromolecular scales, where properties change much from those at the micro-scale. As researchers use more advanced control over the molecular level organization, the morphology of materials is a very important factor. Hence, the evolution from micro-

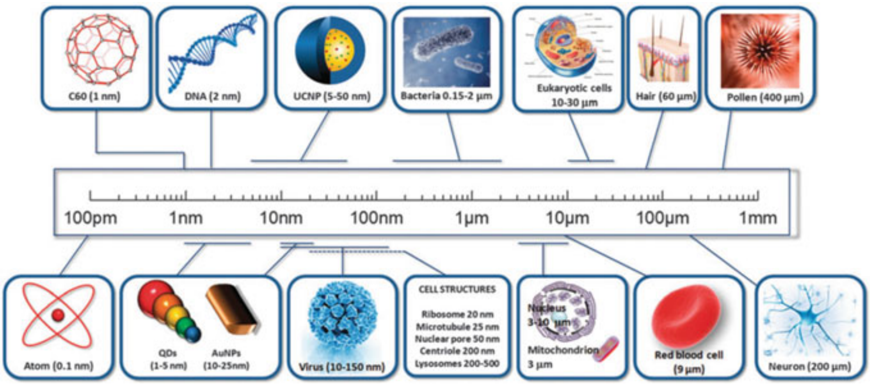

Fig. 1 The size of lanthanide-doped nanoparticles referenced to other, biologically. [Reprinted with permission from Ref. [1]. Copyright (2011) The Royal Society of Chemistry

to nanoparticles led to a desirable alteration in its physical and chemical properties. The properties of nanoparticles can arise from two important factors: first the increase in the ratio of the surface area to volume (S/V) and second, the size of the particle. The higher surface to volume ratio decreases the particle size thereby affects the overall material properties and this higher S/V ratio of nanoparticles also enhances the reactivity with the other particles which results in the increases in strength, heat resistance, etc. K. Eric Drexler was the first person to popularize this technology in the early 1980s [13]. Thereafter, nanotechnology becomes a key thrust area to enable new science and exploration of new technologies. Recently, NCs emerged as a new field of research and maturity in the physical as well as in medical sciences which intended the foundation of new and smart materials for use in advanced technological applications. NCs material has categorized appreciably to include a number of systems such as 1-D, 2-D, 3-D, and amorphous materials, made of clearly different components and blended at the nanometer scale. The noteworthy attempt is centered around the ability to deal with the nanoscale structures via novel synthesis methods. The properties of NCs depend not just on the properties of their every counterpart but also on their morphology and interfacial features. This fast-growing field is creating many fascinating novel materials. There is also the prospect of interesting properties that are unidentified in their parent counterparts. Polymer-based NCs have been a sign of a new paradigm for materials since the 1960s [14].

To date, a large number of published papers on polymer-based NCs have increased progressively since 2000 (Fig. 2) [15–34]. However, PNCs based on carbon nanotubes (CNTs) have been the subject of many types of research for decades. Now a day's these are the most widely used nanofillers in various matrices. Numerous studies on NCs elaboration and characterization have been observed with all polymer families. CNTs-reinforced PNCs have good attraction properties, but this depends on their fabrication method. The shape, size, interaction, dispersion, and orientation of CNTs play an important role in the production of CNTs-reinforced PNCs [35].

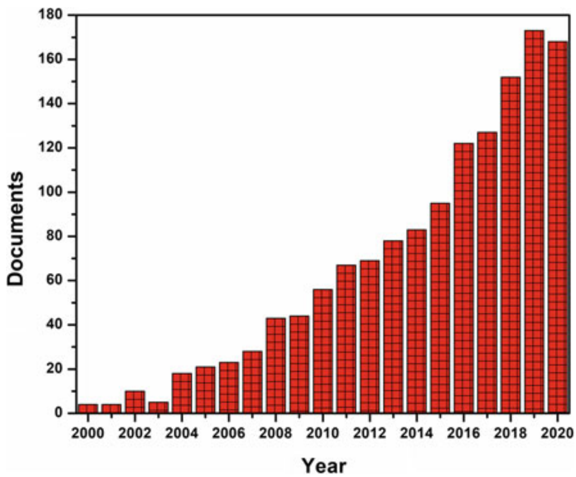

Fig. 2 Number of research papers available on the use of various PNCs for wide range of applications per year from 2000 to 2020 extracted from Scopus with the keywords "Polymer-based nanocomposites"

The extensive use of polymers benefits from their excellent blending properties, lightweight, and ease of handling. In any case, many substances are added to the polymer matrix and polymer compounds are formed to develop specific properties, such as heat resistance and mechanical resistance. The NCs are characterized by a blend of at least two materials with different physical and chemical properties and a recognizable interface. Compared to many metallic compounds, important concerns of composites are high specific stiffness and strength, high hardness, corrosion resistance, low density, and thermal insulation properties [36–38]. Thus, the focus of this chapter is to describe the recent progress in polymer (viz. polystyrene, polyethylene, and polyvinyl) NCs based on CNTs. In this section, a meticulous discussion of the developments made in the polymer/CNTs NCs is presented. A portion of the properties of these materials that have specific importance in the utilization of these materials in the biomedical fields is also summed up. The motive of the introduction part is to give adequate information to comprehend the importance of CNTs and their NCs with polymeric materials. The aim of this chapter is not to cover all the documented literature, but rather to check some of the most important accomplishments in this direction. The second part deals with the structure and properties of CNTs. In the third section, various synthesis methods of CNTs such as arc discharge, laser ablation, and chemical vapor deposition are included. The fourth part comprises brief information about polymer matrix and the related properties suitable for the synthesis of NCs with various nanomaterials. In the fifth section, the preparation of CNTs-based PNCs is discussed. The sixth section will incorporate the biomedical applications (viz. drug delivery and tissue engineering) of polymer/CNTs nanocomposites. Conclusion and opportunities for the future improvement of polymer/CNTs-based NCs are presented at the end.

2 Structure and Properties of Carbon Nano Tubes (CNTs)

CNTs have drawn the attention of many scientists due to their important physical, chemical, and biological properties [39]. CNTs consist of a moving graphite sheet covered with a hemispherical structure whose curves are formed by pentagonal and hexagonal rings. CNTs have a hollow cylindrical one-dimensional structure. CNTs immobilize carbon molecules within the ring, which gives them high strength and mechanical properties Nanotubes have excellent electrical conductivity. Due to the dislocation of electrons in the hexagonal structure of the carbon ring, the charge of electrons in the nanotubes can develop freely. CNTs also have tremendous thermal conductivity due to the vibration of the carbon atoms in the CNTs, which allow them to conduct heat [40]. CNTs can be classified into two types based on the number of carbon layers they contain. CNTs are also classified as single-walled nanotubes (SWNTs) and multi-walled nanotubes (MWNTs). Single-walled carbon nanotubes (SWCNTs) consist of a single-walled graphite layer with a diameter of 0.4–2 nm, usually in the form of a hexagonal bundle. In 1960, Roger Bacon first discovered micron-sized carbon tubes that were structurally equivalent to the new multi-walled carbon nanotubes (MWCNTs). The first nanotubes observed were multilayer MWCNTs consisting of circular hollow graphite sheets of at least two concentric shells arranged coaxially around a hollow core, with graphitic (0.34 nm) spacing between the layers. MWCNTs consist of two or more cylindrical graphite flakes. Their diameters range from 1 to 3 nm [41] and as a result, they have a high aspect ratio (132 000 000:1). This makes them excellent candidates for various applications. There are three unique geometries of CNTs and these are also called flavors [40]. The three flavors are armchair, zigzag, and chiral. Because nanotubes can be both semiconductors and metallic subject to the diameter and "twist" they exhibit extremely fascinating characteristics. CNTs are classified into three categories: zigzag, armchair, and chiral, depending on the arrangement of the contained carbon and the properties of CNTs differ according to these arrangements [40].

CNTs have outstanding chemical and physical properties such as tensile strength, ultra-lightweight, special electronic structure, and high chemical and thermal stability. Researchers have shown great interest in these nanomaterials due to some special properties [42]. CNTs have excellent electrical, mechanical, and thermal properties due to their high S/V ratio. Theoretical and experimental results show that CNTs has Young's modulus of up to 1500 GPa/1.5 TPa and very high tensile strength of 50–500 Gpa unusual mechanical properties of CNTs [43, 44]. Experimental techniques such as SEM, TEM, AFM, and Raman spectroscopy, as well as theoretical models such as ab initio models, molecular dynamics, and continuum models were used to explain the various properties of CNTs [45–50]. The young modules of SWNTs and MWNTs were measured by micro-Raman spectroscopy in the temperature range of 2.8–3.6 TPa and 1.7–2.4 TPa by various authors [45, 46]. They found to have high stiffness, high modules, very low density of 1.3 gm/cm^3, higher strength than iron, and at the same time lighter weight [45, 46]. Besides their mechanical properties, the theoretical electrical properties of the SWNTs were also studied. It was concluded that the diameter and chiralities of the tubes and SWNTs can be metallic or semiconducting [51, 52]. CNTs can be semiconductors or metals depending on their shape. It has been shown that about 66% of them are semiconductors and the rest are metals; the conductivity of CNTs depends on the shape of the tube [51–55], and the conductivity of each CNTs ranges from 10^4 to 10^7 s/m [56, 57], and the conductivity of MWNTs has been measured with four probes in the 10^7–10^8 s/m range [53]. The resistivity of metal CNTs is usually in the range of 10^{-8}–10^{-7} Ωm [58]. SWCNTs form a network of contact blocks [59–61]. The formation of the contact block depends on the length, diameter, and structural configuration of the nanotube. Connecting molecules within the framework of the medium or matrix increase the adhesion of the molecular center. In both cases, the electrical tunneling effect leads to a stronger grounded shell and decreases the electrical conductivity of the material. Nanotube corrugations play a vital role in controlling the electrical conductivity of CNTs [54], and they have some benefits over other carbon materials in terms of electrical and thermal properties.

3 Synthesis of CNTs

CNTs are mostly fabricated by three primary techniques: arc discharge, laser ablation, and chemical vapor deposition [62]. However, researchers are exploring more cost-effective ways to synthesize these structures. A brief overview of each synthesis method is given below.

3.1 Arc-Discharge Method

The arc-discharge technique is the one by which CNTs were first synthesized and documented [63]. The historical backdrop of CNTs is firmly identified with the large-scale manufacturing of fullerenes produced by Krätschmer and coworkers [64]. This method uses two carbon electrodes connected to an energy source of roughly 50–100 A and a potential difference of approximately 20 V. The best results were obtained in the presence of an inert gas, which is probably due to the high ionization potential of helium (He). In the presence of the inert gas, the helium accelerates the deposition of carbon and once the pressure is adjusted, the current is switched on. The positive electrode near the negative anode fills the electrical curve. This causes the electrode to turn red and hot, producing plasma. As the arc settles, the rods remain slightly apart and the carbon nanotubes gather at the negative electrode. When a certain distance is touched, the current is cut off and the discharge is cooled. The process allows the formation of MWCNTs and SWCNTs. The resulting MWCNTs are measured over a wide range of different microns. At the preferred position, the framework MWCNTs crystallize anomalously and are surrounded by van der Waals forces [65–68].

3.2 Laser Ablation Method

The synthesis of SWNT by laser ablation method was described for the first time by Smalley and his colleagues in 1995 [69]. Laser ablation is the vaporization of graphite containing a few metal catalysts (Co, Ni) in an electric laser furnace at an extremely high temperature of 1200 °C and the constant pressure of 500 Torr in the presence of inert gas. The resulting SWCNTs were formed in uniform strands or bundles with diameters of 10–20 nm, while the length of the tubes was about 100 μm and evolved into multilayered thin films using a high vacuum ablation layer. The properties of the formed CNTs depend on the catalyst, laser power, inert gas, temperature, and pressure; the byproducts formed during the synthesis are fullerenes and graphite polyhedron [70, 71].

3.3 Chemical Vapor Deposition Method (CVD)

CVD is normally used for large-scale production of CNTs [63]. The disintegration of hydrocarbons into CNTs requires high temperatures at atmospheric pressure (500–750 °C) and enough energy, but with the use of hydrocarbons such as CH_4, C_2H_2, and CO as carbon sources, CNTs can be obtained [53]. The chemical deposition of C_2H_2 using cobalt or iron as catalysts and silica as support allows the synthesis of many CNTs. A mixture of hydrogen and CH_4 dispersed in a metal catalyst with MgO as solid support was used to produce CNTs in high yields. This synthesis was carried out by CVD using microwave energy [72]. Subsequently, other changes were made to this method. Plasma-enhanced chemical vapor deposition (PECVD) is a commonly used technique for the synthesis of CNTs [73].

4 Polymer Matrix

A "composite material" is a mixture of materials with unique physical and chemical properties at the macroscopic level [74]. The resulting materials generally have properties that are not identical to those of the individual components. Through the use of composites, properties such as mass, resistance to high temperatures, consumption, extreme temperature conditions, and the required coefficient of thermal expansion can be achieved. Composites consist of two phases, the matrix is usually a continuous phase, and the different phases incorporated into this matrix are called "reinforcements". Several interesting mixtures of these meshes (e.g., polymers, carbon, metals, and ceramics) and reinforcements (e.g., particles, wires, and laminates) have been used to combine different composites and NCs [75]. Polymers are generally reinforced with different dosing levels to ease the expansion of applications to include specific polymers. The use of nanofillers to improve the mechanical and physical properties of polymers has led to the extreme neglect of traditional polymer composites. In any case, nanoscale fillers are characterized by the desired size at the nanoscale, which is fundamentally different from isotropic and planar or highly anisotropic needle-like forms. Nanoscience and nanotechnology have generated special opportunity costs for the production of progressive mixtures of nanocomposite fillers and polymers to get attractive PNCs [76, 77].

The establishment of PNCs as nanotechnology has generated extraordinary logical interest and found widespread applications from toys to aircraft. By definition, PNCs are polymers that are reinforced with nanomaterials, such as nanofillers. In the case of polymer nanocomposites, the most important consideration is the dispersion of the nanofillers in the polymer matrix. The uniform cycling of the nanofillers improves the properties of the resulting polymer nanocomposites. However, the solid van der Waals forces between the particles tend to agglomerate the nanomaterials, which lead to degradation of the polymer nanocomposites. It has been established that nanomaterials can be functionally modified on the surface to improve the dispersion of nanomaterials in the polymer matrix. Surface modification and functionality of nanomaterials improve the interfacial cooperation or similarity between the polymer matrix and the filler, resulting in better dispersion and thus producing high-performance nanocomposite applications [78]. Polymer nanoscience is the field of research and use of nanoscience's identified by polymeric nanoparticle matrices. These types of compounds consist of polymers dispersed in a polymer matrix with nanofillers. The development from a small scale to the nanoscale has proved to be one of the properties due to the extreme changes in the physical and composite structure of each molecule. When these particles react with other molecules, this has an extraordinary effect on their properties. Since nanoparticles have a large surface area, they are more likely to cooperate with different types of particles within them. This increases their mechanical resistance, thermal resistivity, and many other confounding factors [79]. Nanoparticles such as graphene, CNTs, MoS_2, and WS_2 are increasingly being employed as reinforcement agents for the synthesis of biodegradable and mechanically strong polymeric NCs for bone tissue engineering [80, 81]. The study of CNTs has unlocked new spaces for the development of polymer matrix compounds with innovative properties and applications. The most commonly reported nanofibers for the fire resistance of polymers are CNTs. The very small diameter and high aspect ratio of CNTs make them ideal for improving the properties of polymer matrices. CNTs have been used to improve the properties of traditional flame retardants and nanoclay. It has gained a lot of consideration as an alternative. It has been documented to enhance the flammability of a large number of polymers by incorporating it at a low loading ratio (<3% by weight) [82, 83]. The fillers are used to reinforce the polymer matrix in a range of sizes. The degree of reinforcement is clear in the molecular size range below 100 nm [84]. Reinforcement materials used to form PNCs can be ranked according to their shape and size [85–88]. The second type of reinforcement consists of a framework of nanotubes with two-dimensional massive shapes and nanoscale elongated structures. Nanoclay, graphene, and cellulose wastes also constitute the second type. The third type of reinforcement is described by a single dimension of nano-regions [89–92]. These nanometers contain extended one-dimensional structures and are reinforced with nanofibers or nanotubes, such as carbon nanofibers and CNTs, or halogen nanotubes as reinforced nanofillers, to make sure excellent properties of the material [93, 94].

5 Preparation of CNTs-Based Polymer Nanocomposites

Polymeric compounds are in high demand in many modern applications, such as electrical protection materials, insulating materials, and high-performance compounds in the automotive and aerospace sectors, although these compounds have certain limitations. CNTs in polymer compounds can achieve better reinforcement when the total amount of CNTs is not compatible, and in general, CNTs need to be well dispersed in the polymer matrix. Several techniques are available to increase the distribution of CNTs in the polymer matrix, such as solution blending, melting and in situ polymerization.

5.1 Solution Mixing

Solution mixing or solvent molding is a process for producing composite materials such as graphite, nanotubes, and polymers. It is the simplest and most widely used process for CNT/PNCs, where CNTs and polymers are mixed with an appropriate solvent, and the solvent is evaporated to a controlled state after forming an NC film on the substrate surface. Thermoplastic and thermoset materials have been produced; a variety of polymers processed in this way include PMMA, polyethylene (PE), polyhydroxy amino ether (PHAE), polyvinyl alcohol (PVA), polystyrene (PS), PEO, and epoxy resins with CNT added [95–99]. It is well documented that it is hard to completely disperse intact CNTs in solvents by simple agitation, and high-power ultrasonic methods are more effective in forming CNT dispersions. Ultrasonic irradiation is widely used for dispersion, emulsification, comminution, and activation of particles. Investigating the multiple effects of ultrasound is effective in decomposing aggregated and entangled CNTs [100]. CNTs in polystyrene matrix were dispersed after ultrasonic stirring at 300 W for 30 min and ultrasonic treatment can be used to get a uniform dispersion of CNTs in PS [97, 101]. Some studies have shown that the surface modification of CNTs is achieved by adding functional groups to obtain the correct dispersion. However, there may be compatibility issues between the functional groups and the polymer matrix. Another approach is electrospinning, which involves treating polymer fibers in the presence of an electric field [102–105].

5.2 Melt Mixing

Solvent blending is one of the most efficient and eco-friendly techniques in the production of composites. The composition is usually performed in a single-screw or twin-screw extruder, where a mixture of polymer and nanoparticles is heated to form a solution. Compounds are subjected to cutting and tensile stresses during processing to help decompose some of the filler aggregates and offer uniform dispersion in the polymer matrix; MWCNTs and SWCNTs provide better dispersion. When mixing solutions, it is always better to use a dissolved polymer matrix. Melting is a general and simple technique that is mainly suitable for thermoplastic polymers such as polystyrene, polypropylene, polycarbonate, thick polyethylene, polyamide, polyoxyethylene, and polyesters [106–111]. This technique involves dissolving the polymers into a viscous liquid, which is then mixed with CNTs. The shear mixture obtained by extrusion or injection improves the dispersion of the CNTs [106, 112, 113]. During softening, a high temperature shear mixer is used to mechanically disperse the CNTs into the polymer matrix [114]. Polymers with suitable sleeves can react with useful sleeves in the nanotubes to improve the dispersion [115]. Tension flow is preferred over shear flow to disperse the nanotubes [116]. One of the obstacles to this technique is that the dispersion of CNTs in the polymer matrix is very low compared to the dispersion that may be obtained by mixing solutions. Due to the high viscosity of the compounds, stacking the CNTs higher should result in lower CNTs [56].

5.3 *In Situ Polymerization*

Several polymerized CNT compounds have been synthesized by in situ polymerization method. This method can be used for both thermoplastic and thermosetting materials. In this method, the CNTs are dispersed in the presence or absence of solvent and then polymerized. Monomers and non-polymers are used as raw materials in this process. The higher content of CNTs is effectively dispersed by this method and can form a strong bond with the polymer matrix. One of the advantages of this method is that the polymer molecules can be grafted onto the walls of the cylinder, resulting in better dispersion coefficients and better coordination between the CNTs and the polymer matrix. This method is useful, such as for placing compounds with polymers that cannot be treated by the matrix or polymers with mixtures of insoluble and thermally unstable polymers that soften the polymer that cannot be treated by the matrix. Several studies have been performed on polystyrene, polyurethane, polyethylene, PMMA, PU, PCL, nylon, and epoxy [117–127].

6 Polymer/Carbon Nanotube Nanocomposites

6.1 *Polystyrene/CNT*

Hill et al. [106] reported the functionalization of SWCNTs and MWCNTs with a polystyrene copolymer. The functionalized CNTs are soluble in various organic solvents, which help to characterize these functionalized CNTs not only by solid-state but also by solution-based techniques. The homogeneous dispersion of CNTs within the PS network provides a model for the general expectation that the dissolution of nanotubes can lead to the desired alignment of CNTs-based PNC [106]. The effect of PS-CNT enhancement on the tribological properties of the NCs was investigated [127]. The improvement of the friction mechanism and wear of the NCs during dry-slip compared to carbon steel alone is also discussed. The CNTs used in this paper were combined with chemically catalyzed vapor deposition. The results show that when the CNT content is less than 1.5 wt%, the microhardness of the NCs is significantly improved; when the CNT content is more than 1.5 wt%, the microhardness value slightly decreases; CNTs significantly improve the wear resistance of the NCs and reduce the coefficient of friction. It is well known that PS-CNT NCs containing 1.5% by weight of CNTs have the lowest wear rate and the lowest coefficient of friction. The decisive improvement in the tribological properties of PS-CNT NCs is due to the excellent mechanical properties of CNTs and the unique cylindrical topology. Due to the structure; the higher strength and durability of CNTs also improve the wear resistance of the NCs [127]. In the year 2005, Zhang and coworkers also reported in situ polymerization of styrene (PS) in the visible region of MWCNTs [128]. Polystyrene and MWCNTs were mixed in different solutions to prepare different types of conductive polymers. The permeation behavior and resistance to natural vapors of the compounds were investigated, focusing on their structures and test conditions to explore the feasibility of MWCNT compounds as chemical vapor recognition sensors. The polymeric filler compounds exhibited significant sensitivity to organic vapors of suitable solvents in the matrix in a short period, and the resistance was quickly restored when the examples were taken to the air and to their unique stimuli. The polymer fillers were more sensitive to organic vapors over a range of MWCNTs than compounds arranged in mixed solutions. However, changes in soluble compound/composition and solvent partial pressure by local temperature to form the most extreme electrical sensitivity of the compound reduce the rate of increase of the reaction; MWCNT/PS compounds could be candidates for gas sensors, and current research is focused on carbon nanotubes. It has the potential to create new applications [128].

The conductivity and mechanical properties of well-dispersed SWNT/PS composite were studied by Chang et al. [129]. They have used a simple technique that gives good dispersion of SWNT in a polymer matrix and studied the effect of SWNT dispersion on conductivity and mechanical properties of composites. The mechanical moduli of the annealed SWNT/PS composites are just expanded marginally and the explanation behind this discrepancy remains unclear [129]. Zhang et al. [130] reported the effect of molten blends on the interaction of MWNTs with PS matrix and discussed the mechanism of the interaction between the polymer and MWNTs. Preparation and characterization of styrene grafted SWCNT and its polystyrene nanocomposite was reported by Nayak and coworkers [131]. They have concluded that the flexural modulus, tensile strength, and electrical properties of the PS/functionalized SWCNTs composite increase by the addition of functionalized SWCNTs. This increase suggests the enhanced compatibility and dispersibility between the SWCNT and PS matrix owing to the existence of 4-vinylaniline on the surface.

The multi-step production of carbon/PS composites by using latex technology is shown schematically in Fig. 3 [132]. The MWCNTs were first ultrasonically dispersed in an aqueous solution of sodium dodecyl sulfate (SDS) sequence and then mixed with different amounts of PS latex. MWCNT/PS compounds were produced from these mixtures by freeze drying and compression molding. The aqueous solutions of MWCNT and SDS were mixed in a bottle and the resulting mixture was rung several times under mild conditions to prepare the dispersions. As SDS is enriched, the maximum absorption plateau increases, the dispersion of MWCNTs at the onset

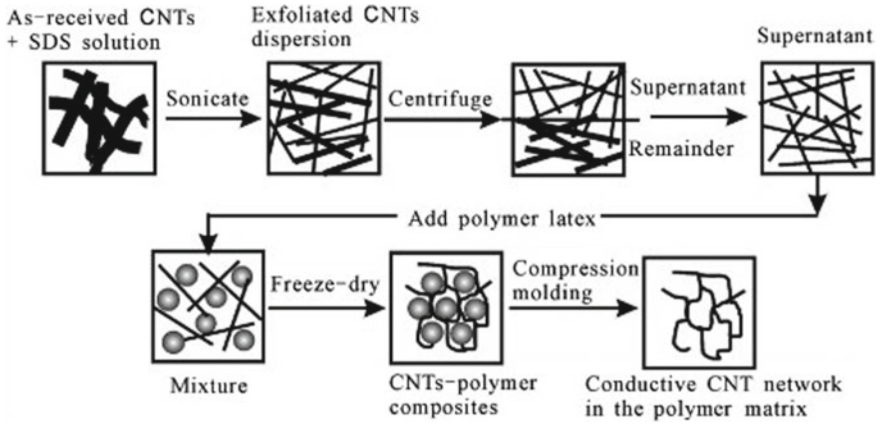

Fig. 3 Schematic description of the multi-step process for preparation of CNT/polymer composites by using latex technology. [Reproduced by permission of Elsevier from Ref. [132]

of sonication increases, and the total energy required obtaining the most extreme dispersion of MWCNTs decreases; an organized MWCNT conductor is placed in the PS network and the amount of MWCNTs decreases. When the percolation threshold is reached, the conductivity of the compound increases extremely [114]. A grafting method was used for the functionalization of CNTs with PS [133]. The polymers were synthesized using a high vacuum anionic polymerization method, which ensures both the subatomic properties of the polymer and the presence of active carbon at the end of the polymer chain. This functionalization has been demonstrated by the reaction of the anion with carbon molecules stretched on the CNT sidewalls. The current approach is based on anionic high vacuum polymerization, which optimally controls the atomic properties of the polymer chains grafted onto the CNTs compared to the near-controlled radical polymerization. All process media were used in a high vacuum to make sure the reliability of the anions and the consistency of the bound polymer chains during the reaction. The separation and dispersion of CNTs are important in the design of PNCs/CNTs [134]. This is the main report to increase the dispersion of CNTs in aqueous arrays using Gemini cationic surfactants. It has been reported in Ref. [134] that in the prepared nanocomposites, individual MWNTs were dispersed homogeneously in the PS matrix as shown in Fig. 4. Strong van der Waals interactions are found to result in a dominant population of huge MWNTs. Here, the dispersion of the twin surfactants from toluene is remarkable, indicating that neither precipitation nor aggregation can be observed. In addition, the PS/MWNT NCs have comparable extended thermal stability to PS without failure by TEM. For the NCs with low MWNT content, an undeniable increase in modulus of 0.25% by weight was observed, which is about 12% more than the full PS at that MWNT content. This study may open up new ways for the dispersion of CNTs, including monolayer CNTs (SWNTs), in various natural solvents and could be extended to the design

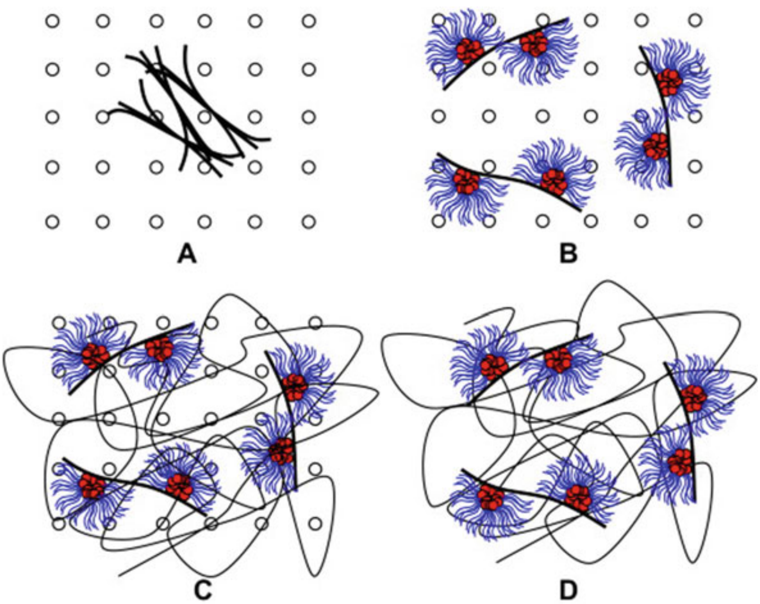

Fig. 4 Schematic representation of the preparation process of the PS/MWNT nanocomposites. [Reproduced by permission of Elsevier from Ref. [134]

of other polymeric nanocomposites/CNTs loaded by Gemini surfactants with ideal atomic structure [134].

In another study, Sun and coworkers reported a homogeneous dispersion of MWCNTs in syndiotactic polystyrene (SPS) by a simple layering technique [135]. The reported SPS/MWCNT composites exhibit significant crystallization behavior and significantly improved thermal and electrical properties. The reported method does not involve surface modifications such as reactive oxidation, fluorination, surfactant coating, and covalent bonding, which can improve the properties, especially the thermal stability and electrical conductivity. This method can be applied to a variety of structures, including monolayer CNTs and other polymeric materials [135]. Martins and coworkers considered the effect of greater flexibility on the flow behavior of polymers and molten compounds [136]. To assess this, shear tests were performed at different temperatures on compounds with different molten polymer and nanotube concentrations. The plastic deformation rate hypothesis was applied to the flow activation in polystyrene castings and compounds with different polyvalent carbon nanotube concentrations. Perturbations induced by varying the shear rate of the monotonic test to bring the melt thickness close to homogeneity were investigated. The response of the material to the perturbation allows assessment of the amount of flow activation. In the case of pure polymers, the volume of the flow trigger is compared to the volume of the tube to which the flow is attached to confirm the validity of the cylinder model. The amount of flow activation was assessed by transient experiments with test flows of polymers and compounds fused

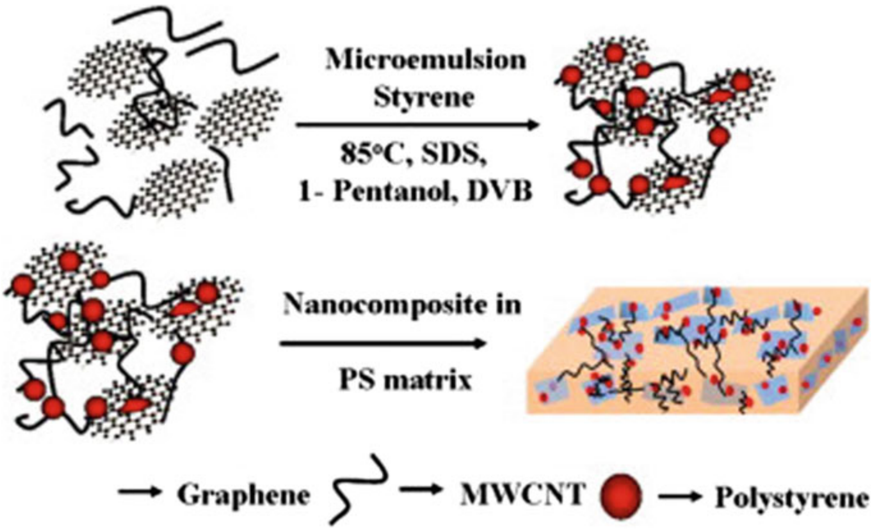

Fig. 5 Multi-step synthesis process for making the conducting graphene/MWCNT/PS films. [Reproduced by permission of Elsevier from Ref. [137]]

to carbon nanotubes. Self-assembled graphene/CNT/PS hybrid nanocomposite by in situ microemulsion polymerization was reported [137]. The complete synthesis and thin film formation are shown in Fig. 5. This new hybrid nanostructure has many prospective applications in various fields.

Raman spectroscopic study of PS nanofibers filled with MWCNTs of different sizes was reported [138]. This study focuses on embedding CNTs into electrospun polymer nanofibers to get better thermal, mechanical and electrical properties. The addition of polyaniline coated MWCNTs and uncoated MWCNTs into the PS matrix were reported by using the solution and melt mixing techniques [139]. PANi-coated MWCNTs have a lower conductivity limit (0.4 wt%) than uncoated MWCNTs (0.7 wt%) because of the better dispersion of PANi-coated MWCNTs in the PS framework. Due to the lower interfacial viability of PANI with PS, the coating resulted in better dispersion of MWCNTs in the PS framework compared to the interfacial viability of MWCNTs with PS. The gel alignment of MWCNT is smoother and rheologically permeable than that of uncoated MWCNT, characterized by interconnected rheological boundaries, such as the ratio (G/G) corresponding to the electroosmotic line [139]. Suemori and coworkers reported the in-plane and out-of-plane thermoelectric characteristics of thick CNT-PS composites prepared by the solution process [140]. They have concluded that the performance of devices that work by thermal flows along the out-of-plane direction is directly related to the orientation of the CNTs in the polymer matrix. Synthesis and mechanical properties of graphene oxide/CNTs aerogel-polystyrene composites were reported [141]. They have concluded that the current preparation technique not only solves the problem of dispersion of graphene oxide in a polymer matrix but also helps in the synthesis of the mechanically strong composite. Conductive polymer composites (CPCs) based on polycarbonate/PS/MWCNT blends with various blend ratios were investigated and evaluated for their morphological and electrical properties [142].

6.2 Polyethylene/CNT

In recent years, CNTs offered exciting opportunities for new materials due to their excellent properties. In particular, one possible application that has attracted a great deal of attention in the materials engineering community is the incorporation of CNTs as nanofiller mainly within a polymeric matrix to form CNTs-based nanocomposites. These materials are expected to show outstanding mechanical, surface, and multifunctional, electrical, and optical properties suited for different applications [143–151]. These properties make CNTs the promising material of the twenty-first century for various applications [31–34]. In 1994, Ajayan and coworkers reported the synthesis of first PNCs using CNTs as fillers [152]. Several methods have already been developed over the last years to achieve an efficient dispersion of CNTs and some of them are already discussed in earlier sections [152–155]. This section mainly deals with polyethylene/CNTs-based nanocomposites. In this regard, Mcnally and coworkers reported the synthesis of polyethylene (PE) MWCNTs with various ratios of MWCNTs ranging from 0.1 to 10 wt% by melt blending using a mini twin-screw extruder [156]. Subsequently, Zhang and coworkers developed a new method for producing compounds in which CNTs were sprayed on the surface of polymer powders in the form of a suspension [157]. They have used HDPE materials containing SWCNTs. At very low SWCNTs concentrations (< 3.4 wt%), the rheological behavior of SWCNTs increases, and the electrical conductivity is improved with a load of only 0.5 wt%. The tensile strength and initial modulus strongly rise with increasing SWNTs content in the polymer matrix. The electrical and thermal conductivity of SWCNT/PE NCs were investigated by considering three factors that enhance thermal conductivity [158]. Kanagaraj and his group reported the use of CNTs to study the mechanical properties of high-density polymeric materials and the use of CNT/HDPE composites to determine the tensile strength of injection molding machines with different volume fractions [159]. The mechanical properties of the nanocomposites, with elastic modulus as tensile strength, hardness, elongation at break, and wear rate, increased proportionally with the addition of CNTs. The temperature was not affected by the addition of CNTs, but the crystallinity increases. The effect of processing parameters on the mechanical and thermophysical behavior of MWNT-reinforced PE compounds was studied [160]. The effect of SWCNTs and MWCNTs on the main melt flow instability of various PE was investigated in detail [161]. A dry state dispersion in linear arrays of LDPE at room temperature without chemical or physical treatment of the nanotubes using a high energy ball milling (HEBM) technique is reported [162]. The mechanical properties showed a significant increase in strength with increasing resistance ratio for all compositional ranges studied. The improvement was most pronounced at low nanotube loading (i.e., 1–3% w/w), reaching a platform value of 10% w/w. The electrical properties are very low at all drag speeds for nanotube loading, and the percolation threshold for solid entrainment due to network failure tends to decrease with increasing drag speed, resulting in an increase in the conductivity of the LDPE insulating substrate by about 9 orders of magnitude [162]. The mechanical properties and oxidation stability of irradiated HDPE reinforced by various concentrations of MWCNTs are studied by Sreekanth and coworkers [163]. They have concluded that the presence of MWCNTs in the PE matrix could successfully confine the loss of mechanical properties because of irradiation. This behavior is observed at high irradiation dose. Various authors investigated the effects of CNTs on the various properties of PE [164–168]. For the first time, a simple and cost-effective surface treatment method was developed to modify the surface roughness of carbon fibers (CFs) by spraying CNTs [169]. CNTs were deposited on the surface of CFs using a spraying technique and then compounded into NTC/CF/HDPE compounds by extrusion and injection molding. The tensile test results of pure HDPE, CF/PEAD compounds, and layered CNT-CF/PEAD compounds with different CF contents showed a significant upward trend in the tensile strength of the CF/PEAD compounds compared to the pure HDPE polymers. This result can be attributed to the high strength and high modulus of CF since higher stiffness loading than the matrix generally improves the mechanical properties of the compounds. They have concluded that spraying CNTs in CF is a promising surface modification method to produce CNT-CF/HDPE compounds with better interfacial properties and it is proved to be an effective method to increase the mechanical properties of hierarchical compounds [169]. A simple and effective method to modify MWCNTs with aqueous cationic epoxy emulsions to improve the dispersion of MWCNTs and increase the interfacial interaction between the substrate and the filler material is reported by Peng and coworkers [170]. Kurup and coworkers developed an LMDPE/EVA-based shape memory NC with improved shape recovery performance by incorporating MWCNTs [171]. They have inferred that the formulation optimization to meet the stability of properties for the development of LMDPE/EVA/MWCNT shape memory NCs in an industry-friendly point of view and could be used in a number of applications from high-performance systems to smart devices.

6.3 Polyvinyl Chloride/CNT

The properties of polymer/CNT NCs are influenced by different components comprising surface contacts, adhesion power, preparation technique, and dispersion of CNT in the polymer matrix. Poor interfacial attachment and un-even dispersion of CNT in polymer structure have become the difficulties for the treatment of polymer/CNT nanocomposite. Broza and coworkers reported the production of NCs of polyvinyl chloride (PVC) with CNTs to investigate the effect of CNTs on electrical and mechanical properties [172]. PVC is a product with various applications, which is rich in natural and chemical resistance, and which has more diverse mechanical properties due to the expansion of plasticizers. The effect of Kevlar coated CNTs for reinforcement of PVC on the mechanical properties was reported by O'Connor and coworkers [173]. These functionalized Kevlar nanotubes can serve as an additional promising material to support PVC. Significant improvements in the stiffness (up to half) and modulus (up to 70%) of the functionalized nanotubes have been observed compared to pure polymer compounds and nanotube-polymer compounds. These nanotube compounds have incredible potential as additives for the production of new ultra-high-strength polymer materials [173]. The influence of CNTs on glass transition temperature, thermal, mechanical, antibacterial, electrical, charge transport, and magnetic properties of PVC was studied by various authors [174–181]. The various property improvements for polymer/carbon nanotube NCs are reviewed in Table 1.

7 Biomedical Applications of Carbon-Based Polymer Nanocomposites

CNTs, discovered by Japanese scientist Iijima in 1991 [209], are now viewed as a cutting-edge research topic in various sectors of science and technology. Since the start of the twenty-first century, CNTs have been used in pharmacies and therapeutic drug delivery systems for pharmaceuticals [41]. The exceptional blend of mechanical, optical, and electrical properties possessed by CNTs has cultivated research for their utilization in various types of applications [210]. The various biological applications of CNTs are shown schematically in Fig. 6 [211–215].

7.1 Drug and Gene Delivery

Several studies have been done in past to demonstrate the use of CNTs in a range of biomedical applications, including drug delivery/quality therapy, biosensors, bioimaging, and therapeutics. The combination of the excellent mechanical, electrical, and thermal properties of CNTs with the properties of polymers makes them suitable for a lot of applications. The blend of gene therapy and chemotherapy has as of late got extensive consideration for cancer treatment [216]. On the other hand, low transfection efficiency and poor endosomal escape of genes from nanocarriers intensely inhibit the realization of the clinical use of small interfering RNA (siRNA). A new pH-responsive, surface-modified SWCNT was developed for the codelivery of doxorubicin (DOX) and survivin siRNA [216]. It was concluded that DOX-functional SWCNTs and survival siRNAs show potent antitumor effects in vitro and in vivo, and thus may represent a substitute approach for the joint use of antitumor drugs and genes. Some of the unique properties of CNTs such as high drug loading limit, huge surface area, high mechanical properties, and chemical stability make them excellent

Table 1 Property improvements for Polymer/carbon nanotube nanocomposites

S. No.	CNT type	CNT wt%	Matrix	Synthesis method	Approach	Properties	Percentage/improvement	Refernces
1	MWCNTs	0.08–0.32	PS	Irradiation	Mechanical	Tensile strength	250%	[182]
2	MWCNTs	0–1	PS	Solution casting	Mechanical	Tensile strength	25%	[56]
3	SWCNTs	0.1–2	PS	Solution mixing	Mechanical, Electrical	Tensile strength, conductivity	Improve M.S and increase E.C	[129]
4	MWCNTs	1–5	PS	Solution mixing, spin casting	Mechanical, Electrical	Tensile strength, conductivity	40% Highly conductivity	[97]
5	MWCNTs	0.2–10	PS	Noncovalent grafting	Mechanical, Electrical	Young's modulus	160%	[183]
6	CNTs	0–4	PS	In situ polymerization	Mechanical	Wear resistance, Tribological	Improve W.R and increase Tribolocigcal	[127]
7	MWCNT/graphene	Various conc.	PS	In situ microemulsion polymerization	Mechanical, Thermal	Tensile strength, stability	Increase T.S and Thermal stability	[137]
8	MWCNTs	0–10	PS	Solution mixing/Electro spinning	Thermal	Thermal stability	Improve Thermal stability	[138]
9	MWCNTs	Diff. conc.	PS/PANi	Solution/Melt mixing	Electrical, Rheological	Dispersion	Good dispersion for both at low E/R percolation.	[139]

(continued)

Table 1 (continued)

S. No.	CNT type	CNT wt%	Matrix	Synthesis method	Approach	Properties	Percentage/improvement	Refernces
10	GOCA	Variou conc.	PS	Self-assembly/Freeze drying/In situ polymerization	Mechanical	Porous, Mechanical, Hardness, Modulus	High porosity, high microhardness/compression modulus, Excellent M.P	[141]
11	MWCNTs	40	PS	Melt mixing	Mechanical	Tensile strength	10%	[114]
12	MWCNTs	40–50	PC/PS	Melt Processing	Electrical	Conductivity	Highest electrical conductivity	[142]
13	MWCNTs	0–5	HDPE	Melt mixing	Mechanical/Punch test	Tensile	12%	[110]
14	MWCNTs	1–10	LLDPE	High energy ball mill	Mechanical, Thermal, Electrical	Strength, Stability, Conductivity	Improved M.S, T.S, E.C	[162]
15	MWCNTs	0.25–1	HDPE	Injection modeling	Mechanical	Mechanical, Irradiation oxidative	Loss of M.P, Oxidation index (−56%)	[163]
16	SWCNT	–	PE	First principal method	Mechanical	Young's modulus	70%	[184]
17	MWCNT	1–18	HDPE	Melt Mixing	Electrical	Conductivity, EMI-SE	Improve with increase MWCNT	[164]
18	CNTs	Diff. CNT	PE	Finite element method	Electrical	Conductivity, EPT	Improve conductivity and current density	[165]

(continued)

Table 1 (continued)

S. No.	CNT type	CNT wt%	Matrix	Synthesis method	Approach	Properties	Percentage/improvement	Refernces
19	MWCNT	1–10	LDPE	Solvent mixing process	Mechanical	Strength	High strength	[166]
20	MWCNT	0.1	LDPE	Screw extruder	Mechanical	Tensile test	Ultimate tensile strength	[167]
21	CNT/CF	–	HDPE	Extrusion and injection molding	Mechanical, Morphological	Tensile, Flexural	70.62 and 40.38%	[169]
22	MWCNTs	–	PE	Solution mixing	Mechanical, Electrical	Tensile, conductivity	Increased tensile and tremendous conductivity	[170]
23	MWCNT	1–3	LMDPE/EVA	Melt mixing	Electrical, Mechanical, Thermal	Conductivity, Strain, Shape memory	High conductivity, >98% excellent S.M	[171]
24	SNWNT/MWCNT	0.1–0.2/5–20	PVC	Solution mixing	Electrical	Conductivity	Increase electrical conductivity	[172]
25	CNT	–	PVC	In situ purification	Mechanical	Tensile, Young's modulus	T.S—50% Y.M—70%	[173]
26	MWCNT	0.2–2	PVC	Pressing method	Mechanical	Microhardness	Microhardness increased	[185]
27	MWCNT	0.5	PVC	Melt mixing	Mechanical, Thermal	Modulus, Tensile strength, Stability,	Modulus increases T.S reduced, increase stability	[175]
28	SWCNTs	–	PVC	Wet phase inversion process	–	Flux, Surface, Antibacterial	Surface/flux Slightly improve, no bacterial	[176]

(continued)

Table 1 (continued)

S. No.	CNT type	CNT wt%	Matrix	Synthesis method	Approach	Properties	Percentage/improvement	Refernces
29	SWCNTs	0–20	PVC	Homogenous solution	Thermal	Stability, Spectroscopic	Increase T.S, Poor spectroscopic result	[186]
30	MWCNT	0–15	PVC	HSBM/HEBM	Electrical, Mechanical	Conductivity, Tensile strength	Improve conductivity and T.S	[177]
31	SWCNT	0.25–1	PVC	Plastisol curing method	Mechanical, Thermal	Tensile, Young's, toughness, elongation, stability	All are high and greatly improve	[179]
32	SWCNT	0.01–0.06	PVC/PU	Homogenous solution	Mechanical, Thermal	Tensile, Modulus, Stability	Increase tensile, elastic modulus and stability	[180]
33	MWCNTs	0.1–2	PVC	Acid ultrasonic method	Mechanical, Thermal	Tensile, Toughness, Stability	Enhance T.S and toughness Excellent stability	[181]
34	SWCNTs	1.8	PP	Solution mixing	Dynamic mechanical analysis	Composite modulus	15% increase	[187]
35	SWCNT	Varying level	PP	Solution process	Mechanical	Tensile, Modulus, Fiber density	All are increase	[188]

(continued)

Table 1 (continued)

S. No.	CNT type	CNT wt%	Matrix	Synthesis method	Approach	Properties	Percentage/improvement	Refernces
36	MWCNT	–	PP	Melt blending	Morphology, Mechanical, Thermal	Strength, Toughness, Stability	Disperse well, all properties are improve	[189]
37	SWCNT	5–20	PP	Solution mixing	Mechanical	Tensile, Young's	Low conc. Both are increase, High conc. decrease	[190]
38	MWCNT	0.1–8	PP	In situ polymerization	Mechanical	Tensile, Young's	Both are increase	[191]
39	MWCNT	0–1 vol.%	PP	Melt mixing	Electrical	Conductivity	Improve E.C	[192]
40	MWCNTs	0.1–3.5	PP	In situ polymerization	Mechanical, Thermal	Young's, Stability	22–37% Increase stability	[193]
41	CNTs	–	PP	In situ graft method	Mechanical	Tensile, Young's	T—110% Y—113%	[194]
42	MWCNTs	0–5	PP	Melt mixing	Mechanical	Tensile, Young's	T—39% Y—69%	[195]
43	CNTs	0–5	PP	Melt mixing	Mechanical, Thermal	Tensile, Elongation, Stability	T and E max. increase at 3% Improve stability	[196]
44	MWCNTs	–	PP	Melt mixing	Electrical	Conductivity	Improve E.C	[197]
45	MWCNT	0–5	PP	In situ polymerization	Mechanical, Thermal	Modulus, Stability	High modulus, T.S—Sharply increase	[198]
46	MWCNTs	0.001–1	PP	Melt processing	Mechanical	Impact strength	Increase 152%	[199]

(continued)

Table 1 (continued)

S. No.	CNT type	CNT wt%	Matrix	Synthesis method	Approach	Properties	Percentage/improvement	Refernces
47	MWCNT	0.1–1	PP	Melt blending	Fracture mechanism	Shear strength (2.2 MPa)	Improve (17.8 MPa)	[200]
48	MWCNT	0.5	PA6	In situ polymerization	Mechanical	Tensile, Modulus	Slightly improve T.S and modulus	[201]
49	MWCNT	–	PA6	Melt mixing	Mechanical	Scratch, Tribological	Both are increase	[202]
50	MWCNT	0.5	PA6	Solution mixing	Thermal	Stability	Better T.S and mechanical, structural	[203]
51	MWCNT	0.1–2	PA6	Melt mixing	Thermal	Stability	Increase T.S	[204]
52	MWCNT	–	PA	Melt mixing	Electrical, Thermal	Van der waals Stability,	Strong van der waals forces and better stability,conductivity	[205]
51	MWCNT	–	PA	Interfacial polymerization	–	Flux, Rejection	Water flux increase and salt rejection decrease	[206]
52	CNT	1–4.5	PA	Melt mixing	Mechanical, Electrical	Tensile, Elongation, modulus, Conductivity	All properties are significantly improved	[207]
53	MWCNT	2.5–20	PA6	Melt mixing	Mechanical	Modulus	Improve modulus	[208]
54	CNT	20	PO	Melt mixing	Mechanical, Electrical	Tensile strength, modulus, conductivity	T.S/T.M are decreases and E.C are High	[182]

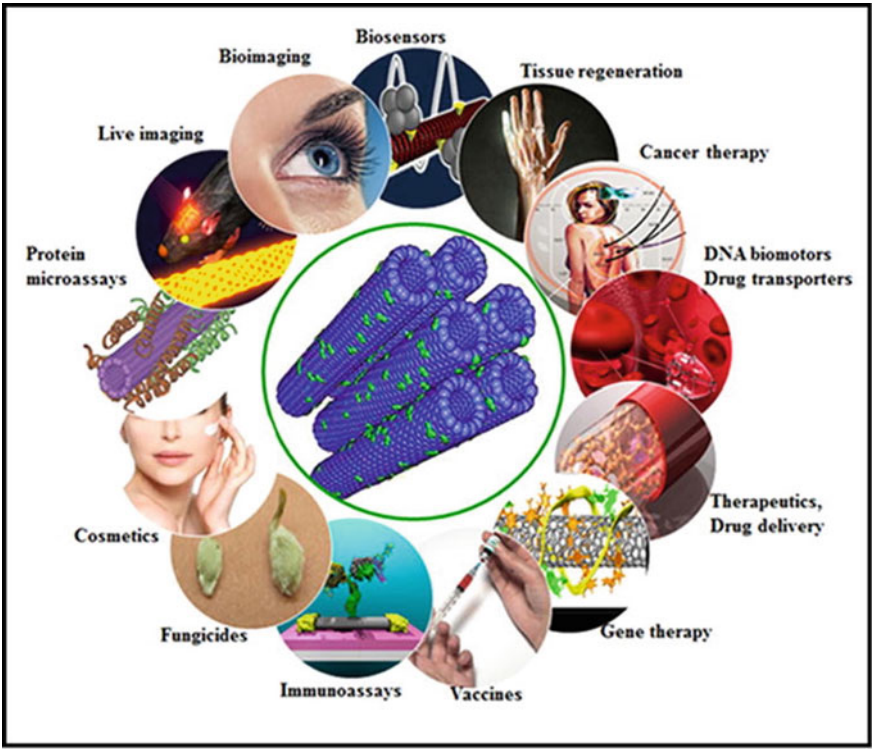

Fig. 6 Carbon nanotubes applications in bio world. [Reproduced by permission of Elsevier from Ref. [211]

materials to be used in drug delivery devices [217–219]. Improvements in peptide-modified SWCNTs have identified the main enemy of tumor action and improved tumor localization [220]; PH dependent efflux also allows drug delivery near tumor tissue [221, 222]. Due to the multi-walled nature of CNTs, there is a force between carbon molecules that forms a cylindrical partition, known as the van der Waals force [223]. The distance between van der Waals forces, static electricity, communication, and nanotubes has been shown to be associated with the encapsulation of drug particles. These compounds play an important pharmacological role in the case of CNT intoxication. MWCNTs and PH-responsive gels pose challenges for the availability of doxorubicin, a nano-hybrid scaffold. The created nano-hybrid structures were designed to deliver drugs to cells with glioblastoma U-87 and it was found that drug delivery with nano-hybrid structures could limit cancer growth [224]. Percutaneous immobilization of electrical properties using multi-walled carbon polymer nanotubes allows the creators to take advantage of the high electrical properties of CNTs; it accelerates drug organization by subjecting it to heat [225]. Cross-linked polymer hydrogels-MWCNTs generate electrolytic reactions through pulsed discharges to sucrose; the discharge curves are evaluated using radioactive sucrose as an electric field as a model for hydrophilic drugs [226]. CNTs were also used as carriers for the inoculation of antigens [227]. Multilayered CNTs are employed for binding to neural tissue cells and are valuable for creating competent drug transport and mass structures [228]. The limitations of SWCNTs in drug binding are more obvious. Exocytosis and enzymatic disruption are two components of CNTs release from the cell [229]. The low cytotoxicity and enhanced anti-Lishman motility of cisplatin MWCNTs show that it enhances low-binding CNTs, highlighting the great ability of CNTs as nano-drug carriers [230]. Due to the unique physicochemical properties of CNT loading on several small drug particles, ciprofloxacin, methotrexate, temozolomide, oxaliplatin, doxorubicin, docetaxel, gemcitabine, cisplatin, epirubicin, amantadine, and lamivudine were studied [231–233]. Sustained release and biodegradation of multilayer carbon nanotubes and biocompatibility the presentation of multilayer carbon nanotubes diclofenac has been improved in enabling the release of diclofenac sodium with an economical release profile [234]. Lofi and coworkers [235] analyzed the noncovalent interactions and various covalent functionalization mechanisms of the anticancer drug cladribine (CDA) with CNTs using quantum computational studies. A group of researchers from the Chinese Academy of Medical Sciences & Peking Union Medical College, PR China reported a concurrent fluorescence imaging monitoring of the programmed release of doxorubicin (DOX) and rhodamine B (RB) as the model drugs from a hydrogel-CNT delivery system (Fig. 7) [236].

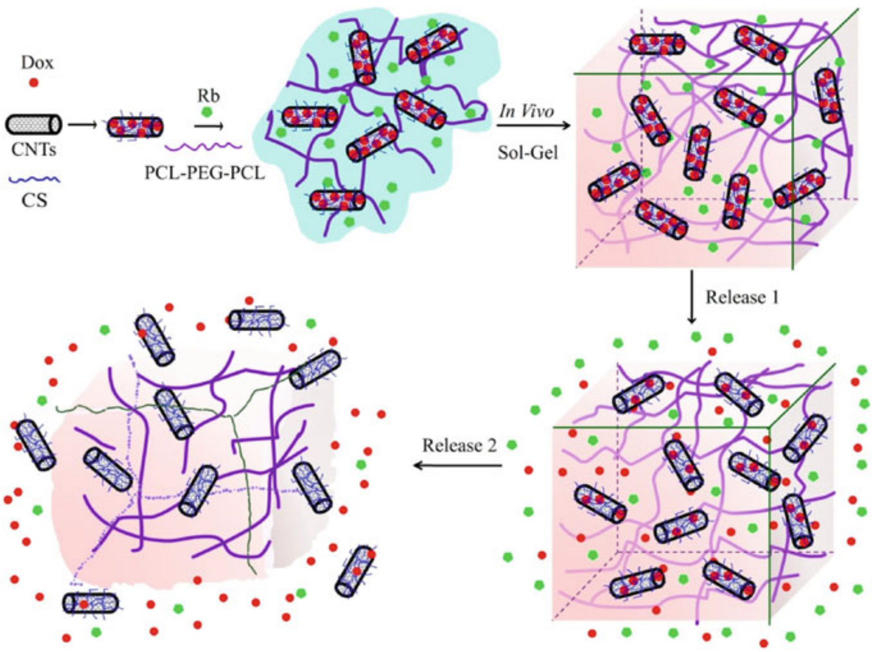

Fig. 7 Scheme of the programmed release of dual fluorescent drugs from hydrogel-carbon nanotubes delivery system. [Reproduced by permission of Elsevier from Ref. [236]

The decoration of MWCNTs with gold TiO_2 nanoparticles to improve their biocompatibility for DOX delivery was reported [237]. They have concluded that this is an effective method for the preparation of carbon-based NCs for practical applications in nanobiomedicine [237]. Mazzagira and coworkers [238] developed a new platform of MWCNTs-cyclodextrin modified with branched polyethyleneimine (PEI) and alloyed rhodium and investigated as a drug delivery system. Mallakpour and coworkers [239] reported the ability to deliver drugs in a hydrophobic model of starch/CNT NCs. The results suggest that MWCNT-G1 plays two important roles: as a stabilizer for nanoparticle production and as a carrier for nanodrugs. Lavado-Gonzalez and coworkers examined several surface oxidative pretreatments to enhance the removal of MWCNTs by macrophages that support biomimetic properties with microtubules and ultimately exert anti-proliferative, anti-migratory, and cytotoxic effects on cancer cells [240]. The biodegradation of these nanotubes could be demonstrated after several days of therapeutic effects. Hesabi and coworkers [241] investigated the interactions between the anticancer drug hydroxyurea and functional zigzag carbon carboxylate (CNT) at the B3LYP and CAM-B3LYP levels in the gas and solvent phases using the functional density theory (FDT) approach. The results showed that all the complexes were favorable, especially in the aqueous phase. In a similar study, Kamel and coworkers [242] investigated the structural and electronic effects of interactions between f-CNTs and FLT molecules in the gas phase and aqueous solution using functional density theory. The results obtained show that the drug adsorption process on the outer surface of functionalized nanotubes is exothermic and all configurations are stable. They have also been observed that the intermolecular hydrogen bonding between FLT drugs and functionalized nanotubes plays an important role in the stability of the physically adsorbed structures. Various authors studied Li and coworkers [243] successfully fabricated the compound FA-CS-CNT(Fe)/HA as a magnetic targeted drug carrier to achieve controlled release of anti-cancer drugs (DOX) by catalyzing in situ CVD of CH4 in HA nanodododers. The synthesized CNTs, Fe, and HA form CNT(Fe)/HA compound structures, which can be used as carriers of targeted magnetic drugs based on acidification, the addition of CS, and AF graft functionalization. The addition of CNTs to hydrogels improved the swelling behavior of mixed hydrogels [244]. Biagiotti and colleagues [245] investigated the formation of metformin salts using oxidized CNTs and their viability testing in three different cell lines with met and ox-MWCNTs with dose-dependent inhibitory activity against PC3 and MCF7 cell cultures. The effect of iron-filled CNTs on the adsorption capacity and sustained release of drugs is reported by Sukhodub and coworkers [246]. They have also studied the mechanical properties of HA-based composite biomaterials formed to create model regions of bone tissue, where the material can resist mechanical stress. This material can be used to model in 3D the regions of bone tissue that need to resist mechanical stress. Rafi and coworkers [247] studied the introduction of pH-sensitive polymer chains into the walls of CNTs to prepare pH-sensitive CNT nano-hybrid polymer systems. The release rate of naproxen was insignificant due to the hydrogen bonding between the polar group and the polymer chain; at pH 7.4, the polar group was deprotonated and the hydrogen bonding was replaced by repulsive electrostatic interactions, resulting in an increased release rate.

Due to their physicochemical properties, CNTs have been developed as transport scaffolds for the treatment of cancer growth [248]. Due to various problems (toxicity, low resistance to excipients, and multidrug resistance, limited cell penetration), the effectiveness of common drug transport systems is limited. Therefore, it is intended to structure an efficient transport system with better cellular development of effective drugs. CNTs can dispense with a large number of cross-linked cell-stretching membranes, and in this sense, the anticancer drugs administered by CNTs are administered in situ at full concentration and, as needed, will be more successful in tumor cells than drugs modulated by standard therapy [248, 249]. Cancer is represented by the uncontrolled development of cells that cause damage to common tissues and organs. Harmful development leads to inadequate cell expansion. Especially in the treatment of cancer growth, the use of conventional chemotherapeutic drugs carries the risk of excessive baseline effects, just as they are less effective in completely destroying cancer cells. Carbon nanotubes comprise a high percentage, have a large surface area, and have a needle-like structure, thus allowing them to bind to drug molecules [250–252]. CNTs are beneficial as nanocarriers in the treatment of cancer [252–254]. Photothermal therapy with CNTs can lead to the cure of tumors [255–258].

7.2 *Tissue Engineering*

Tissue engineering (TE) is a multidisciplinary discipline that focuses on reincarnation events and uses information from science, materials science, design, life science, and clinical science to solve fundamental medical issues such as tissue loss and organ failure [259]. Tissue design, which originated from the field of biomaterial modification, is the combination of bioactive scaffolds, cells, and particles to create functional tissue [260–262]. The purpose of tissue design is to bring together practical entities that repair, support, or enhance damaged tissues and entire organs. CNTs can be used for tissue engineering, cell tracking, and nomenclature, and to improve their performance [263–265]. Advances in tissue engineering have demonstrated new methods for unprecedented evaluation of cultured tissues; CNTs can improve cell screening, biosensors, transfection experts, and platform placement. Also, CNTs can be fused to scaffolds to help structural reinforcement and provide platforms with new properties, such as electrical conductivity, to promote cell development [266]. Recently, the carbon nanotube method has been used for tissue engineering and regenerative medicine culture [267]. In this technique, cells are encapsulated in appropriate biomaterials to allow the continued development of new tissues [267–269]. Carbon nanotubes are valuable in improving the mechanics and electrical aspects of scaffolds, allowing cells to feel microscopic sensations and responses of intracellular substances. Tissue engineering applications are twofold, such as the repair of damaged tissues and the development of in vitro human models. In vitro 3D models, however, could change the way we understand cancer. Lovat and coworkers [270] investigated the use of CNTs as a possible device to improve the transmission of neural signals while promoting dendritic outgrowth and cell adhesion. The results strongly suggest that the growth of neural circuits in CNT networks is associated with a significant increase in network activity. The improved efficiency of neural signal transmission may be linked to the particular properties of CNT materials, such as high electrical conductivity.

It was reported that poly(2-hydroxyethyl methacrylate) pHEMA/mWCNTs lead to the increased electrical conductivity of the material, which activates SHSY5Y cells as neurotransmitter channels when transferring electrical potentials [271]. mWCNTs seem to contribute to improved cell adhesion and conductive nerve potentials. It may be preferable to align the mWCNTs on the membrane before seeding the cells and then applying lower potentials than the alignment used in this study. Armentano and coworkers [272] provided an overview of current research trends in tissue engineering-related nanocomposites: including biodegradable polymers, organic/inorganic nanostructures, matrix interactions between nanostructures, and strategies for fabricating interconnected pore nanocomposite scaffolds. The combination of biodegradable polymers with nanostructures opens up new perspectives in the field of NCs for biomedical applications with tunable mechanical, thermal, morphological, and electrical properties [272]. The preparation and characterization of the collagen/CNT matrix and the cellular response of PC12 cells when cultured in this composite membrane in the absence and the presence of electrical stimulation was studied [273]. Compared to collagen membranes, the electrical conductivity and electroactivity of the composites were greatly improved by the deposition of CNTs, even at less than 1% charge. It was also found that the concentration of CNTs added to composite membranes altered cellular activities such as adhesion, metabolic activity, and neuronal expansion. Cells grown on SWCNT were damaged, chromatin concentrated, and apoptosis occurred after SWCNT acid treatment [274]. The hypothesis that CNT-based electrospun PLA scaffolds that could be produced for tissue engineering applications were tested by Mackle et al. [275]. Ogihara and coworkers [276] were the first to investigate the biocompatibility and bone tissue compatibility of CNT/alumina composites with alumina ceramics in vivo and in vitro. The results showed that the compound exhibited good bone histocompatibility in bone grafting studies and was directly transformed into new bone. The production of unidirectional bioglass fibers (BGF) (13–93) and reinforced polylactic acid (PLA) compounds that have improved the mechanical properties of bone plates for healing weight fractures of tubular bone were reported by Mehboob et al. [277]. The mechanical and microscopic properties, in vitro degradation, and bioactivity of the BGF/PLA compounds, were also evaluated. Badhe et al. [278] studied a bilayer macroporous vascular scaffold based on a chitosan-gel compound with many desirable properties that accurately mimic the morphology and mechanical properties of biological blood vessels. The macroporous layer offers a huge surface with strong cell adhesion and proliferation properties. The non-porous outer layer delivers cell protection and gives extra flexibility and elasticity to the reinforcement. Biomedical applications of different CNTs polymer-based NCs are summarized in Table 2.

Table 2 Biomedical applications of CNTs/polymer-based nanocomposites

S. No.	Backbone	Drug	Application	Ref.
1	Hydrogel combine with CNTs	doxorubicin, rhodamine B	Release dual drug delivery system, demand on combination chemotherapy	[236]
2	CNT Fe/Hydroxyapatite [(CNT(Fe)/HA)]	Anticancer drug doxorubicin	Realizing magnetic targeted delivery	[243]
3	pH-responsive Polyethylenimine–betaine functionalized SWCNTs	siRNA and DOX	Effective antitumor effects in vitro and in vivo, codelivery of anticancer drugs and genes.	[216]
4	Antibody-conjugated CNTs	Anti-P-glycoprotein	P-glycoprotein-targeted photo thermal therapy	[278]
5	Starch nanocomposite films containing MWCNT	Zolpidem	Drug/gene delivery	[239]
6	Hydroxypropyl-β-cyclodextrin (HP-β-CD) modified carboxylated SWCNT	Formononetin	Drug delivery	[279]
7	Biodegradable MWCNTs	oxidized MWCNTs (o-MWCNTs)	Anti-tumoral effect	[240]
8	Carboxylation of CNT	Hydroxyurea	Improved drug loading	[241]
9	Functionalized SWCNT	Flutamide	Improved drug loading	[242]
10	MWCNTs modified with cyclodextrins and polyethylenimine	Cidofovir	Antiviral drug delivery	[238]

8 Conclusion

In this chapter, an attempt has been made to combine all the crucial information about CNTs, polymer matrix, CNTs-based PNCs including synthesis methods, properties, and biomedical applications. Arc discharge, laser ablation, and chemical vapor deposition techniques for CNTs have been briefly discussed. Biomedical applications of CNTs and their NCs with polymeric materials have also been discussed in detail. The remarkable properties of CNTs viz. mechanical, electrical, and thermal makes them ideal candidates as fillers in the various polymer matrix. The field of NCs has been one of the most encouraging and growing at a fast pace. They find exceptional consideration because of the novel properties, for example, lightweight, ease of synthesis, and flexibility. A characterizing highlight of PNCs is that the nano size of the fillers prompts an enormous increase in the interfacial area when contrasted with customary composites. CNTs-based NCs possess novel properties to use them in various applications. CNTs are a particularly alluring class of fillers for polymers on account of their high mechanical and thermal properties. They stand for one of the strongest and toughest materials known. In short the field of nanocomposites, developing mechanically strong, electrically conducting, and novel materials based on CNTs and the polymer is just the foundation and hoping to become a very charming sector in science and technology in near future.

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Molecularly Imprinted Polymer—Carbon Dot Composites for Biomedical Application

Monika Sobiech and Piotr Luliński

Abstract Carbon dots, a kind of materials discovered nearly two decades ago, have attracted attention due to unique properties such as bright fluorescence emission, facile synthetic ways, high chemical and photostability, low cytotoxicity, good biocompatibility and environmental friendliness. The tunable fluorescence features caused widespread applicability in different scientific fields but mainly in biomedicine. However, the analytical methods that based on carbon dot fluorescence measurements are characterized by insufficient selectivity, weak anti-interference ability and moderate sensitivity. Thus, prior to utilization to highly complex biomedical samples, those materials have to be functionalized. Here, molecularly imprinted polymers are the class of materials, synthesized in the presence of template molecules, that provide sufficient selectivity, high cleanup capabilities as well as satisfactory enrichment potential. In this chapter, the biomedical application of molecularly imprinted polymer-functionalized carbon dots will be presented. The brief characterization of carbon dot synthetic approaches together with summarized overview of imprinting process and its limitations followed by detailed discussion of the current state of the art of the carbon dot molecularly imprinted polymer conjugates for biomedicine will provide insight into the future prospects of those advanced materials.

Keywords Carbon dot · Molecularly imprinted polymer · Biomedical application · Biomolecules · Material characterization

1 Introduction

Molecular imprinting technology enables production of materials called molecularly imprinting polymers (MIPs) with unique, desirable properties of specific recognition of target molecules or similar objects. MIPs are paying researchers' attention

M. Sobiech · P. Luliński (✉)
Department of Organic Chemistry, Faculty of Pharmacy,
Medical University of Warsaw, Banacha 1, 02-097 Warsaw, Poland
e-mail: piotr.lulinski@wum.edu.pl

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due to high selectivity and specificity toward the chosen analytes. Additionally, the MIPs are characterized by satisfactory stability and resistance to chemicals and mechanical treatment as well as simpler and less expansive preparation process when compared to the biological selectively recognized systems such as enzymes or antibodies [1]. Described advantageous qualities of MIPs paved the way for application in various fields including: extraction [2], chromatography [3] and electrophoresis [4] as stationary phases, different types of sensors (electrochemical, optical, mass-sensitive, etc.) as recognizing elements [5], drug delivery systems [6], catalysis [7], synthesis [8] (Fig. 1).

The wide spectrum of MIPs applications imposes defined structural format of the material suitable to the chosen purpose from monoliths and membranes to beads and films. The most common way of MIPs synthesis is bulk polymerization as a simple and low-cost method. However, obtained product possesses some drawbacks such as the template leakage, mass transfer limitation and wide range of particle size [9]. To overcome the problems, the production of nano-sized MIPs that enables their application in biomedical areas such as diagnostics, imaging and drug delivery is an alternative. Among nano-sized MIPs, the core–shell nanoparticles can be distinguished. The above-mentioned MIP form includes the imprinted shell and the core built from different kinds of materials that could affect MIP core–shell optical, magnetic, electronic or mechanical properties.

One of the core materials utilized for MIPs core–shell production is carbon dots (CDs). Due to their optical properties, CDs enable to execute fluorescence measurements. The advantages of the fluorescence analysis such as cost-effectiveness,

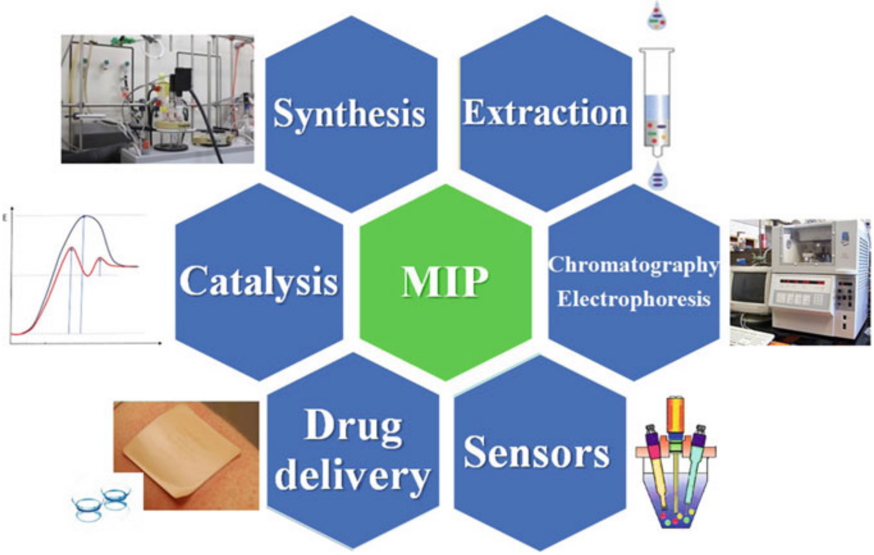

Fig. 1 Application of MIPs

simplicity and satisfactory sensitivity made this instrumental technique highly attractive. Nevertheless, the direct fluorescence analysis of target analytes is not frequently proceeded because of the limited group of compounds, possessing fluorescence properties. For that reason, CDs could be a valuable alternative. Additionally, CDs possess satisfactory chemical stability and controllable optical properties. The designability and functionability of the fluorescent CDs resulted with unprecedented biomedical applicability. However, taking into account biomedical applications of such devices, the complicated sample matrix could affect the measurements. Thus, the fabrication of materials that possess ability to reduce the sample matrix effect or to enhance the cleanup capabilities is highly required. Here, merging CDs with MIPs could result with selective and sensitive fluorescence probes that can be utilized in the biomedical analysis.

In this chapter, we will focus on CDs synthesis and functionalization processes with the use of specific precursors and we will highlight principles and limitations of MIPs prior to discussion of recent advances in the fabrication and the biomedical application of CD–MIPs conjugates. Finally, the current limits and future prospects for the CD–MIPs conjugates will be pointed out.

2 Carbon Dot (CD)—Synthetic Approaches

The CDs are new fascinating zero-dimensional carbon-built nanomaterials with the size less than 20 nm [11, 12]. The structure of CDs is composed of the core, consisting of the sp^2-hybridized carbon embedded in the sp^3 carbon matrix or graphene nanosheets consisting of stabilized shell of functionalized groups such as carboxyl, hydroxyl, amine, ether, carbonyl or polymeric aggregates [13, 14]. The core provides fluorescence properties of CDs, and the shell is responsible for control of optical properties, water solubility, biocompatibility and the capability for compositing with other materials [14–16]. The term of CDs ranges four carbon nanostructures: carbon quantum dots (CQDs), carbon nanodots (CNDs), graphene quantum dots (GQDs) and carbon polymeric nanodots (CPDs) which are classified according to the crystal core structure, properties and surface groups. The CQDs are spherical nanoparticles composed of the multilayered crystalline graphitic structures with chemical groups on the surface, the CNDs do not possess crystal lattice structure, the core of GQDs is built from small graphene sheets, and the CPDs possess polymer/carbon hybrid structure including multiple functional groups and/or polymer chains on the surface and a carbon core [14, 15, 17].

The discovery of CDs in 2000 was related to the observation of green luminescence of polymer-bound carbon nanotubes in the solution [18]. The phenomenon was explained, concerning the presence of luminescent impurities or small aromatic species from the solubilization reactions. The first synthesis of CDs was performed accidentally in 2004 during the purification process of single-walled carbon nanotubes derived from arc-discharge soot [19]. However, the term of CDs was used for the first time in 2006 by Sun et al. [20] during the studies that aimed to explain the impact of the surface passivation on the luminescence properties of CDs. Ever since, there has been growing interest in the preparation of fluorescent CDs as an attraction for toxic quantum dots because of desirable properties of CDs, including superior optical properties (e.g., strong absorption, bright photoluminescence, excellent light stability and resistance to light bleaching) [21, 22], low toxicity [23, 24], eco-friendliness [25, 26], good biocompatibility [27, 28], high water solubility [29, 30], easy synthesis [31], and functionalization processes [32, 33] or tunable fluorescence and quantum yield (QY) [34, 35]. The syntheses of CDs were performed, showing wide spectrum of possible CDs application fields such as bioimaging [36, 37], disease therapy [38, 39], gene and drug delivery [40, 41], energy conversion (e.g., light-emitting diodes) [42], photovoltaic solar cells [43], sensing [44, 45] and catalysis [46, 47] (Fig. 2).

Taking into account the kind of starting carbon source, the synthetic strategies of CDs could be divided into two main types, so-called top-down and bottom-up. In the "top-down" strategy, a large-sized carbon material is destroyed or dispersed into nano-sized CDs by chemical or physical treatment. On the other hand, in the "bottom-up" method, the small molecular structures are converted into CDs, mainly by the chemical reaction [33, 48, 49].

During the CDs formation by the "top-down" method, the bulky or sheet carbon sources such as graphene [50], graphene oxide [51], carbon soot [52], carbon fibers [53], carbon nanotubes [54], activated carbon [55], graphite [56], oxidized graphite [57], carbohydrates [58], fullerene [59] and coal [60] are breaking down, using mainly arc discharge, laser ablation/passivation, chemical oxidation or electrochemical methods [33, 61, 62]. In the green synthesis as carbon precursor candle, tire or natural gas soot could be used as well [44].

Fig. 2 Application of CDs

In the first report that documented the synthesis of CDs in 2004, the **arc-discharge** method was applied. Xu et al. [19] obtained water-soluble, green–blue, yellow and orange fluorescent CDs from arc-discharged single-walled carbon nanotube soot. Unfortunately, the product possessed wide size distribution. In another example, Dey et al. [63] prepared undoped and B-doped GQDs from graphite in two-step process. Firstly, gas-phase arc-discharge method with carbon and boron sources was applied followed by the chemical cutting. Resultant particles had average diameter of 5–6 nm and strong blue emission related to the excitation wave. The B-doped CDs were prepared because the heteroatom doping is a preferable way to improve the performance of CDs.

In the pioneering study in which the name "carbon dots" in 2006 was used for the first time, Sun et al. [20] obtained CDs from appropriately prepared mixture of graphite powder and cement via **laser ablation** method. In the presence of water vapor in an argon atmosphere and acidic treatment, the CDs were produced prior to passivation by organic and polymeric agents. Resultant products had the average diameter of about 5 nm and the QY between 4 and 10% that depended on the effectiveness of passivation process. Yu et al. [64] formed CDs by laser irradiation with the use of toluene as the carbon source. Nguyen et al. [65] applied double-pulse femtosecond laser ablation for synthesis of ultra-small CDs from graphite powder dispersed in ethanol. The size of CDs varied between 0.5 and 2.0 nm, and it was smaller than the size of CDs obtained by single-pulse ablation.

The **chemical oxidation** method treats the carbon material with the use of strong oxidants such as nitric and sulphuric acids, potassium permanganate or hydrogen peroxide. This easy, fast and repeatable technique gives the possibility of large-scale production and enables to introduce hydrophilic groups (such as carboxyl or hydroxyl) on the surface [33, 49]. Liu et al. [66] applied acidic oxidation during the synthesis of multicolor fluorescent CDs from candle soot. Obtained products were small with the size under 2 nm and were characterized by water solubility but possessed relatively low QY value of 0.8 or 1.9%. In another example, Zhou et al. [59] produced yellow-emissive CDs from fullerene soot with the use of oxidizing mixture of nitric and sulphuric acids. The structures had a diameter of 2–3 nm. The QY of the co-produced CDs was in the range of 3–5% which is relatively satisfactory value, considering the CDs obtained by "top-down" methods. Coal was used as a carbon source to produce CDs by "top-down" method which combined carbonization and acidic oxidation techniques [67]. The size and QY value of resultant CDs were dependent on the carbonization temperature and varied between 2 and 3.1 nm and 0.55 and 1.1%, respectively. After the CDs reduction, the QY increased to 8.8%. The product was successfully applied for Cu^{2+} ion sensing in water samples.

The **electrochemical method** is a simple operation carried out under the normal pressure and in room temperature. Together with readily available setup, this method is the most frequently used among "top-down" approaches. Zhou et al. [68] demonstrated the first electrochemical synthesis of blue fluorescent CDs from multi-walled carbon nanotubes that covered carbon paper. Obtained product had the size of about 2.8 nm and QY of 6.4%. Li et al. [69] prepared CDs from graphite rods with the use of aqueous salt solution as electrolyte. The addition of salt to distilled water used as electrolyte shortened operation time from 7 days to 20 min only, giving the product with comparable size (1–3 nm) and easy access for the scale-up production. In another example, the GQDs were produced from graphite rods with the use of natrium hydroxide and citric acid mixture as the electrolyte in the electrochemical exfoliation process [70]. The average size of blue to green fluorescent GQDs was ranged between 2 and 3 nm. Other methods that could be used during cutting process of carbon materials into CDs include: nanolithography [71], hydrothermal [72], solvothermal [73], microwave-assisted [74], sonication-assisted [75] or photo-fenton reaction [76, 77]. The "top-down" methods were applied preferably in the early stage of CDs investigations. However, the high cost of carbon sources, harsh reaction condition, long reaction time, uncontrollable CDs parameters (size and optical properties), sophisticated equipment, complicated post-processing treatment and low QY hampered the application of "top-down" methods for CDs production [32, 62, 77, 78].

In the "bottom-up" strategy, small molecular or oligomeric carbon precursors are aggregated in four-step synthetic process which includes: condensation, polymerization, carbonization and passivation. The small precursors are condensed to form intermediate polymeric chain prior to aggregating using covalent or non-covalent interactions. In the next step, the polymers are carbonized in high temperature to create CDs core. Finally, the surface passivation process is performed to improve CDs optical properties [44, 79].

The most common method used during CDs synthesis is **hydrothermal/solvothermal** approach. It is a simple, low-cost, eco-friendly strategy with non-complicated setup. Additionally, resultant products are uniform in size and are characterized by high QY [49, 62, 80]. During hydrothermal/solvothermal process, a carbon source is dissolved in an appropriate solvent and heat up to the temperature between 100 and 200 °C in autoclave, forming nano-sized CDs [13]. This method was firstly applied for CDs synthesis in 2010. Zhang et al. [81] obtained CDs with the size of 2 nm and QY of 6.75% from L-ascorbic acid as a carbon source. Carbon sources used in the hydrothermal procedure could provide other elements such as N or S atoms that are doped to regulate fluorescence behavior of CDs. Guo and Zhao [82], in one-pot synthesis, prepared blue-emissive N-doped CDs with a size of about 3 nm and a high QY equal to 84.8%. Citric acid was applied as a carbon source and diethylenetriamine, as a source of nitrogen. Resultant product was successfully utilized to detect ellagic acid in urine samples. Yu et al. [83] used dopamine as carbon precursor to synthesize CDs with the size of 3–5 nm and QY of 6.4%, which showed broad emission spectra. The material was applied for the determination of Fe^{3+} ions in real water samples and dopamine in human urine and serum samples. In the CDs hydrothermal synthesis, different organic small molecules such as glucosamine [84], glucose [85], sucrose [86], ampicillin [87], amino acids: serine, histidine and cysteine [88], tryptophan [89], alanine [90], lysine and glutathione [91], folic acid [92], glycerol [93], organic acids and their salts: succinic acid [93], maleic acid [94], perfluorooctanoic sulfonate [95], phenols: catechol [96], p-hydroquinone [97], hexadecylpyridinium chloride [98], carbamaldehyde [99], ethanediamine [100] as well as the polymeric compounds (hyaluronic acid [101] and polyacrylic acid [102]) could be applied as carbon precursors. In order to make CDs synthetic process environmentally benign, the renewable natural products are used in hydrothermal/solvothermal method, as carbon sources, including tragacanth [103], gardenia fruit [104], lemon and grape fruits [105], Seville orange [106], fungi of *Thelephora ganbajun* [107], seeds of *Lens culinaris* [108], seeds of *Azadirachta indica* [109], seeds of *Nigella sativa* [110], leaves of grass [111], ginkgo [112] or guava [113], *Hibiscus sabdariffa* leaves [114], wheat straw [115], flowers of *Magnolia liliiflora* [116], water hyacinth [117], potatoes [118], green alga of *Dunaliella salina* [119], fungi of *Cryptococcus* [120], chocolate [121], cornflour [122], apple juice [123], chitosan [124], human urine [125], chicken blood [126] and others [44, 61, 79, 127, 128].

The general rules and types of carbon precursors that are applied in **microwave-assisted** "bottom-up" CDs synthesis are similar to the hydrothermal method, but heating process is replaced by the microwave irradiation. This method is popular according to cleanness, easiness, short time of reaction and low cost. Zhou et al. [129] prepared fluorescent CDs via 2–10-min microwave pyrolysis of polyethylene glycol and saccharide solution. The QY of resultant product ranged from 3.1 to 6.3%, and the diameter was from 2.65 to 3.75 nm. Recently, Pajewska-Szmyt et al. [130] synthesized N- and S-doped CDs from citric acid and glutathione or urea with microwave-assisted method set for 5 min. The average diameter and QY of CDs was equal to 14.6 nm and 26%, respectively. The product was used for the mercury ion detection in river and wastewater samples. Fast (3 min only) microwave-assisted method was developed for preparing CDs with strong solid-state fluorescence, using phthalic acid and piperazine as precursors [131]. The powdered CDs with the diameter of 1.5 nm were characterized by high QY equal to 48.7% and emission of bright yellow-green solid-state fluorescence. The CDs were successfully used in rapid latent fingerprint detection. In the microwave-assisted methods, natural renewable carbon sources such as linter [132], kelp [133], eggshells [134], sewage sludge [135], human fingernails [136] and others [44, 61, 79, 127, 128] were also utilized.

Another group of the "bottom-up" methods for CDs production is the **thermal** routes such as thermolysis/pyrolysis/carbonization or combustion. Thermal pyrolysis route of CDs obtained was firstly described by Bourlinos et al. [137] who used ammonium citrate salts and 4-aminoantipyrine as the carbon source. Fabricated CDs were characterized by QY of 3% and size of 5–9 nm. Carbonization process is oftenly employed in the CDs synthetic process with the use of natural products as carbon sources. Sha et al. [138] described the CDs synthesis by a single-step pyrolytic treatment of chia seeds. The CDs had the size of 2–6 nm and were applied to form platinum nanoflower CDs composite. In the next example, the photoluminescent carbon nanoparticles were prepared by a simple confined combustion of aromatic compounds (benzene, toluene, xylene) or their mixture in air [139]. The size and QY were 50 nm and 12–13%, respectively.

Apart from the "bottom-up" synthetic methods that were mentioned above, other examples include: ultrasound-assisted method [140], anchor/supported methods [141], microplasma process [142] or fullerene cage opening [143]. The "bottom-up" approach is usually low cost and efficient, the operation is simple, and the conditions are easily controllable, giving the possibility for producing fluorescent CDs on a large scale. Those materials are characterized by excellent optical properties and high QY—the properties highly required for the production of novel materials for biomedical applications [32, 72].

The pristine CDs do not have any specific groups which can interact with the analytes. Thus, the detection process is not sensitive in the presence of interferences. Moreover, the emission of only blue fluorescence, low QYs (less than 5%) and unsatisfactory interactions with biological systems hamper potential application of CDs. Thus, the functionalization of CDs is a powerful tool to improve photophysical and photochemical properties of CDs as well as broaden their application potential [12, 144]. Two main approaches of CDs functionalization include heteroatom doping and surface modification [12]. Heteroatom doping is achieved through the application of appropriate synthetic method and precursors during CDs fabrication. This process modifies inner structure and electron distribution of CDs, resulting in the change of optical, electronic and chemical properties of CDs as well as moderates QY, emission spectra shifting, charge transfer ratio, orbital structures, surface and local chemical features, and others. The CDs doping could be realized using nonmetals and metals [12, 145]. The most often nonmetal-doping includes N- [146], S- [147], P- [148], Si- [149], B- [150], F- [151], Cl- [152], I- [153] and Te-doping [154]. Additionally, the examples of co-doping were described such as: N,S- [155], N,P- [156], N,B- [157], N,Si- [158], N,Cl- [159], N,F- [160], S,Cl- [161] and N,I-doping [162]. It provides significant improvement of the QY of CDs. Metal atom doping can be used to modulate the band structure of CDs; therefore, moderate color-emitting QY could decay CDs lifetime [12, 35]. Different examples of metal-doping were reported: Au- [163], Cu- [164], Al- [165], Ga- [165], Sn- [166], Zn- [166], Ag- [166], Ca- [167], Mo- [168], Hf- [169], Er- [170], Zn- [171], Mn- [172], Co- [173], K- [174] and Fe-doping [175]. Also, the examples of co-doping of metal and nonmetal were reported such as N,Cu- [176], N,Fe- [177], N,Mg- [178], N,Gd- [179], N,Mn- [180], N,Zn- [181], N,Tb- [182] and S,Gd-doping [183] as well as tri-doping of different atoms: Se,N,Cl- [184], N,S,I- [185], N,S,P- [186], N,Zn,Cu- [187], S,N,F- [188] and B,N,S-doping [189].

The surface modification functionalizes CDs to vary the surface states and to increase the number of active sites. The typical functional ligands include ions, organic molecules, polymers, DNA or proteins. Those could be combined to CDs via covalent or non-covalent interactions with the functional group that is present in the CDs shell. The whole process is more complicated than doping but provides unique properties to CDs as well as significantly improves QY (i.e., surface passivation) [12]. Ions attached to CDs give the new reactive sites in CDs, changing the physical and chemical properties such as catalytic ability as well as could enhance fluorescence properties. Here, metal ions, e.g., Fe^{3+} [190], Cu^{2+} [191], Ca^{2+} [192] or lanthanides [193], could be applied in the surface modification of CDs [12]. Small organic molecules linked to the CDs surface improve the QY, supporting unique properties, enhancing hydrophilic properties and making recognition functional group. Many examples of small molecules modifying CDs surface were reported, including: imidazole [194], 4-aminoantipyrine [195], 3,4-hydroxypyridinone [196], L-cysteine [197], β-cyclodextrin [198], 4-naphthalenyl-3-thiosemicarbazide [199], hexadecyl trimethyl ammonium bromide [200] and triphenylphosphonium [201]. The CDs could be conjugated with biological molecules to create the possibility of CDs application in the field of biomedicine. Biomolecules that were attached to the CDs surface or were conjugated during synthetic process include: ribonuclease A [202], DNA fragments (aptamers) [203] or antibodies [204]. However, the most common surface passivation agents are polymers, enhancing QY dramatically due to the cutoff of other non-emitting ways and amplification of the emission intensity caused by irradiation [205]. Among the polymers commonly used in the passivation process are: polyethylene glycol [206], polyethylene imine [207], poly(diphenylbutadiene) [208], a four-arm star polymer [209], polyvinylpyrrolidone [210], polydopamine [211] and polyacrylic acid [212]. Table 1 summarized exemplary methods of synthesis, precursors and application of CDs.

Here, the MIPs could be considered as attractive candidates for surface-modified agents [213].

3 Molecular Imprinting Process

The MIPs are characterized by the high level of selectivity and specificity because of the presence of specific recognition sites in the polymer network formed by the template-tailored synthesis. In the MIPs synthetic process, three main stages could be determined. Firstly, the prepolymerization structure, often named as the prepolymerization complex, is formed from functional monomers and the template molecules in the presence of appropriate solvent. It is a crucial stage, because it determines creation of specific sites responsible for MIPs selectivity and specificity. This step could be realized by the covalent or non-covalent approaches. Two above-mentioned methodologies differ from the type of interactions formed between the template and monomers. The covalent approach is executed via creation of chemical bonds, and as a result new prepolymerization compound is obtained [214]. In the non-covalently created prepolymerization complex, weak molecular interactions, such as hydrogen bonds, ionic forces, π-type interactions or van der Walls forces, are responsible for stability of the system [215]. In the next step of imprinting process, the polymerization reaction is performed, mostly with the addition of chosen cross-linker and the initiator to fix the prepolymerization structure and to form polymeric matrix with recognition sites and the template bound inside of them. In the last stage, the template is removed from the polymer by appropriate reaction (in covalent approach) or with the use of eluting solution (in the non-covalent approach). As a final product, highly cross-linked polymeric matrix is obtained with three-dimensional recognition sites complementary in terms of the size, shape and chemical functionalities to the template molecule. Preferably, the MIPs are synthesized using the non-covalent approach because of the simplicity and reversible interactions with the template and analytes. In the covalent strategy, the template removal is mostly a time-consuming process carried out as the chemical reaction. Nevertheless, the footprint of template

Table 1 Exemplary methods of synthesis, precursors and application of CDs

	Precursors	Method	QY	Size	Application	References
Top-down methods	Carbon nanotube	Arc discharge	1.6%	–	–	[19]
	Hot-pressed graphite powder and cement	Laser ablation and HNO_3 treatment	4–10%	5 nm	–	[20]
	Toluene	Laser ablation	18%	4 nm	–	[64]
	Graphite	Laser ablation	–	1 nm	–	[65]
	Natural gas soot	Acid treatment	0.4%	5 nm	–	[52]
	Carbon nanotubes and graphite	Acidic oxidation	3–6%	3–4 nm	In vivo imaging	[54]
	Activated carbon	Acidic oxidation	12.6%	4.5 nm	Cell imaging	[55]
	Fullerene carbon soot	Acidic oxidation	3–5%	40–50 nm	–	[59]
	Candle soot	Acidic oxidation	1.9%	1 nm		[66]
	Coal	Carbonization and acidic oxidation	5–30%	2–3 nm	Cu^{2+} detection in water	[67]
	Graphite rods	Electrochemical	–	2 nm	–	[69]
	Polyamide resin	Ultrasonic-assisted	28.3%	3 nm	Light-emitting diode	[75]
Bottom-up methods	Citric acid and phenylalanine	Hydrothermal	–	2–3 nm	Fe^{3+} detection in tap water	[21]
	Piper betel leaf	Hydrothermal	12%	3–6 nm	Cell imaging; writing/drawing on the filter paper	[22]
	Tapioca	Hydrothermal	–	3–4 nm	Adsorption of Pb^{2+} ions from aqueous solution	[26]
	Duck breast	Hydrothermal	38%	2–3 nm	Cell imaging	[27]

(continued)

Table 1 (continued)

	Precursors	Method	QY	Size	Application	References
	Sorbic acid and proline	Hydrothermal	8.5 and 4.5%	2 nm	Cr(VI) detection in water samples; cell imaging	[30]
	Ascorbic acid	Hydrothermal	6%	2 nm	–	[81]
	Citric acid and diethylenetriamine	Hydrothermal	85%	3 nm	Ellagic acid detection in urine	[82]
	Dopamine	Hydrothermal	6.4%	4 nm	Fe^{3+} detection in real water samples; dopamine detection in human urine/serum samples	[83]
	Citric acid, glucosamine, polyethylenimine, urea	Hydrothermal	13%	1–15 nm	Cell imaging and Cu^{2+} detection in drinking water	[84]
	Seville orange	Hydrothermal	13.3%	5 nm	Fe^{3+} detection in tap and ground water	[106]
	N-methyl-1,2-phenylenediamine hydrochloride	Solvothermal	18%	1–2 nm	Lysosome imaging in cells	[24]
	Citric acid, 1-(2-pyridylazo)-2-naphthol	Solvothermal	46.7%	3 nm	Light-emitting diode	[34]
	m-Phenylenediamine, maleic acid	Dehydration and condensation reaction at room temperature	42% (blue) 35% (green)	3 nm (green) 5 nm(blue)	Tetracycline detection in urine; cell imaging; light-emitting diode	[23]
	Glucose	Microwave-assisted	-	2 nm	Photocatalyst	[28]

(continued)

Table 1 (continued)

	Precursors	Method	QY	Size	Application	References
	Polyethylene glycol and saccharides	Microwave-assisted	3.1–6.3%	3–4 nm	–	[129]
	Citric acid, glutathione, thiourea	Microwave-assisted	26%	14 nm	Hg(II) detection in tap water and wastewater	[130]
	Phthalic acid and piperazine	Microwave-assisted	48.7%	1–2 nm	Latent fingerprint detection	[131]
	Waste cotton linter	Microwave-assisted	-	10 nm	Cell imaging	[132]
	Sewage sludge	Microwave-assisted	21.7%	4 nm	p-Nitrophenol detection in river/tap water	[135]
	Albumin	Alkaline hydrolysis	16.8%	3 nm	Cell imaging	[31]
	Citrate salts 4-Aminoantipyrine	Thermolysis Pyrolysis	–	7 nm 5–9 nm	–	[137]
	Glucose	Alkali/acid-assisted ultrasonic treatment	7%	5 nm	–	[140]
	Resols	Silica-supported method	14.7%	1.5–2.5 nm	Cell imaging	[141]
	Citric acid and ethylenediamine	Microplasma-assisted	9.9%	6 nm	–	[142]

molecule is better defined. Historically, it was the pioneering methodology of MIPs obtaining [214].

Despite the advantages derived from the template-tailored synthesis, the heterogeneity in the population of specific adsorption sites on the MIPs surface remains the main problem. The origins of MIPs heterogeneity could be related to the diversity of the functional monomer and cross-linker interactions with the template, while the non-covalent approach is considered. Moreover, additional aspects should be considered in order to identify the factors affecting the recognition behavior of polymers: the conformational stability of the template, the dimerization or self-complexation of templates, and the chemical stability of the template.

In an excellent paper published by Karlsson et al. [216], it was stated that despite being weak, the cross-linker forms interactions with template molecule which becomes a significant factor during the formation of shape-specific recognition sites in the imprinted polymer. It was underlined that the change in the population of template conformations could be driven by the changes in the local microenvironment of the template governed by the presence of the cross-linker molecules. This hypothesis explains the fact that more functionalized cross-linking agents can be successfully used to provide highly specific MIPs. The problem was also investigated by other groups [217–219]. Finally, it should also be pointed out that the polymerization reaction is under kinetic control, making the nature of template complexation of great importance for the recognition process and distribution of binding sites in the resulting MIPs.

The problem of template conformational stability was also discussed by Olsson et al. [220]. The template–template complexation could occur, resulting with the heterogeneity of the MIP because of the population of highly specific binding sites for two molecule complexes in the specific adsorption site. In such a site, the adsorption does not rely only on the interactions with positioned residual functional groups from monomers but also from template–template contacts. Moreover, additional complexes were postulated such as those involving two molecules of templates interacting with one molecule of the functional monomer acting as a bridge between them.

Another reason underlying the formation of the heterogeneous population of adsorption sites was also identified. Martin et al. [221] revealed unexpected selectivity of a propranolol imprinted polymer toward a compound of tamoxifen. The mechanism, explaining the behavior of MIP, was not discussed, but it was concluded that a rigorous screen of MIPs should be conducted in order to determine fitting to their targeted analyte. In another example, Klejn et al. [222] investigated the structural transformations of the template during the imprinting process of 3,3-diindolylmethane, resulting in the MIP, providing high binding capacity toward a structurally related compound, indole-3-methanol. A cascade of free radical reactions promoted the transformation of the template molecule of 3,3-diindolylmethane to indole-3-methanol, the compound that was subsequently present in the prepolymerization system and was imprinted into the resulting polymer matrix. In the consequence, a highly heterogeneous population of adsorption sites was formed. It was concluded that the presence of a free radical initiator and elevated temperature could be responsible for the structural changes of the template. However, it should be underlined that most of the template molecules are stable enough to survive the polymerization conditions notably when the free radical thermal process is carried out.

The main parameter, showing the efficacy of imprinting process, is the imprinting factor (IF). The IF is commonly defined as a ratio of the binding capacity of the template on the MIP to the binding capacity of the template on the reference non-imprinted polymer (NIP). Hence, the conditions of NIP synthesis have to be identical as MIP production with omitting the addition of the template molecule [223].

4 Molecularly Imprinted Carbon Dot Conjugates—Biomedical Application

In the subsequent part of the chapter, the most valuable and interesting papers devoted to CD–MIP conjugates will be discussed, emphasizing the fabrication process of MIP layer as well as the analytical performance of the material and the application in biomedical field. In order to systemize the section, firstly CD conjugated with inorganic MIP shell obtained by ionic mechanism of the reaction will be described followed by the CD–MIP organic materials prepared by the free radical polymerization. Those latter materials were fabricated, for instance, for cell targeting and imaging, making their biomedical purpose highly attractive.

4.1 CD–Inorganic MIP Shell Conjugates

The solgel hydrolysis and condensation approach to fabricate CD–inorganic MIP shell conjugates proceeds in mild and controllable synthetic condition, even at room temperature. The mild polymerization conditions are advantageous during MIP synthesis because elevated temperature could diminish the prepolymerization complex between the template and the functional monomer, affecting the efficacy of the imprinting process. However, the excessive amount of the functional monomer or cross-linker could result with thick MIP layer, deteriorating both, the efficiency and the responsive time of fluorescent probe. In contrary, the insufficient amount of functional monomer and cross-linker leads to decrease of sensitivity and selectivity of the material.

Xu et al. [224] developed a new and sensitive method for fluorescent determination of caffeic acid, using the silane-functionalized CDs coated with MIP. The target analyte of caffeic acid is characterized by unique antioxidant properties responsible for anti-inflammatory, anti-tumor or immunomodulatory actions. Caffeic acid is used in the therapy of leucopenia, thrombocytopenia and hemostasis. Nevertheless, the compound could be also responsible for carcinogenic effects. Thus, the monitoring of caffeic acid levels is necessary in order to make the therapy safe. For that purpose, the new method was proposed. The material was composed of CD synthesized in the presence of 3-aminopropyltriethoxysilane and tetraethoxysilane as well as caffeic acid acting as the template molecule. The post-synthetic process was optimized, aiming to remove entirely the template. The process of template removal is crucial because it results with the formation of spatial geometries on the MIPs surface commonly described as the three-dimensional cavities. Here, different eluents, including methanol, methanol–acetic acid (90:10 v/v), methanol–water (90:10 v/v), ethanol, ethanol–acetic acid (90:10 v/v) and ethanol–water (90:10 v/v), were analyzed to wash out the template effectively. The highest fluorescence of CD–MIP conjugates was revealed when methanol–acetic acid (90:10 v/v) was used as the eluent. It means that the template was efficiently removed, resulting with only limited quenching of fluorescence. The comprehensive characterization of resulted material is compulsory to prove its morphology and structure. Here, transmission electron microscopy revealed the appearance of CD–MIP conjugates as the spherical structures with a mean diameter of about 140 nm. The Fourier transformation infrared spectroscopy could confirm the presence of characteristic vibrations derived from specific bonds in the material, such as absorption peak at 1667 cm^{-1} assigned to the stretching vibration of amide carbonyl group and peak at 1576 cm^{-1}, attributing to secondary amine R-NH-R bending vibration. These two different signals confirmed the formation of amide type of bonding, demonstrating the successful acylation reaction of 3-aminopropyltriethoxysilane. The peaks at 1061 cm^{-1} and at 792 cm^{-1} were ascribed to Si–O–Si asymmetric stretching and Si–O vibrations, and the peak at 1488 cm^{-1} was assigned to the aromatic ring stretching vibration of template molecules of caffeic acid. This latter peak disappeared in the spectrum of the material after the template extraction process, confirming the effectiveness of the template removal step. The X-ray photoelectron spectroscopy is another versatile technique to confirm the structure of conjugates. The elemental composition revealed four peaks at 101.62, 398.65, 283.94 and 531.43 eV, which were assigned to Si2p, N1s, C1s and O1s, respectively. Finally, the most important is the spectroscopic analysis of optical properties of CD–MIPs conjugates. The fluorescence excitation and emission spectra of conjugates revealed symmetric fluorescence emission peak at $\lambda_{em} = 450$ nm obtained after excitation at $\lambda_{ex} = 360$ nm. It indicated that the silica coating at the surface did not restrict the photoluminescence properties of CD since the silica layer was optically transparent and inert. The photoluminescence stability is also a very important parameter that should be taken into account when the material is considered for practical application. Here, the results showed that the relative standard deviation of the material fluorescence intensity changes within six hours was equal to 3.1% only. It confirmed great stability of CD–MIPs conjugates against photobleaching. In order to confirm the specificity of the material, the adsorption of caffeic acid from standard solutions was carried out and the fluorescence quenching was quantitatively determined employing the Stern–Volmer equation. The linear relationship was studied in the concentration range between 0.5 and 200 $\mu mol\ L^{-1}$. The calculated limits of detection and quantification were equal to 0.11 and 0.34 μmol L^{-1}, respectively. The selectivity of the material is another important parameter that should be analyzed before application because it allows to assess cleanup capabilities in the presence of competing components of the complex matrix. Here, various interfering components in plasma, including ions (Na^{+}, Ca^{2+}, Mg^{2+}), hydrocarbons (glucose and galactose), amino acids (glycine and cysteine) and proteins (bovine serum albumin), were investigated to evaluate the practical applicability of proposed biosensor platform for caffeic acid detection. The results confirmed high selectivity of obtained material with very low non-selective adsorption of tested compounds. Finally, in order to prove the utility of new material, the analysis of caffeic acid in human plasma sample was carried out. The recoveries for spiked concentration between 5 and 150 μmol L^{-1} varied from 98.4 to 107.6% with relative standard deviation between 3.6 and 10.2%. It was concluded that the probe was easy to synthesize and simple to operate, merging the virtue of the high sensitivity of CDs and the high selectivity of MIPs.

Shariati et al. [225] constructed CD–MIPs conjugates for determination of phenobarbital. This drug is used in the pharmacotherapy of epilepsy. However, if overdosed, it may cause sedation and hypnosis and may promote symptoms such as shallow breathing, drowsiness, decreased urination and level of consciousness. Thus, the monitoring of the drug levels in plasma during the pharmacotherapy is crucial not only to make therapy effective but also to avoid adverse effects. Authors used pulverized Cedrus as a source of CDs, obtaining the material characterized by relatively high QY of 18.6% when compared to CDs obtained by other ways (QY of ca. 5%). The QYs of the CDs are low when compared to QYs of semiconductor nanocrystals which are characterized by values of ca. 50–60%. Here, the main problem responsible for unsatisfactory QY is related to the presence of surface defects. The material was composed from CDs coated by 3-aminopropyltriethoxysilane and tetraethoxysilane in the presence of phenobarbital template. The transmission electron microscopy and Fourier transformation infrared spectroscopy were employed together with X-ray diffraction and dynamic light scattering. The X-ray diffraction measurements are valuable technique to reveal the crystalline structure of materials. Here, the X-ray diffraction pattern exhibited a broad peak at $2\Theta = 24.7°$, corresponding to the (002) diffraction planes of carbon materials. It indicated the amorphous structure. The data obtained from the dynamic light scattering measurements revealed the average size of CDs equal to 5.5 nm. The zeta potential of CDs was calculated as − 10.5 mV. It was underlined that the negative charge of nanoparticles prevented their accumulation and increased their stability. The adsorption process was optimized in terms of temperature and time of contact. It was found that the fluorescence intensity of the CD–MIP conjugates decreased with increasing temperature. The response time increased with time up to 10 min and then remained almost unchanged. The analytical performance of the sensor was evaluated in the concentration range between 0.4 and 34.5 nmol L^{-1}, and the limit of detection was equal to 0.1 nmol L^{-1}. The selectivity tests were carried out in order to measure the mutual competition of phenobarbital and other compounds (dopamine, tryptamine, tryptophan, cysteine, ascorbic acid, primidone or selected ions). The highest competing capability to phenobarbital was revealed by biogenic amines and primidone, a structural analog of the template compound of phenobarbital. Among tested ions, the interfering capability of K^+ was recognized as the most important. Finally, the applicability of the sensor was confirmed by the analysis of human blood plasma samples. The recoveries of phenobarbital from spiked samples were between 96.5 and 109.5%. In conclusion, it was mentioned that new sensor had advantages of being simple, cost-effective, rapid, independent of organic solvents and easy to use.

Apart from the above solgel method, the reverse microemulsion is another approach to generate the silica-based CD–MIPs conjugates. The reverse microemulsion is an oil-in-water stable micelle system. The oil phase is a continuous phase, and the water phase is inside the reverse micelle formed by the surfactant. Usually, cyclohexane and Triton X-100 are selected as the oil phase and surfactant for the preparation of CD–MIPs conjugates. Thus, the Triton X-100 diffuses and stabilizes water droplets, containing CDs in cyclohexane.

Ensafi et al. [226] used above-mentioned technique to fabricate an optical sensor for selective fluorescent determination of promethazine. This compound is an antihistamine drug that is used to treat allergy symptoms such as rash and itching or to prevent nausea and vomiting. However, the side effects include endocrinal, cardiac and reproductive alterations. The material was synthesized from CDs coated by 3-aminopropyltriethoxysilane and tetraethoxysilane in the presence of promethazine hydrochloride, acting as the template. Additionally to transmission electron microscopy, Fourier transformation infrared spectroscopy and X-ray diffraction analyses, the dynamic light scattering diagrams were studied to characterize the material. The results revealed that the average sizes of CD–MIPs and CDs–NIPs particles possess a diameter of 62.1 and 64.5 nm, respectively. The higher polydispersity indices for CD–MIPs and CD–NIPs conjugate (0.391 and 0.362, respectively) than to CDs (0.189) showed that both conjugates were characterized by the broader size distributions than CDs alone. The obtained material was also characterized by very fast adsorption kinetic and equilibrium time equal to 2 min. The regeneration capability of the sensor was analyzed. The recovery efficiency was remained almost unchanged after five sorption cycles. Therefore, the sensor could be used up to 5 times without loss of its sorption properties. The analytical performance for adsorption of promethazine was analyzed in the range between 2 and 250 μmol L^{-1}, and the limit of detection was calculated at 0.5 μmol L^{-1}. The selectivity tests were carried out toward a group of competitors such as cysteine, lysine, ascorbic acid, tryptamine or histamine and ions (K^+, Mg^{2+}, Ca^{2+}). The interfering role of ions was most significant to affect the adsorption capabilities of new sensor toward promethazine. Finally, the recoveries from promethazine spiked human blood plasma were investigated in order to prove the utility of the material. The recoveries of promethazine from spiked samples were between 96.4 and 102.3%, showing that the proposed method was suitable for detection of promethazine in real samples.

In order to enhance the sorption properties of imprinted layer, Bhogal et al. [227] described the fabrication of CD–MIPs conjugate using N-(2-aminoethyl)-3-aminopropyltrimethoxysilane that acted as the coordinating solvent, providing passivation of the surface of CDs as well as the functional monomer for the polymerization reaction. The presence of tetraethoxysilane ensured cross-linking of the ketoprofen, a template molecule. Ketoprofen is frequently used nonsteroidal anti-inflammatory drug. Prolonged administration of ketoprofen and overdosing could cause intestinal bleeding, ulceration and heart failures. Thus, due to widespread use of the drug, it is important to possess a rapid, simple and efficient method for analysis of ketoprofen. The Fourier transformation infrared spectroscopy was employed to analyze the composition of material. The spectrum of silane-functionalized CDs showed peaks at 1588 and at 1656 cm^{-1} that correspond to the amide N–H and $C = O$ stretching vibrations, respectively. A broad peak at 3276 cm^{-1} was assigned to N–H stretching, confirming the formation of amide group during passivation reaction. The stretching vibration of Si–O–CH at 1080 cm^{-1} and the asymmetric stretch of Si–CH_2 at 747.9 cm^{-1} as well as the bands at 2933 cm^{-1}, corresponding to C–H stretching, were the evidence of the presence of aminoalkyl group derived from N-(2-aminoethyl)-3-aminopropyltrimethoxysilane. The spectra of CD–MIP and CD–NIP conjugates revealed strong peak at 1078 cm^{-1} from Si–O–Si asymmetric stretching and peaks at 755 cm^{-1} and 417 cm^{-1} from Si–O vibrations. The bands at 2922 and 2965 cm^{-1} were assigned to aliphatic C–H stretching, and the bands at 3452 and 1591 cm^{-1} were assigned to N–H stretching, indicating the presence of aminoalkyl groups. The detailed Fourier transformation infrared spectroscopy analysis proved the presence of MIP layer. The fluorescence stability of the sensor was obtained by twelve-repeated detections after every 5 min up to a total of one hour from 1.9 μmol L^{-1} ketoprofen standard solution with relative standard deviation below 4%. The response time of the sensor was set for 5 min. The analytical characterization was as follows: the linear response of the sensor to ketoprofen was performed in the range of 0.039–3.9 μmol L^{-1} with limit of detection equal to 0.01 μmol L^{-1} and limit of quantification equal to 0.33 μmol L^{-1}. To validate the applicability of the sensor, the proposed method was employed for analysis of ketoprofen in real human urine samples. Recovery studies were carried out by spiking the samples with ketoprofen in the concentration range between 0.4 and 2 μmol L^{-1}, revealing the total recoveries between 96 and 104%. In conclusion, it was emphasized that the robust, inert and non-toxic CD–MIP conjugates were fabricated with high specificity and faster adsorption kinetics, enabling for development of a new highly sensitive and selective analytical method for ketoprofen determination in real samples.

The effective imprinted shell layer for the analysis of low molecular weight organic compounds is relatively easy to proceed. In contrast, the imprinting of macromolecular proteins is still challenging: firstly, because those molecules are fragile for usage of organic solvents, and secondly, due to conformational instability of these structures. Wang et al. [228] described the fluorescent nanosensor for determination of ovalbumin, applying imprinting process to form a silane MIP shell that was further conjugated with fluorescein isothiocyanate to enhance the fluorescence signal. Ovalbumin is a glycoprotein present in egg albumen but also in fetal bovine serum and human serum. Transmission and scanning electron microscopies were employed to analyze the surface of materials. The micrographs revealed that the surface of CD–silane nanoparticles was velvety and uniformly dispersed with an average particle size of 70 nm, but the size increased to 90 nm after MIP coating and conjugation with fluorescein isothiocyanate, showing a highly rough imprinting layer. It was underlined that thin imprinted layer benefits from unique advantages such as better accessibility and facile mass transfer of the analyte. It also hinders stereo avoidance for the large target protein to enter MIPs' cavities. The working conditions of the sensor were optimized in terms of pH of adsorption and stability. The acidic pH significantly reduced the emission intensity of fluorescein isothiocyanate because of the dominant formation of neutral and cationic forms responsible for lower intensity fluorescence. However, the amine groups of 3-aminopropyltriethoxysilane at pH value below 6 adopt proton and change the charge to positive, leading to decrease of affinity toward ovalbumin. At pH above 7, the amine groups are in the neutral form, increasing the affinity of the material to ovalbumin. The stability of the nanosensor did not significantly change over 2 h, indicating that the system was characterized by acceptable fluorescence stability. The selectivity tests were carried out toward bovine serum albumin, phycocyanin, lysozyme and bovine hemoglobin. The adsorption of all tested compounds on CD–NIP material was very similar, but nearly double amount of ovalbumin was adsorbed on CD–MIP conjugates when compared to adsorption capacity of other competitors. Finally, the material was placed on the test papers, presenting the obvious color responses to the different concentrations of ovalbumin. The method was applied to human urine spiked with ovalbumin, revealing total recoveries between 92 and 104% with relative standard deviation between 3.3 and 3.9%. In summary, it was stated that the developed strategy was facile and convenient. The sensitive and selective detection of ovalbumin can be displayed on fluorescent test papers by unaided eye observation. Consequently, it should be expected that the work will pave the way for the growing sensing of biologically important macromolecules.

To sum up, the potential of CD–silane-based MIPs conjugates was revealed. The methods for CD surface modification were presented, showing the utility of new devices in the biomedical analyses. However, a few limits should be outlined here. Those are characterized by limited adhesive properties, moderate porosity and difficulties in controlling of the particle size, morphology and monodispersity. The limited number of silane precursors of modifiable chemical functionalities is another disadvantage. The commercially available silane functional monomers are strictly limited to several compounds, providing a scarce choice of functional groups to interact with the template molecules when compared to organic monomers that could be used in the synthesis of MIP layers.

4.2 CD–Polydopamine MIP Shell Conjugates

In order to overcome the above-mentioned problems, a polydopamine, an organic copolymer that is characterized by an excellent biocompatibility, hydrophobicity, biodegradability and the unique adhesive properties, could be considered for the formation of a film on a wide range of substrates such as CDs.

Jalili and Amjadi [229] described a novel fluorescent sensor for determination of 3-nitrotyrosine. The sensor consisted of green-emitting CDs as a signal transducer conjugated to imprinted layer of polydopamine. The synthesis is based on previous observations that a simple immersion of semiconductor nanocrystals in a dopamine alkaline solution leads to spontaneous deposition of polydopamine layer. Here, the deposition was carried out in the presence of 3-nitrotyrosine, a template molecule. 3-Nitrotyrosine could indicate the elevated levels of reactive nitrogen species in cells. Those species lead to damage of cellular components, provoking pathological actions. In proteins, most of nitration reactions appear on a tyrosine aromatic ring which is easily nitrated to 3-nitrotyrosine, a compound characterized by higher acidity than tyrosine, affecting the conformational stability of protein. As a result, an accelerated degradation of modified proteins is observed and higher levels of free 3-nitrotyrosine are detected. The analysis of nitratively altered macromolecules in biological samples attracted attention due to their role in the pathogenesis of various diseases. Higher levels of 3-nitrotyrosine were reported in the pathogenesis of asthma, diabetes or neurological disorders. Here, the analytical performance of the sensor was comprehensively analyzed. The relative intensity after adsorption of 3-nitrotyrosine on CD–MIP polydopamine material increased up to 8 min, reaching equilibrium. Thus, the sensor was characterized by the extraordinary rapid response derived probably from the high porosity of imprinted polydopamine layer. The impact of pH on the effectiveness of the adsorption process was evaluated further, revealing the maximum fluorescence quenching between pH values of 6 and 7. This fact could be explained by the analysis of pK_a values of 3-nitrotyrosine that are equal to 2.2, 7.2 and 9.1, and pH value at the point of zero charged of polydopamine that is equal to 4. Polydopamine is negatively charged at pH values higher than 4, and 3-nitrotyrosine has also a net negative charge above pH 7. Therefore by increasing the pH of standard solution, the repulsion between polydopamine layer and 3-nitrotyrosine would prevent the latter compound from penetration to adsorption sites, resulting with the decrease of fluorescence quenching. The linear range of the regression line was between 0.050 and 1.85 $\mu mol\ L^{-1}$. The limit of detection was equal to 17 $nmol\ L^{-1}$. The relative standard deviation for five replicate determinations of 3-nitrotyrosine at 0.45 $\mu mol\ L^{-1}$ was 3.6%, indicating the good repeatability of the sensor. The stability tests proved that the CDs–MIP polydopamine material was stable for more than two weeks when stored at 4 °C and that the fluorescence intensity was 95.1% of its initial value after three weeks. The selectivity tests revealed that glucose, lactose, uric acid, ascorbic acid, cysteine, glycine and creatinine as well as Na^+, K^+, NH_4^+, Ag^+, Ca^{2+}, Zn^{2+}, Mg^{2+}, Fe^{2+}, Cd^{2+}, Pb^{2+}, Ni^{2+}, Cu^{2+}, Fe^{3+} and Al^{3+} ions did not affected the fluorescence response significantly. The recovery studies were carried out on spiked human serum samples, revealing the values between 95.6 and 101.2% with relative standard deviation in the range of 1.4–3.9% for the concentration range between 0.4 and 1.0 $\mu mol\ L^{-1}$. In summary, it was stated that the sensor showed a bright green emission which was selectively quenched by 3-nitrotyrosine adsorption probably via photoinduced electron transfer mechanism. The sensor was characterized by the comparable sensitivity to immunoassays and revealed increasing sensitivity when compared to selected chromatographic techniques.

Current challenges in biomedical analysis aimed to simplify the analytical process. New analytical methods should be elaborated in order to make them available for complex matrices as well as to facilitate and to make them faster. One of the excellent tools to fulfill above-mentioned demands is to merge CDs–MIPs conjugates with magnetic susceptible materials. Those materials could be easily employed in the dispersive mode of solid phase extraction, resulting with the reduction of time and costs of the sample preparation process. For that purpose, Lv et al. [230] proposed magnetic CDs-based MIP system for fluorescent detection of hemoglobin. The synthetic approach involved hydrothermal method to obtain carbon–magnetite hybrid material. Then, the hemoglobin, the template, was immobilized on the surface of material which was subsequently coated with a polydopamine thin layer. The presence of magnetite core allowed for fast and easy separation of the material from sample environment by the simple application of external magnetic field. The characterization of material included Fourier transformation infrared spectroscopy, X-ray electron-dispersive spectroscopy and vibration sample magnetometry. The elemental analysis revealed the atomic percent of C, O and Fe in the magnetic CD–MIP equal to 45.80%, 34.87% and 18.43%, respectively. The saturation magnetization value of the magnetic CDs was equal to 56.4 emu g^{-1} but decreased to 44.1 emu g^{-1} for magnetic CD–MIP. It means that the magnetic properties of magnetic CD–MIP were decreased due to the additional presence of polydopamine layer. The analytical characterization was carried out, revealing linearity in the range of 0.05–16.0 μmol L^{-1} and calculated limit of detection for hemoglobin equal to 17.3 nmol L^{-1}. The selectivity tests were performed toward the group of competing proteins such as ovalbumin, bovine serum albumin and human serum albumin. The results of adsorption of magnetic CDs–MIP revealed that only halved amounts of competitors were adsorbed when compared to the adsorption of hemoglobin. It means that the sensor was characterized by satisfactory selectivity. The recoveries from spiked bovine urine samples were in the range between 102.0 and 102.7% and from spiked bovine serum between 99.0 and 104.0%. It was concluded that the high selectivity and sensitivity of the material make new method promising for specific recognition and determination of proteins in biological fluids.

4.3 CD–Acrylate-/Methacrylate-Derived MIP Layer

In order to address the limits derived from silane-based MIPs such as moderate porosity and a lack of modifiable chemical functionalities, the organic acrylate- or methacrylate-based functional monomers are attractive alternative. Those compounds possess different functional groups that can interact via various types of non-covalent interactions with the templates, strengthening the stability of the prepolymerization complexes. Unsaturated double bonds from acrylate- or methacrylate-based functional monomers make possible free radical polymerization process after thermal or photo-initiation. The free radical process suffers from randomness and limited scale-up. Moreover, the thermal conditions could destabilize the prepolymerization complex resulting with heterogeneous population of adsorption sites. For that purpose, Li et al. [231] proposed additional component present in the organic imprinted layer, viz. thiolated calix[6]arene to increase the stabilization of prepolymerization complex between methacrylic acids, acting as the functional monomer and L-DOPA, the template. The above-mentioned approach was called as dual recognition strategy, and it was recognized as very effective to provide MIPs for chiral resolution. L-DOPA is a prodrug, frequently used in the treatment of neurological disorders, which possesses ability to cross the blood–brain barrier to increase dopamine concentration. Here, the CDs composite with iridium–gold fluorescent nanoparticles was fabricated followed by addition of thiolated calix[6]arene with L-DOPA. Then, methacrylic acid and N,N'-methylenebisacrylamide were added to form, after polymerization, an organic MIP layer. The transmission electron microscopy, X-ray diffraction, X-ray photoelectron spectroscopy, porosity measurements and inductively coupled plasma atomic emission spectroscopy were used to characterize the material. The X-ray diffraction pattern revealed the peak at 25.8° attributed to the C atom, the diffraction peaks at 37.8°, 43.0°, 62.8° and 76.0° were assigned to the (111), (200), (220) and (311) planes, respectively, of the gold lattice, whereas the diffraction peaks at 40.5°, 47.8° and 69.2° were corresponded to the (111), (200) and (220) planes, respectively, of the iridium lattice. These results indicated that iridium and gold on the surface of CDs were characterized by good crystallinity. The X-ray photoelectron spectrum showed intense peaks assigned to Au (4f2/5) at 84.08 eV and Au (4f2/7) at 87.08 eV, the characteristic peaks for zero valence state of gold atom as well as peak assigned to Ir(4f) at 64.08 eV characteristic for zero valence state of iridium. The mass fractions of gold and iridium, determined by atomic absorption spectroscopy, were equal to 23.4% and 22.8%, respectively. The porosity measurement using Brunauer–Emmett–Teller isotherm revealed the specific surface area of CD–Au/Ir–MIP composite equal to 285.8 m^2 g^{-1} and the total pore volume equal to 0.32 cm^3 g^{-1}. The analytical performance was analyzed in the range between 0.5 and 120 nmol L^{-1}. The limit of detection was set at 0.145 nmol L^{-1}. To study the chiral resolution of the material, the adsorption capabilities of both enantiomers, viz. L-DOPA and D-DOPA, were carried out. The results revealed that the fluorescence intensity of the imprinted material did not change significantly even after using a 100-fold excess D-DOPA. This observation confirmed high ability of MIPs to discriminate enantiomers. It could be explained by the spatial geometry of the specific adsorption site that was imprinted by the specific enantiomer. The selectivity tests were carried out toward selected biomolecules such as ascorbic acid, L-tyrosine, epinephrine, endorphins, phenylethylamine, pyrocatechin and amphetamine as well as ions such as Cl^-, NO_3^-, SO_4^{2-}, Na^+, K^+, Cu^{2+}, Hg^{2+}, Fe^{2+}, Mn^{2+}, Mg^{2+}, Co^{2+}, Zn^{2+}, Fe^{3+}, Ni^{2+}, Al^{3+} and Ca^{2+}, revealing minor impact on the L-DOPA adsorption. It was concluded that the combination of thiolated calix[6]arene and MIP provided more recognition sites for chiral recognition and, thus, effectively eliminated enantiomer interference. The integration of CDs with iridium–gold formed fluorescent nanoparticles that possessed potential to be a versatile sensor. However, the adsorption capacity and stability of the material needed further improvement, and the preparation process was relatively complicated.

Another interesting modification of the composition of CD–MIPs conjugate was presented by Zhao et al. [232] who described the synthesis of silanized CDs based on thermo-sensitive MIP. The fluorescence sensor was dedicated to determine bovine hemoglobin in urine samples. The MIP layer was composed from methacrylic acid acting as the functional monomer, N-isopropylacrylamide, the monomer sensitive to temperature and N,N-methylenebisacrylamide, the cross-linker. Bovine hemoglobin was used as the template. The thermo-sensitive element was introduced into the material to increase the binding capacity and to improve the imprinting efficacy. In the lower temperature, the material was transformed into hydrophilic pellets, making the adsorption of hemoglobin more favorable and therefore increasing the fluorescence signal. The analytical performance was analyzed in the range between 0.31 and 15.5 μmol L^{-1}, revealing the limit of detection at 0.155 μmol L^{-1}. The determination of hemoglobin in real sample revealed the recoveries between 98.6 and 100.5% with the relative standard deviation between 0.85 and 2.6%. It was underlined that one could expect that the material would serve in the future as a promising sensor for special recognition and detection of hemoglobin, and this strategy could also be expended to the detection of other proteins in biological fluids. However, the non-specific adsorption still could be a problem, while the MIP organic hydrophobic layer was formed on the composite.

To overcome the problem, Liu et al. [233] proposed a fabrication of CD-restricted access MIP for selective detection of metronidazole in serum. Metronidazole is an antiprotozoal and antibacterial drug used frequently to treat pelvic inflammatory disease, endocarditis and bacterial vaginosis. The material containing CD–MIP conjugates was composed from acrylamide (the functional monomer), ethylene glycol dimethacrylate (the cross-linker) and glycidyl methacrylate, the monomer, possessing epoxy ring which could be used as the copolymerization functional monomer, providing dual surface configuration. After the polymerization, the epoxy ring is hydrolyzed to obtain hydroxyl groups which makes the materials hydrophilic and eliminates the interference of biological macromolecules. The characterization of material consisted of transmission electron microscopy, Fourier transformation infrared as well as the specific measurement of the water contact angles to prove the hydrophilic character. Those experiments were carried out of CD–MIP material without and with glycidyl methacrylate (after the hydrolysis). The results revealed that the static state contact angle was equal to 102.0° and 73.8°, respectively. It means that the latter material possessed more hydrophilic properties. Such material was ideal for protein exclusion, improving the selectivity of MIP. The selectivity tests toward ornidazole, dimetridazole (the structural analogs of metronidazole) as well as toward chloramphenicol confirmed satisfactory selectiveness. The analytical performance was carried out in the range between 50 and 1200 μg L^{-1} with limit of detection at 17.4 μg L^{-1}. The applicability of material was studied by detecting the content of metronidazole in serum samples. The total recoveries from spiked samples varied between 93.5 and 102.7%, and relative standard deviations were between 1.9 and 3.6%. In conclusion, it was stated that the sensor possessed a capability to detect trace substances in the biological applications. However, the complicated synthetic process of CD-restricted access MIPs limits usage of the method.

Finally, fluorescent probes based on CD–MIP materials were used for cell targeting and imaging. Fang et al. [234] presented a novel fluorescent probe for lysozyme detection and cell imaging. The material was constructed from acrylamide (functional monomer), N-isopropylacrylamide (thermo-sensitive monomer) and N,N-methylenebisacryamide (cross-linker). The most important part of investigation was dedicated to assess the cytotoxicity of novel material since its application required direct contact with living cells. Thus, the MTT assays were carried out. It was revealed that the cell viability was at the level of 80% when the CD–MIP conjugates were applied to HepG-2 cells at test concentrations between 1.56 and 100 mg L^{-1}. It means that the material had a minor cytotoxic effect on HepG-2 cells. In conclusions, it was emphasized that combining the fascinating optical properties of CDs with the favorable selectivity of MIPs, a new type of fluorescent probe for lysozyme detection and cell imaging was constructed for the first time. The material was characterized by low cytotoxicity, providing a new way to detect lysozyme in vitro and to probe intracellular lysozyme in vivo.

To sum up, CDs exhibit unexpected advantages in fluorescence sensing and imaging because those materials are characterized by high chemical stability, biocompatibility and low toxicity. Merging CD with MIPs opens a way to selective adsorption of analytes and targeted cell therapy or imaging. Table 2 summarized biomedical application of CD–MIP conjugates.

Those profits make CD–MIP conjugates attractive materials for the biomedical and clinical applications as well as highly desirable tools in the field of nanomedicine.

Table 2 Exemplary biomedical application of CD–MIP conjugates

MIP type	CD functionalization	Analyte	Sample	LOD	References
Silane-based	Silane	Caffeic acid	Plasma	0.11 μM	[224]
	Silane	Phenobarbital	Plasma	0.1 nmol L^{-1}	[225]
	–	Promethazine hydrochloride	Plasma	0.5 μmol L^{-1}	[226]
	Silane	Ketoprofen	Serum/urine	0.01 μM	[227]
	-	Ovalbumin	Urine	15.4 nM	[228]
Polydopamine	-	3-Nitrotyrosine	Serum	17 nM	[229]
	Fe_3O_4	Bovine hemoglobin	Bovine urine/blood	17.3 nM	[230]
Acrylate/methacrylate	Ir/Au	L-DOPA	Urine/chicken meat	0.145 nmol L^{-1}	[231]
	Silane	Bovine hemoglobin	Urine	0.155 μM	[232]
	–	Metronidazole	Serum	17.4 μg L^{-1}	[233]
	Silane	Lysozyme	Chicken egg	0.55 mg L^{-1}	[234]

5 Current Status and Future Prospects

Carbon dots exhibit unexpected advantages in fluorescence sensing and imaging due to high chemical stability, biocompatibility and low toxicity. Merging carbon dot with molecularly imprinted polymer provides materials capable to selective adsorption of analytes for biomedical purposes because of high selectivity, sufficient cleanup and enrichment abilities of imprinted layer. It also opens a way to targeted cell therapy and tissue imaging. However, a few drawbacks shall be addressed at this point. Firstly, low quantum yields of carbon dots could limit their practical use. Thus, novel synthetic approaches for fabrication of carbon dots shall be developed to reduce surface imperfections and improve quantum yield of those nanoparticles. Secondly, the heterogeneity of imprinted layers shall be minimized in order to improve the selectivity of materials. Finally, the alternative ways to apply carbon dots–molecularly imprinted polymers for biotargeting and tissue imaging could result with progress in tumor identification and cancer therapy. To sum up, above-mentioned profits make carbon dot–molecularly imprinted polymer conjugates attractive materials for the biomedical and clinical application as well as highly desirable tools in the field of nanomedicine.

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Magnetic Polymer Nanocomposites: Manufacturing and Biomedical Applications

Hüsnügül Yilmaz Atay

Abstract Magnetism and magnetic materials have been used in plenty of medical applications for many years. Advances in the synthesis and characterization of magnetic particles, especially nanomagnetic particles, have also helped the use of magnetic biomaterials. The combination of magnetic nanoparticles and a biocompatible material has led to the production of a multifunctional and remotely controlled platform that is useful for a variety of biomedical problems. Biocompatible materials such as magnetite (Fe_3O_4) are among the most widely used biomaterials for different applications, from cell separation and drug delivery to hyperthermia. Apart from that, numerous magnetic materials in bulk as well as nanoparticles have been used for various medical applications. In this section, the current explanation of magnetic nano composites from basic science to the latest innovations is given. Starting with the introduction of magnetism and magnetic materials, characterization of magnetic biomaterials, synthesis techniques, production methods, and application areas were studied. An easy way to understand new techniques emerging in this field is presented to the reader. In addition, more current processes and practices are briefly mentioned.

Keywords Magnetism · Biomaterials · Magnetic nanoparticles · Hyperthermia · Drug delivery · Magnetic bioseparation

1 Introduction

Magnetism is a unique property found in every atom, and it has an important effect on living organisms. If we look at the hemoglobin in our blood, it is seen that it is also an iron complex and is magnetic in nature. Magnetotactic bacteria appear to be perhaps the first living organisms to orient themselves according to the earth's magnetic field. These bacteria contain chains of magnetite particles that are variously aligned. In

H. Yilmaz Atay (✉)
İzmir Katip Çelebi University Department of Materials Science and Engineering Çiğli, İzmir, Turkey
e-mail: husnugul.yilmaz.atay@ikcu.edu.tr

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fact, there is substantial evidence that all living organisms contain magnetic particles and act as magnetic receptors, including animals and humans [1, 2].

Magnetism and magnetic materials have been found to play a powerful role in health and biological applications. We first see these practices as the removal of metal objects found in animals and humans' bodies. Recently, a combination of magnetism is used in materials designed as biomaterials. Examples of these are sophisticated biomedical applications such as cell separation, drug delivery, and magnetic intracellular hyperthermia treatment of cancer. Similarly, purification of bone marrow cells from tumor cells using immuno-magnetic beads is an important method used in clinical therapy [1–4].

When we look at the materials that allow the use of magnetism, we come across composite materials. Especially nano composites are at the forefront in this area. More precisely, the inclusion of magnetism in the development of nano technology has opened up new windows of sophisticated biomedical applications [1].

Materials known as nanocomposites are composites where at least one of the phases has dimensions in the nanometer range (1 nm = 10–9 m). These materials have emerged as viable alternatives to overcome the limitations encountered in microcomposites and monoliths. These materials are considered twenty-first-century materials because they have a combination of design uniqueness and properties not found in conventional composites [5]. Magnetic nanocomposites, on the other hand, emerge as nanocomposite materials, which are increasingly important and developed due to their potential applications in biomedicine, information technology, magnetic resonance imaging, catalysis, telecommunication and environmental improvement [6].

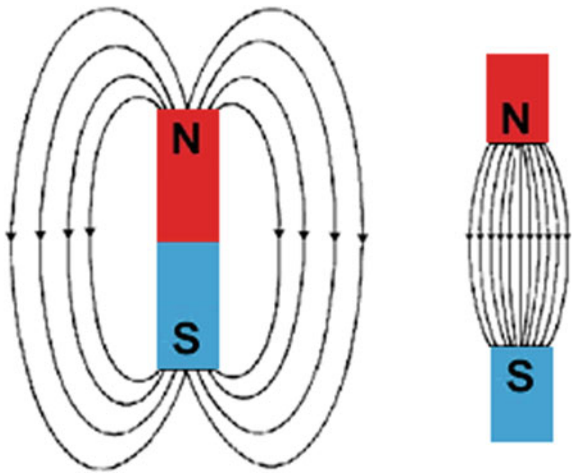
1.1 Magnetism and Magnetic Materials

We have known magnetism since childhood as the phenomenon in which some material pulls and repels other materials from a distance. Examples of such materials include iron, lodestone, and some steels. In general, it can be said that magnetic forces are generated by moving charged particles and this leads to magnetic fields (Fig. 1) [7].

For example, consider a material placed in an external magnetic field. The atoms in this material have an atomic moment that reacts to this outer field. Magnetic dipoles found in magnetic materials can be thought of as dipoles, small north, and south poles bar magnets. Dipoles have a magnetic dipole moment that can react to the external magnetic field. It can be better understood with some field vectors: external magnetic field strength is denoted by H (units A/m), magnetic induction in the material by B (units of tesla), and magnetization by M (units of A/m).). B, H and M associated with (Eq. 1) [7]

$$B = \mu_o(H + M) \tag{1}$$

Fig. 1 Magnetic field lines



where μ_o is the permeability of free space (its magnitude is 1.257×10^{-6} H/m) and M is the magnetic moment m per unit volume of the material. Depending on the type of material and temperature, the value of M may vary. Also, it can be related to the field H through the volumetric magnetic susceptibility χ by the relation (Eq. 2) [7]

$$M = \chi H \tag{2}$$

1.2 *Categories of Magnetic Materials*

There are several types of magnetism that basically depends on the orbital motions of electrons, their rotational motion, and how the electrons interact with each other. The best way to show the different types of magnetism is to describe how materials react to a magnetic field. Although all materials have magnetic properties, the basic distinction between these materials can be explained as follows: Some materials do not have a total atomic magnetic moment interaction, whereas in other materials there is a very strong interaction between their atomic magnetic moments [7].

Accordingly, the magnetic response that occurs in terms of the behavior of atoms causes materials to be classified as diamagnetic, paramagnetic, or ferromagnetic (Fig. 2). Ferromagnetic materials exhibit a long-range magnetic pattern under a certain critical temperature. They are substances such as iron, cobalt, nickel that can be magnetized in the same direction as the magnetic field lines of any magnet while in the magnetic field of that magnet.

Diamagnetic substances are composed of atoms that do not have a net magnetic moment (i.e., orbital shells are all full and no unpaired electrons). Diamagnetism is very weak and not permanent; it continues only as long as there is a magnetic field

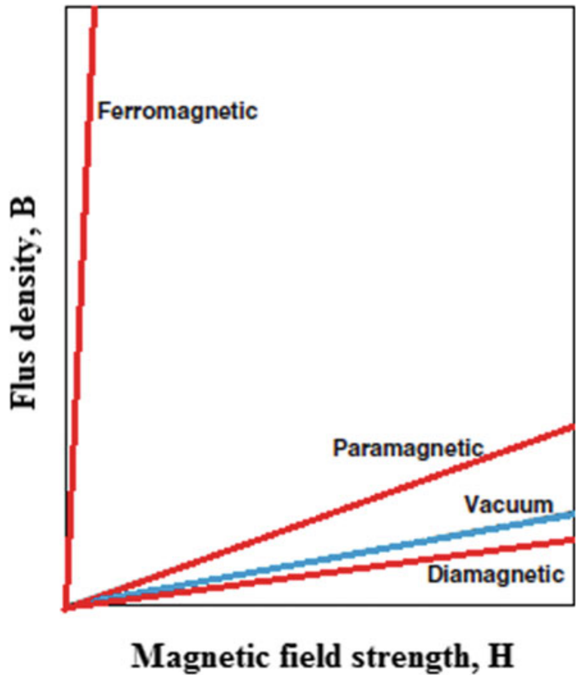


Fig. 2 Schematic of the flux density *B* as a function of *H* for various materials [7, 8]

outside. Occurs due to a change in the orbital motion of electrons due to the external field, the direction of the induced magnetic moment is the opposite of the field. In an inhomogeneous area, such materials are attracted towards areas where the area is weak.

Paramagnetic materials have a net magnetic moment due to the unpaired electrons of some of the ions or atoms in the material in their partially filled orbitals. For this reason, every atom has a permanent dipole moment. When a field is applied, these atomic dipoles tend to align with the discrete field, just like a compass needle aligns with the earth's magnetic field [7].

Diamagnetic and paramagnetic materials show magnetization properties only in the presence of an external field. Because the magnetic induction is very weak in these materials, they have low sensitivity values. Typical sensitivity values for diamagnetic copper at room temperature are -0.96×10^{-5}, for paramagnetic aluminum 2.07×10^{-5}, and paramagnetic manganese sulphate 3.7×10^{-3}[8].

The strongest type of magnetism is ferromagnetism. For example, it occurs in many alloy compositions based on iron (b.c.c.), cobalt, nickel. Ferromagnetic materials can exhibit permanent magnetic moments even in the absence of an external field. However, the property is not seen in dia- and para-magnetic materials. Sensitivity values can reach up to 106, which is very high compared to money and diamagnetic materials. Magnetic moments in such materials are mainly due to atomic spin magnetic moments. In these materials, the interactions between atoms cause the spin magnetic moments to align with each other in a cooperative manner. Because of this,

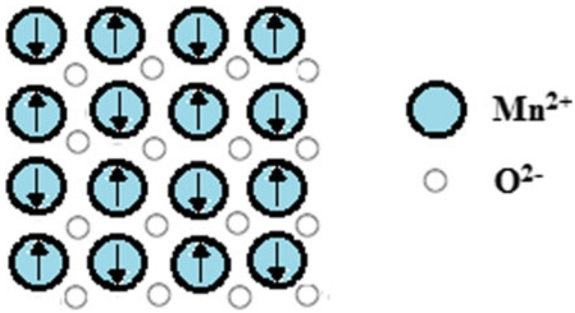


Fig. 3 Schematic of antiparallel alignment of spin magnetic moments in antiferromagnetic MnO [7, 8]

large regions in a crystal can have atoms with rotations aligned with each other. When all magnetic dipoles are aligned, the magnetization reaches its saturation value Ms, for example, the magnitude of Ms for nickel is 5.1 × 105 A/m. [7]

Ferromagnetism results from the cooperatively parallel alignment of the spins. The magnetic moment connection between atoms (or ions) results in the rotational moments of neighboring atoms aligned in opposite directions. Such materials are antiferromagnetic. In the case of MnO, the moments of adjacent Mn^{2+} ions are antiparallel, so the material has no net magnetic moment (Fig. 3).

Hexagonal ferrites and garnet are ceramic materials with ferrimagnetic properties. Cubic ferrites such as magnetite can be represented as MFe_2O_4 where *M* is a metal. In the Fe_3O_4 state, Fe ions exist in both +2 and +3 valence states. The magnetic moments of the two types of Fe ions are different. In this case, however, there is a net magnetic moment because the rotational moments of the solid material as a whole are not completely canceled. Although the rotation moments of Fe^{3+} ions cancel each other out, magnetization occurs due to the parallel alignment of the moments of Fe^{2+} ions (Fig. 4). By adding other ions such as Ni^{2+} and Co^{2+} to Fe_3O_4, it is possible to produce ferrite materials with various magnetic properties. This flexibility can be used to adjust magnetic properties for hyperthermia applications by creating a cubic mixed ferrite material [7, 8].

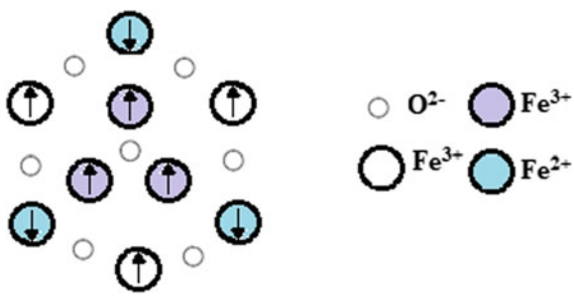


Fig. 4 Schematic depicting the spin magnetic moments for $Fe^{3+}$and Fe^{2+}in Fe_3O_4 [7, 8]

1.3 Fundamentals of Magnetic Nanoparticles

The large number of electrons in a substance are designed to annihilate each other's magnetic moments. Electrons are therefore combined to form pairs according to the Pauli exclusion principle. In this case, one electron absolutely destroys the magnetic moment of the other electron. In addition, even when electron sequences are made and an unpaired electron is seen, the material is deprived of its magnetic properties due to the contribution of various electrons in the solid to the magnetic moment in different directions [9].

The magnetic moments of the atoms in ferromagnetic materials make these materials behave like small permanent magnets. These moments are interconnected and are in a very smooth or irregular orientation called the magnetic domain or Weiss domain. When a domain contains a large number of molecules, it becomes unstable and splits into two domains oriented in opposite directions. Thus, they are connected to each other in a more stable way.

When exposed to a magnetic field, the domain boundaries act to magnify the magnetic field and dominate the structure. Once the magnetic field is removed, the domains may not revert to their de-magnetized state. This situation causes ferromagnetic materials to turn into permanent magnets.

The domain that is magnetized sufficiently powerfully overcomes all other consequences results in a single domain. This substance is magnetically saturated. When a ferromagnetic substance is heated to the Curie temperature, the molecules begin to vibrate and begin to lose the magnetic domain organization and magnetic properties. If this substance is cooled, the orientation of this domain suddenly returns.

When the particle size shrinks to a single domain and is above the hot blocking temperature, the particle becomes superparamagnetic. If the particle is small enough and the temperature is high enough, the thermal energy overcomes the anisotropy energy and directs the moments randomly.

If we reduce the size of any ferromagnet we will eventually reach a size. The resulting thermal energy at this size (kBT ~25 meV at 300 K) and anisotropy will make the magnetization direction random. In other words, such materials present no coercion (HC = 0), similar to paramagnets but behave with very large moments and are called superparamagnets. In practice, the randomization of the magnetization direction occurs by excitations on an energy barrier, $B = KV$, given by the product of the anisotropy constant K and the volume, V [9].

Similar to a paramagnet, the magnetization response M (H) of a superparamagnet can also be explained by the Langevin function. The relaxation time, τ, is exponentially dependent on M. With $D = 2\ R$ where the nanoparticle is split into multiple areas, there is a balance between the additional energy cost of placing the field wall and the reduction-gain in magnetostatic energy.

Considering previous studies, it was seen that the characteristic dimension D in which the single domains are stable was determined by using simple models and mass properties for the domain stability of fine particles found. With $D = 2\ R$ where the nanoparticle is split into multiple regions, M is a balance between the additional energy cost of placing the field wall and the reduction in magnetostatic energy-gain. [9–11].

1.4 Role of Magnetism in Biomaterials

When we look at the usage areas of magnetism, we see that some special biological applications come to the fore. For example, in the sorting of cells, interactions occur between biological cells and magnetic nanoparticles leading to separation under the influence of the magnetic field gradient. The properties of hard and soft magnetic materials have been used for different bio applications. Also, particle size dependent properties are also used in this field. As mentioned above, magnetic properties change significantly when particle size falls beyond a critical limit and moves to single area and subfield regions. It was also mentioned above that it shows superparamagnetic (SP) properties when it falls below a critical size [1]. This entity is widely used for magnetic biodiscrimination, MRI contrast agent and drug delivery. From the point of view of biological applications (eg MRI contrast agent, biological separation, etc.), Superparamagnetic particles have been found to be superior to ferro/ferri magnetic particles due to the absence of remanence. Since the material exhibits magnetic properties in the presence of a magnetic field, it can be removed from the suspension by applying a magnetic field in the biological separation process. After this process is completed, it is redistributed in a homogeneous mixture in the absence of magnetic field [12].

In magnetic hyperthermia, it is beneficial to use the ferro, ferri, and superparamagnetic properties of the particles. The losses from magnetization and reorientation of these particles depend on the type of magnetization process, which is determined by its intrinsic properties such as magnetocrystalline anisotropy, and external properties such as particle size and microstructure. Magnetic hysteresis is an important property of the material. Hysteresis loss is the representation of the energy consumed when rotating a material between positive and negative fields. The area within the second quarter of the cycle is used to determine the energy consumed in a cycle. The delayed power loss of an AC device can be obtained by multiplying the frequency by the hysterical loss per cycle. Power loss can be dissipated as heat for hyperthermia applications. When there is a reduction in size, this magnetic nanoparticle behaves superparamagnetically. These SP particles will not exhibit hysteresis losses. However, the Neel relaxation in them will be equally useful in generating and dissipating heat [1].

2 Magnetic Nanoparticles

2.1 Types of Magnetic Nanoparticles

2.1.1 Metal and Metal Oxide Nanoparticles

Transition metals such as iron (Fe), Ni and Co are among the magnetic materials that have been studied in many studies. When we look at these magnetic metals, we see that they exhibit ferromagnetism at low temperatures (and room temperature). Besides, they show paramagnetism features at high temperatures. In the literature, there are studies on the synthesis and magnetic properties of Fe, Ni, and Co nanoparticles, as well as studies investigating the magnetic behavior of the oxides of these metals.Looking at these materials, it is seen that iron oxide nanoparticles stand out due to their biomedical and industrial applications. Iron oxides are compounds consisting of Fe along with O and/or OH. There are 16 iron oxides available in the form of oxides, hydroxides, or oxide-hydroxides. The three main types of iron oxides are hematite, magnetite, and maghemite. Among these, magnetite has the highest saturation magnetization [6, 13].

2.1.2 Ferrites

Ferrites (α-Fe_2O_3) are ferromagnetic materials derived from metal oxides such as magnetite (Fe_3O_4). They do not show conductive properties. These materials have three different structural symmetries: garnet, hexagonal, and cubic or spinel ferrites. The balancing of the charge and relative amounts of oxygen ions is adjusted according to the size and charge of the metal ions. Various studies have been carried out on the diameter and magnetic fields of ferromagnetic particles. When looked specifically at magnetic nanoparticles, it is seen that superparamagnetic iron oxide nanoparticles (SPIONs) are the most exploited. SPIONs have unique magnetic properties thanks to their atomic composition, crystal structure, and size. SPIONs have the ability to generate heat with the loss mechanism obtained from the rotation of magnetic moments in overcoming the energy barrier. The energy is generated by the relaxation of the MNP moment to the equilibrium orientation (ie Neel relaxation). It was said above that the hysteresis is zero for superparamagnetic materials. But in real SPION ensembles, a hysteresis loop with negligible remanence and coercion occurs. This is probably due to some large particles and agglomerates in the community [14, 15].

2.1.3 Dilute Magnetic Semiconductors

Dilute magnetic semiconductors (DMS) appear as a group of materials with both semiconductor properties and magnetic properties. In these materials, some of the cations in the lattice are replaced by magnetic ions. Atomic spin on these magnetic additives interacts with carriers within the lattice. In this way, a spherical ferromagnetic order is created in the material. Thus, the unusual magnetic properties of these materials are due to the presence of isolated magnetic ions in the semiconductor lattice. Studies on these materials started in the 1980s and still continue. As candidate materials for DMS, we see simple oxides such as SnO_2, ZnO, TiO_2 or mixed oxides doped with various transition metals (Fe, Co, Ni, Mn) or rare earth metals (Dy, Eu, Er) [16–20].

2.1.4 Polymer Magnets

A polymer magnet or plastic magnet is a nonmetallic magnet made from an organic polymer. This is a new class of magnetic materials which has gained the interest of researchers. Torrance et al. [21] synthesized poly(1,3,5-triaminobenzene)which when oxidized with iodine was reported to show a ferromagnetic phase up to 400 °C. After that, Rajca et al. [22] reported synthesis of organic pi-conjugated polymer with very large magnetic moment and magnetic order at low temperatures below 10 K.

In 2004, Zaidi et al. [23] reported the synthesis of a novel magnetic polymer PANiCNQ produced from polyaniline (PANi) and an acceptor molecule, tetracyanoquinodimethane (TCNQ), the first magnetic polymer to function at room temperature. In the structure of PANiCNQ, the fully conjugated nitrogen-containing backbone is combined with molecular charge transfer side groups. This combination creates a stable structure with high density localized spins. Looking at the magnetic measurements, it is seen that this polymer has a curie temperature above 350 K and a maximum saturation magnetization of 0.1 $JT^{-1}\ kg^{-1}$. This indicates that the material is ferri or ferromagnetic. In addition, Crayston et al. [24] have studies examining the synthesis of organic magnets and other developments in the field of organic magnets [6].

2.2 *Synthesis of Magnetic Nanoparticles*

It is possible to encounter the synthesis of magnetic nanoparticles (MNP) in a wide variety of literature. We see different compositions and phases, including iron oxides such as Fe_3O_4 and α-Fe_2O_3, pure metals such as Fe and Co, and spinel ferrites such as $MgFe_2O_4$, $MnFe_2O_4$, $CoFe_2O_4$ and $NiFe_2O_4$, $ZnFe_2O_4$. In addition, alloys such as $CoPt_3$ and FePt, dilute magnetic semiconductors and polymer magnets are also among the topics studied in this sense. Various methods have been tried over the past few years in studies that aim to synthesize magnetic nanoparticles with a stable structure and monodisperse shape control. When we look at the methods studied in this sense, it is seen that methods such as hydrothermal techniques, sol-gel processing, surfactant-assisted synthesis, co-precipitation, microemulsion techniques and solution burning come to the fore for the synthesis of high quality magnetic nanoparticles [25–27].

2.2.1 Hydrothermal Technique

It is known that the solvothermal methods mentioned in the literature are also used for the synthesis of ultrafine powders for the synthesis of MNPs. Generally, this technique relies on reactions taking place in reactors or autoclaves in an aqueous environment. The reactions are carried out at high vapor pressure (usually in the range of 0.3–4 MPa) and at high temperature (usually in the range of 130–250 °C). In fact, this process can also be used to grow single-crystal particles that do not contain dislocations. In this way, grains having a better crystallinity than other methods can be obtained. This is why highly crystalline magnetic nanoparticles can be obtained using the hydrothermal technique [28].

Hydrothermal synthesis of magnetic nanoparticles can be carried out with or without specific surfactants. For example, Wang et al. [29] performed one-step hydrothermal synthesis of highly crystalline magnetite nanoparticles (40 nm) without using surfactants. Togashi et al. [30] performed a surfactant-assisted hydrothermal synthesis of superparamagnetic magnetite nanoparticles (20 nm) at 200 °C in the presence of 3,4-dihydroxyhydroxycinnamic acid. Phumying et al. [31] reported a new hydrothermal method for the synthesis of spinel ferrite MFe_2O_4 (M = Ni, Co, Mn, Mg, Zn) nanocrystalline powders with different morphologies. On the other hand, hydrothermal synthesis of various other magnetic nanoparticles (such as iron oxide nanoparticles, ferrites and dilute magnetic semiconductors) has also been reported by the researchers [6].

2.2.2 Sol–Gel Method

Sol–Gel is in principle a solution chemistry-based technique used to synthesize pure, stoichiometric, and monodispersed oxide nanoparticles (including iron oxide nanoparticles). In this technique, liquid precursors are primarily hydrolyzed, followed by colloidal sols [6]. Metal precursors, metal or metalloid elements are used as starting materials. These are surrounded by a variety of reactive ligands. They undergo slow hydrolysis and polycondensation reactions to form a colloidal system. The name of this colloidal system is Sol. After sol development, a network is formed that includes a liquid phase called a gel. With this method, which is carried out at low temperatures, large-scale nanoparticles with a relatively narrow size distribution can be produced. There are successful studies in which magnetic nanoparticles are synthesized in this way. Examples include synthesis of Er-doped SnO_2 and Ni-doped ZnODMS nanoparticles [32].

We have used the sol–gel technique many times in the production of magnetic nanoparticles as it is relatively easy, inexpensive, and applicable at low temperatures [33, 34]. In one of our studies, barium hexaferrite nanoparticles were produced by the Sol-Gel method. In another study, we produced magnetic ZnO particles with the same method. In another study where we produced Co-doped ZnO nanoparticles, we examined how the amount of Co-doped affects the magnetic properties.

Figure 5 shows the flow chart of the Co-doped ZnO synthesis using Sol-Gel method. The used precursors are zinc acetate dihydrate ($C_4H_6O_4Zn.2H_2O$, Aldrich) and cobalt(II) acetate (CH_3CO_22Co, Aldrich), and a complexing agent is citric acid monohydrate ($C_6H_8O_7$ _H_2O, Aldrich). The following compositions of $Zn_{1-x}Co_xO$, with x¼0, 0.03, 0.06, 0.09, and 0.12 mol, were prepared. Zinc acetate and cobalt acetate were separately dissolved off in distilled water. The solution was mixed by magnetic stirrer until the transparent solution was obtained. The solution was kept at 80_C in the air until wet gel with high viscosity was obtained. The wet gel was treated at 130_C for 12 h in the oven to prepare dry gel. The dry gel was exposed to the sintering process at 500_C for 3 h to evaporate impurities and in the air in an ash furnace (Fig. 5).

Figure 6 shows XRD patterns of Co-doped ZnO powders ($Zn_{1-x}Co_xO$, with x¼0, 0.03, 0.06, 0.09, and 0.12 mol) synthesized by Sol-Gel method. It revealed that all peaks corresponding to (100), (002), (101), (102), (103), (110), and (112) planes related to the hexagonal wurtzite crystal structure the wurtzite structure ZnO with Co-doped concentration up to 12%. There is no sign-related cobalt metal, oxides, or any binary zinc iron phases. All the powders showed peaks similar to pure ZnO, which indicates that no structural deformation occurred in ZnO lattice upon Co-dope. This supports the successful substitutional replacement of Co ions in Zn lattice sites in the ZnO matrix.

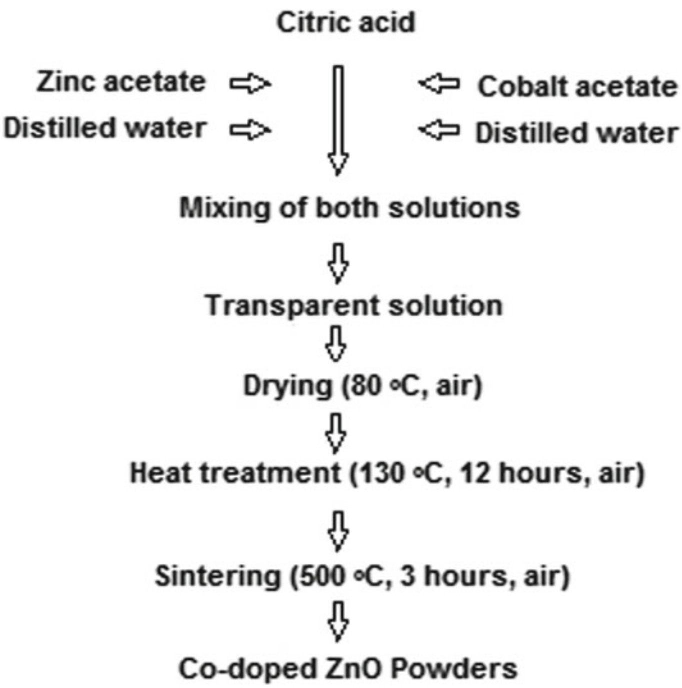


Fig. 5 The flow chart for producing Co-doped ZnO powders [34]

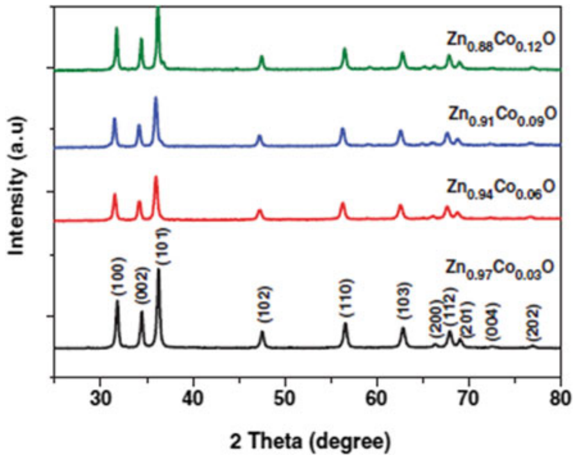


Fig. 6 XRD patterns of Co-doped ZnO powders produced by the Sol-Gel method [34]

The magnetization (M) versus the applied magnetic field (H) for $Zn_{1\_x}Co_xO$ (x¼0–12) powders and different weights of Zn 0.91 Co 0.09 O powders at room temperature are shown in Fig. 7. The magnetic properties of samples were measured by VSM measurements, putting into PTFE Teflon Tape. Room temperature ferromagnetism was observed for all the samples. Co-doped ZnO powders show high saturation magnetization as compared to un-doped ZnO. It is known that pure ZnO shows paramagnetic behavior, Co is the reason for the observed ferromagnetism in the Co-doped ZnO samples. However, in this study, even undoped ZnO powders showed ferromagnetic behavior. This effect comes about owing to the presence of oxygen vacancies that is the critical role in appearance of the ferromagnetic phase has been proved in recent studies. During the calcination process, the presence of carbon ions from precursor substances gives rise to the formation of oxygen vacancies in our samples. In the case of doped diluted magnetic semiconductors, it can be certain that the observed ferromagnetism in the $Zn_{1-x}Co_xO$ powders originate from the Co substitution for Zn in ZnO. Another possible explanation is related to a nanoscale

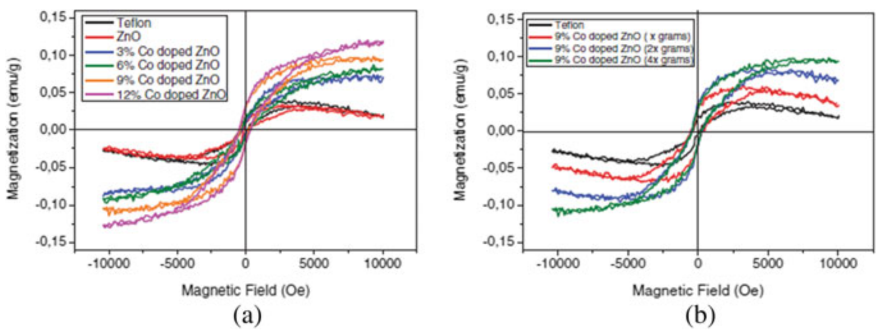


Fig. 7 **a** M–H curves of $Zn_{1\_x}Co_xO$ and **b** different weight of Zn0.91Co0.09O [34]

phase separation responsible for the presence of Co-rich magnetic nanoparticles. Figure 7 shows that with increasing Co content from 0 to 0.12, saturation magnetization was increased from 0.035 to 0.12 emu/g, coercivity Hc is 3.4_104 A/m, and loop area is 18.1_103 Oe_emu/g. The effect of the amount of Co-doped ZnO powders in Teflon composite is shown in Fig. 7. The highest Co content ZnO powders (Zn0.91Co0.09O) were used at different weight ratios in composite to compare the amount effects of these powders. With increasing weight ratios of 9% Co-doped ZnO powders in composite, saturation magnetization increased from 0.05 to 0.1.

2.2.3 Solution Combustion Method

Solution combustion technique (SCT), in other words self-leveling high-temperature synthesis, is a method used in the production of magnetic materials. This method, which is used in the production of the most advanced materials, is very energy efficient. In this technique, a self-sustaining reaction takes place between an oxidizer (eg, metal nitrate) and a fuel (eg, Glycine, hydrazine). First of all, the substances to be reacted are solved in water. Then the solution obtained is thoroughly mixed in order to achieve molecular level homogenization of the reaction medium. After the water is heated to boiling point and evaporated, the solution can ignite, while the temperature rises rapidly (up to 104 °C/s) to 1500 °C. With these self-sustaining reactions, transformation of the initial mixture into the desired fine crystal powders occurs simultaneously [6].

When we look at the studies in this area, Patil and Sureh [35] are the first scientists to publish the instantaneous synthesis of maghemite (γ-Fe_2O_3) by the combustion process. Deshpande et al. [36] and Erri et al. [37] applied the solution combustion approach using various fuels for the direct synthesis of different iron oxide phases (α- and γ-Fe_2O_3 and Fe_3O_4) for the first time in the literature; such as glycine, hydrazine, and citric acid. After that, many studies were carried out on the synthesis of iron oxide nanomaterials and magnetic nanoparticles used for different purposes by solution burning technique [38, 39].

2.2.4 Co-precipitation

Co-precipitation is one of the easiest and most convenient ways to synthesize MNPs (metal oxides and ferrites). With this technique, MNPs are synthesized from aqueous salt solutions by adding a base at room temperature or under an inert atmosphere at high temperature. The size, shape, and composition of these nanoparticles depend on the type of salts used, e.g., chlorides, sulfates, nitrates. In addition, Fe^{2+} /Fe^{3+} ratio, reaction temperature, pH value, and ionic strength also affect these properties [40]. In the chemical reaction below, the reaction of iron oxide nanoparticles (either Fe_3O_4 or γ-Fe_2O_3) and ferrites prepared in an aqueous environment is shown (Eq. 3) [6].

$$M^{2+} + 2Fe^{3+} + 8OH \Rightarrow MFe_2O_4 + 4H_2O \quad (3)$$

The symbol M that appears in this reaction can be elements such as Fe^{2+}, Mn^{2+}, Co^{2+}, Cu^{2+}, Mg^{2+} , Zn^{2+} and Ni^{2+} [41]. Iwasaki et al. [42] synthesized Fe_3O_4nanoparticles by co-precipitation in a new technique he developed. They did not use any additives such as surfactants or oxidizing/reducing agents in this process. They used chilled ball mills as synthesis reaction space. In this way, they both prevented the synthesis reaction and stopped the progress of particle growth due to heat energy. An initial suspension of iron hydroxide and goethite colloids was prepared. The formation of Fe_3O_4nanoparticles was achieved by grinding this suspension in a ball mill. Simultaneously the crystallization was allowed to proceed without heating.

2.2.5 Microemulsion Technique

In the synthesis process, obtaining particles of similar size is an important parameter. Often times it is a difficult feature to obtain. However, it is possible to synthesize similarly sized MNPs with microemulsion method and it is widely used. Basically, this method uses a thermodynamically stable isotropic dispersion of two immiscible liquids. The process begins with the stabilization of the microdomain of surfactant molecules in one or both liquids by an interfacial film. When the surfactant molecule reduces the interface tension between water and oil, a transparent solution occurs. In this solvent mixture, surfactant molecules self-assemble in various structures. These structures are formed in forms such as micelles, bilayers or vesicles. This process takes place depending on relative concentrations.

When we look at the structures most commonly used in nanoparticle synthesis, we come across micelles in reverse (water in oil) or normal (oil in water) form. In both micelles, the monodisperse droplets forming the dispersed phase range in size from 2–100 nm [6].

Thanks to this dispersed phase, a limited environment for the synthesis and formation of nanoparticles can be provided. Lee et al. [43] carried out the large-scale synthesis of uniform magnetite nanoparticles from reaction salts in microemulsion nanoreactors. In addition, these nanoparticles had a highly crystalline structure. In the presence of iron salts, it was possible to vary the relative concentrations of surfactant and solvent. Thus, the particle size could be controlled between 2 and 10 nm.

Barium ferrite ($BaFe_{12}O_{19}$) nanoparticles were synthesized by Pillai et al. Microemulsion process [44]. In this method, aqueous cores of water-cetyltrimethylammonium bromide-n-butanol-octane microemulsions were used as a microreactor. With these cores, which are typically used in 5–25 nm size, the precursor carbonates (typically 5–15 nm in size) were co-precipitated. The precipitated carbonates were dried and calcined to form barium ferrite nanoparticles.

In addition, nickel-zinc ferrite nanoparticles ($Ni_{0.20}$ $Zn_{0.44}$ $Fe_{2.36}$ O_4) were synthesized using reverse micelle process in the study conducted by Morrison et al. In this study, the procedure took place at room temperature without calcination [45]. Liu et al. Obtained $CoFe_2O_4$ nanoparticles using sodium dodecyl sulfate (NaDS) as a surfactant to form normal micelles in their microemulsion method [46].

2.2.6 Other Methods of Synthesis

In addition to the above, there are many other methods for the synthesis of magnetic nanoparticles. Among these, methods such as thermal decomposition, sonochemical, electrochemical, bacterial synthesis, polyol method can be counted. Zhao et al. [47] synthesized water-soluble superparamagnetic Fe_3O_4nanoparticles by thermal decomposition of Fe (acac) 3 in methoxy poly (ethylene glycol) (MPEG). The mean diameter of the particles was around 9.5 ± 1.7 nm. In this study, MPEG functioned as a solvent, reducing agent, and modifying agent. The production of uniform size $CoFe_2O_4$ ferrite nanoparticles was performed by Mazario et al., who synthesized in one step using an electrochemical technique [48]. In a new study, superparamagnetic $ZnFe_2O_4$ nanoparticles with a size range of 28–38 nm were synthesized. In this study, polyol process based on the use of varying chain length glycols (diethylene and polyethylene glycol) as solvent was used [49].

3 Fabrication of Hybrid Magnetic Composites

With the development of sophisticated multi-functional composite materials, hybrid structures consisting of magnetic nanoparticles embedded in a polymer matrix have been produced as magnetic nanocomposites. The good mechanical properties of these materials as well as their magnetic field sensitive behavior have led them to attract attention as a new class of smart materials. There are extensive studies in the literature on the development of high performance magnetic polymer composites [9]. These were applied in a variety of applications in structural materials engineering and biosciences. These studies posed some scientific difficulties in understanding the physics behind the magneto-elastic properties of composite materials. These difficulties are generally due to the cumulative effect of various factors such as the chemistry of the material, nanostructure morphology, and interface interactions [50, 51].

When the performance of magnetic composite materials is examined, it is seen that this depends not only on the microstructure but also on the processing techniques. It is even known that the effect of the micro-environment during certain applications has an effect on the material [9]. In this sense, while the examination of structure–property relationships in magnetic nanoparticle reinforced composites continue, this requires high-level characterization tools.

Elastomers, an important class of soft polymeric materials that exhibit low modulus of elasticity, are an important material used as matrix in composites. Magnetic polymer composite is produced by mixing magnetic nanoparticles with an elastomeric matrix such as a poly (vinyl alcohol) based hydrogel or silicone rubber.

As a result of being under the influence of an external magnetic field, it is possible for this material to show controlled stress, contraction, and bending deformations. It can be performed by adjusting the direction and magnitude of the external magnetic field to control the tensile strength of the composite material, deformation movements, and variations in the compression and shear modulus. The materials developed in this way are ideal materials to be used as dampers in the automotive industry, as rotating tools for machines or as mixing pumps in microfluidic devices.

When viewed as a composite, we generally see two types of magnetic nanoparticle loaded elastomers. The first is an isotropic magnetic polymer composite, in which the magnetic nanoparticle fillers have a uniform spatial distribution. The second contains uniaxial in-line filling nanoparticles, which are anisotropic composites [52]. The production of an anisotropic system can be achieved by using elastomer and magnetic nanoparticles under a uniform magnetic field. Increasing the elastic modulus may be possible by parallelizing the direction and compression force of the magnetically aligned nanoparticle chain. This data has been demonstrated by Filipcsei et al. [52], suggesting that strong mechanical anisotropin can be affected by such incorporation of nanoparticle chains. The spatial distribution of magnetic nanoparticles has an important effect on the stress–strain dependence of composites. A deviation from ideal mechanical behavior may occur depending on the direction of the pressure force. Because the effect of the applied force varies according to whether the force is parallel or perpendicular to the magnetic nanoparticle chain structure [9].

In a similar study, ultra-low magnetic fields were used bu using superparamagnetic iron oxide coated reinforcement particles. In this way, the position and direction of the reinforcing particles in the polymer matrix can be precisely controlled. It is also possible to lead to numerous properties including out-of-plane global or local increase in composite hardness, strength, wear-resistance , and shape memory effects [52].

The full incorporation of magnetic nanoparticles in the polymer matrix is crucial to the performance of the composite. Several well-known polymerization techniques have been optimized to facilitate this process. These techniques used for grafting various polymer brushes on magnetic nanoparticles consist of click chemistry techniques, ring-opening polymerizations, and grafting-polymerization methods using controlled radical polymerization methods.

It is seen that the production of well-defined and dispersed polymer coated magnetic nanoparticles is possible, especially with controlled radical polymerization approach such as reversible addition-fragmentation chain transfer polymerization (RAFT) and atom-transfer radical polymerization (ATRP) [9]. The grafting of polymer brushes on various nanoparticle types have been successfully achieved using RAFT and ATRP methods. These methods are also methods that facilitate the use of a wide variety of polymers such as poly (methyl acrylate), poly (methyl methacrylate), poly (acrylic acid), and poly (poly (acrylic acid) [53].

A sudden end of the polymer branching or polymerization process is not desirable. Through surface-initiated ring-opening polymerization (ROP), both this was avoided and polymer brushes such as poly-ε-caprolactone and poly (lactic acid) were successfully grafted and grown on the surface of magnetic nanoparticles [54].

In addition, in different studies, Cu-catalyzed click chemistry techniques, block copolymers with alkyne termination groups have been used to impregnate magnetic nanoparticles containing azide functionality onto the surface. The creation of higher order hierarchical structures was achieved by coating polymer shells on the surface of magnetic nanoparticles. The seeded emulsion polymerization technique enabled the encapsulation of magnetic nanoparticle clusters in polymer matrices. It is necessary to adjust the concentration of the emulsifier in order to control the size of the emulsion micelle containing monomers and magnetic nanoparticles. Following polymerization of the monomers by the addition of an initiator and a crosslinking agent, crosslinking occurs in a stable polymer shell that locks the magnetic nanoparticle cluster in place. Magnetic nanoclusters produced in this way have been reported to give successful results in optimized MRI contrast enhancement and magnetic hyperthermia applications [55].

On the other hand, magnetic composites made of thermoplastic polymers are also manufactured using various processing methods. Examples include compression molding, melt bonding, solution casting, and melt extrusion. When looking at the manufacture of magnetic thermoplastics, we see that particle agglomeration is a general challenge. To overcome this challenge and prevent aggregation, core–shell magnetic polymer nanoparticles with increased stability have been used [9].

The inclusion of iron oxide nanoparticles in an ultra-high molecular weight polyethylene (UHMWPE) matrix as a platform allowed us to study the effects of inter-particle interaction on the AC magnetic field response of iron oxide nanoparticles.However, the use of UHMWPE as a matrix in composites is extremely difficult due to the difficulties in machining. Because this polymer has an extremely high molecular weight, it is not possible to process it with conventional thermoplastic processing techniques. Also, due to the extremely high viscosity of the polymer, there is a problem in the distribution of magnetic nanoparticle fillers.

It is a known feature that the surface/volume ratio increases with decreasing particle size. This affects the surface properties, interfacial properties, and agglomeration behavior from the perspective of nanoparticles. It is, therefore, necessary to adapt the magnetic nanoparticle surface as well as adjust the interface layer between the particle and the polymer matrix. Because, both the machinability and performance of the composite material depend on these properties.

The use of dispersants in the composite affects the rheology. In this case, the degree of particle agglomeration can also change due to the reduction in inter-particle friction. For example, Bin et al. have used decaline and paraffin as MWCT dispersants in their study where they produced multi-walled carbon nanotube (MWCT) reinforced UHMWPE and achieved high hardness and electrical conductivity in composites [56]. Rong et al. after grafting styrene monomers onto the surface of 7 nm sized SiO_2 nanoparticles, they mixed it with a polypropylene matrix. In their work, they were able to produce cluster-free samples, and the particle-polymer matrix interface interactions were greatly increased [57]. Guoliang et al., in their study, produced composites with better mechanical properties than dry powder direct mechanical dispersion method with liquid–solid mechanical dispersion method [58].

Adapting a liquid–solid mechanical mixing approach, this system was used to produce magnetic UHMWPE composite films with good particle distribution. In this system, blending magnetite nanoparticles with UHMWPE in the presence of an organic solvent dispersant was first achieved by using a high-speed blade mixer. With this method, processing of Al_2O_3 nanoparticle reinforced PEEK polymer and production of carbon nanotube reinforced UHMWPE has been successfully applied. The magnetic composite films were produced by compressing the UHMWPE and iron oxide magnetic powder mixture under 7 metric tons of pressure between PTFE plates and iron steel plates. The mechanical properties of the magnetic polyethylene composite manufactured using the optimized liquid bonding method were compared with the mechanical properties of the composite manufactured using the typical dry mixing method. In this comparison, it was seen that there is a significant increase in elastic modulus with the liquid compounding method. This may be due to improved nanoparticle dispersion in the polyethylene matrix due to the presence of organic solvent during mixing [9].

The study also found that magnetic nanoparticles were superparamagnetic at room temperature even after embedded in the UHMWPE matrix. As a result, it can be said that the morphology and superparamagnetic properties of nanoparticles are preserved in the composite film. This can be stated as an indication that the flexibility of the nanoparticles is subjected to high temperature and pressure conditions during the compression molding stage.

Inclusion of different amounts of magnetic nanoparticles into the UHMWPE matrix is possible by the liquid–solid processing method [59]. Improved heating profile was observed that can be used for magnetic hyperthermia applications. This was accomplished by increasing magnetic nanoparticle loading upon AC field stimulation. However, it was also observed that the elastic modulus and tensile strength of the magnetic polyethylene composite decreased with increasing nanoparticle loading. Overcoming this challenge may be possible by improving polymer-iron oxide nanoparticle interface interactions, for which a hydrothermal carbon coating approach has been applied.

Antiferromagnetic FeO nanoparticles were used as co-reagents in the hydrothermal carbonization of glucose. The reason for this is to create carbon-coated iron oxide nanoparticles. Enhanced magnetic bipolar inter-particle interactions occurred due to the slow oxidation of FeO precursor nanoparticles to ferrimagnetic Fe_3O_4 nanoparticles. This is a situation that facilitates the formation of short iron oxide nanoparticle chain [9]. Finally, after the carbon-coated iron oxide chains were obtained, it was also possible to reveal the improved mechanical properties of the magnetic polymer composite by mixing it into the UHMWPE matrix.

Magnetic polymer composites exhibit superparamagnetic properties at high temperatures. This can be shown to be similar to the behavior of ferrofluids [9]. However, since the positions of the magnetic nanoparticles embedded in the polymer are rigidly fixed, particle movement is also prevented. This is in contrast to magnetic fluids. Therefore, in magnetic polymer composites, Brownian rotation becomes limited as a result of the nanoparticles being held by the polymer network. In this case, the predominant magnetic relaxation mechanism is Néel relaxation. If we assume that the particles do not interact, the magnetic behavior of the superparamagnetic material can be described by the Langevin function [9]. From this point of view, it would not be wrong to say that the magnetic UHMWPE composite can be used to study magnetic relaxation effects that counteract Brownian motions [60].

4 Biomedical Applications

4.1 Magnetic Bioseparation

Bioseparation techniques are becoming more and more important as they are important to the success of many biological processes. Promising among bioseparation techniques, magnetic separation has long been used for applications other than biological separation. Examples of this are separation of magnetic colored impurities from kaolin clay, enrichment of low-grade iron ore, removal of ferromagnetic impurities from boiler water. Although the application of these techniques was limited until 1970, then magnetic separation became useful for some interesting applications in the bioscience and biotechnology fields, especially due to the development of innovative ideas and the improved properties of magnetic materials [61]. So much so that today, we see that the separation technique is regularly used in molecular biology, cell biology, and microbiology. It is also known that the magnetic separation technique has many advantages over other techniques used for the same purpose [1].

Looking at the mechanism of magnetic separation of cells and bio molecules, it appears that it works due to the contrast of magnetic susceptibility between discrete (magnetic) and ambient (other non-magnetic) materials. As mentioned above, some cells or biomolecules have intrinsic magnetic properties. If we classify the magnetic bio-separation method into two modes, in the first case the separator may have sufficient intrinsic magnetic moment, for example, red blood cells and magnetotactic bacteria. In this case, it is possible to separate directly by applying magnetic fields. In the latter case, cells or biomolecules that are not magnetic in nature may be present. In this case, it is possible that these cells and biomolecules can be changed by adding the magnetic-sensitive entity. So it can be manipulated using an external field [1].

Separation of cells or compounds can be accomplished using direct and indirect methods. The process of immobilizing the ligands on magnetic particles and incubating the cells or compounds in the environment for a while is a direct method. The target cells are first bound by these ligands, and then the complex formed can be separated by a magnetic field. On the other hand, we see that in the indirect mode, the target cell initially interacts with the ligand (primary antibody). In the next step, the secondary antibody is immobilized on magnetic particles and added to the medium containing cells. It is suggested that indirect methods work better when antibodies with poor affinity or antigen are less accessible [62]. Finally, separation of the magnetic complex is achieved using a magnetic separator. Since superparamagnetic materials exhibit magnetic properties only in the presence of a magnetic field, magnetic separation of cells or bio molecules can be done more effectively with these materials.

In addition, ferromagnetic and superparamagnetic particles coated (or encapsulated) with polymers or liposomes can be used for magnetic labeling. It is known that magnetite (Fe_3O_4) or hematite (Fe_2O_3) magnetic minerals are widely used as carriers for this purpose [63].

Another application of magnetic separation is to remove cancer cells from bone marrow. Tumor cell separation from peripheral blood was achieved by immobilization of the antibody on silica-coated with superparamagnetic iron oxide [64, 65]. On the other hand, for biological fluid detoxification methods, there is a study in which ferro-carbon particle is used as magnetic absorber. First, the suspension of absorbent particles is injected into an extracorporeal system, and then it is allowed to absorb low, medium, and high molecular weight toxic substances in the bloodstream during movement. Then the magnetic particles are removed with a high gradient magnetic separator and the purified blood returns to the organism [66].

4.2 Drug Delivery

Magnetic drug delivery technique is performed in such a way that the drug can be encapsulated in a magnetic microsphere or nanosphere. This can also be achieved by conjugating the micro/nanosphere to its surface. If the magnetic carrier is administered intravenously, deposition can occur within the area where the magnetic field is applied. In this case, it increases with magnetic agglomeration. Local delivery of the drug can be achieved by the deposition of the carrier in the target area. The efficiency of magnetic carrier deposition depends on physiological parameters. Examples of this include particle size, surface properties, field strength, and blood flow velocity [67, 68].

Site-directed drug targeting is used in local or regional antitumor therapy. One way to increase the effectiveness of chemotherapy treatment is by magnetic-assisted delivery of the cytotoxic agent to the specific site. Multiple magnetic delivery systems can increase drug concentration efficiency at the tumor site [69]. The therapeutic applications of drug targeting, which are still in the research phase, continue in some clinical trials. Results from these studies show that magnetic drug targeting is a promising area. It signals that new and cost-effective clinical protocols will be developed in the near future. Undoubtedly, together with nano-biotechnology, "magnetic drug distribution" will play an important role in improving human health [1].

4.3 Magnetic Resonance Imaging Contrast Agents

One of the most reliable techniques used in diagnostic, clinical medicine and biomedical research is magnetic resonance imaging (MRI). The acquisition of magnetic resonance images is provided by placing the area of interest in a strong and homogeneous static magnetic field. This static magnetic field allows most of the protons to align with the field because there are abundant hydrogen nuclei (single proton) in the body. These protons then move out of alignment due to the application of an alternating magnetic field generated by the radio frequency coil near the sample. The nuclei move from a low energy state to a higher energy state by absorbing energy from the oscillating magnetic field. If the alternating magnetic field is switched off, the nuclei return to equilibrium. In this case, they emit energy at the same frequency as previously absorbed. On the other hand, this phenomenon induces a signal in the coil, which is the source of the alternating magnetic field. This nuclear magnetization that occurs is converted into diagnostic images by a series of algorithms [70].

MRI can provide information that differs from other imaging modalities. Its major technological advantage is that it can characterize and discriminate among organ using their physical and biochemical properties. The ability of MRI techniques to get images in multiple planes offers special advantages for radiation or surgical treatment. Though MRI can provide definite noninvasive diagnoses, the sensitivity or the specificity of such processes can be improved by the addition of contrasting agents. Difference in proton density as well as in the relaxation process of protons in their physiological environment is the source of tNo. contrast. This can be enhanced with the help of contrasting agents. These may be paramagnetic macromolecular compounds, superparamagnetic iron oxide, or rare earth metal ion (Gd) complexes. Paramagnetic metal ions reduce the T1 relaxation of water protons and enhance the signal intensity, hence images are brighter. Superparamagnetic iron particles (SPIO) are more effective than monomolecular or macromolecular Gd contrast agents for this purpose [1].

When we look at the most commonly used superparamagnetic materials in this field, we see Fe_3O_4 with different coatings such as dextrans, polymers and silicon. Due to the superparamagnetic iron particles, a significant shortening occurs in T2 relaxation. This is due to the decrease in signal intensity (SI) that occurs in MR images [71].

4.4 Hyperthermia for Treatment of Cancer

Hyperthermia is basically a form of heat therapy based on the principle that the temperature of the organs is raised to 42–46 C and in this case the viability of cancerous cells is reduced. The basis here is the fact that tumor cells are more sensitive to temperature than normal cells. In hyperthermia, only tumor cells need to be warmed or inactivated, preventing the organs from being affected. For this, it is important to create a heat distribution system. There are various hyperthermia techniques depending on the heating methods used. However, each has advantages and limitations compared to each other. We can say that the boundary effects limit the microwave, ultrasound and RF hyperthermia. The depth penetration of high-frequency microwave rays is poor. In contrast, low-frequency microwaves are difficult to focus on target areas. In the use of ultrasound, its high penetration and focusing capabilities are good. However, in applications, it is seen that it is limited to high reflection due to strong absorption by the bone and air-filtered spaces (lungs, etc.). Also, it has been observed that it is difficult to heat targets with high perfusion areas to the desired temperature using this technique. The reason for this is that there is continuous heat dissipation [1].

We know that magnetic materials are widely used in the hyperthermia of biological organs. This application is based on the principle that the magnetization process determines the magnetic losses. It is possible to distribute these losses in the form of heat, which raises the temperature of the environment. This can be applied depending on the thermal conductivity and heat capacity of the surrounding environment. The losses can be of different types. These types are determined by intrinsic/extrinsic properties and particle sizes. Besides the hysteresis losses, we see that different losses are exploited for hyperthermia; eddy current losses, and relaxation losses for superparamagnetic particles (Neel relaxation) and friction losses of particles (Brownian motions).

In an external AC magnetic field, heating of magnetic oxides with low electrical conductivity can occur if the particles are able to rotate in a sufficiently low viscosity environment. This is either due to loss processes or friction losses during magnetization redirection [72]. The inductive heating of the magnetic oxides is negligible. Particle size and particle microstructure of the powders used greatly affect their magnetic properties. The magnetic reorientation that causes losses in ferro- or ferri-magnetic particles is dependent on the demagnetization process. This is determined by intrinsic magnetic properties such as magneto crystalline anisotropy and magnetization. At the same time, external properties such as particle size and shape have a great influence.

Induction of magnetic hyperthermia can be applied in different ways. One method is based on the surgical placement of finite-sized magnetic implants into the tumor site. In this way, energy is absorbed from the externally applied AC magnetic field and dissipates in the form of heat. Many glass and glass-ceramic materials have been used for this type of research. These bioactive and biocompatible materials form a bond by forming an apatite layer on the surface. However, due to the difficulty of obtaining homogeneous heat distribution with this method, an increase in temperature should be observed in areas close to the implanted material [1].

Instead of needles or rods as heat mediators, an alternative approach has emerged to use fine particles such that hyperthermia becomes noninvasive. It has been observed that when fluids containing magnetic particles with a size of 1–100 nm are injected, these particles can be easily incorporated into cells since their diameters are in the nanometer range. Heating occurs when these magnetic particles bind the AC magnetic field to the targeted magnetic nanoparticles.This process, in which the whole tumor can be heated evenly in this way, is called intracellular hyperthermia. Studies have reported that malignant cells occupy nine times more magnetic nanoparticles than normal cells. Consequently, the heat produced in malignant cells is expected to be higher than in normal cells [1].

As for the magnetic fluids used in Hyperthermia processes, these materials can be defined as liquids composed of ultramicroscopic particles. These particles are stabilized using surfactant to prevent their agglomeration, as well as to make a stable colloidal suspension in suitable media such as water or hydrocarbon. These fluids behave like true homogeneous fluids and are highly sensitive to magnetic fields. Ferrofluids, which are among these magnetic fluids, consists of superparamagnetic particles of Fe_3O_4 and other magnetic particles. It is known that those modified or coated with different types of biopolymers or synthetic polymers are used for hyperthermia applications [73].

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Jackfruit Seed Starch-Based Composite Beads for Controlled Drug Release

Amit Kumar Nayak, Saad Alkahtani, and Md Saquib Hasnain

Abstract Jackfruit (*Artocarpus heterophyllus* Lam.; family: Moraceae) seed starch (JSS) is a natural starch candidate, which is already reported as a potential pharmaceutical biopolymeric raw materials/excipients in various pharmaceutical dosage forms, such as binding agent and disintegrants in pharmaceutical tablets, emulsifier in emulsions, suspending agent in suspensions, and mucoadhesive agent in biomucoadhesive dosage forms. Recent years, JSS has been utilized to prepare controlled drug-releasing composite beads, when processed with other biopolymers like sodium alginate, gellan gum, and low methoxy pectin. All these JSS-based composite beads demonstrated in vitro controlled releasing of encapsulated drugs over a longer time and significant in vivo hypoglycemic actions in the treated alloxan-induced diabetic Albino rats *over prolonged time interval* after administration through oral route. The current chapter presents a comprehensive review of various JSS-based composite beads for controlled sustained releasing of encapsulated drugs.

Keywords Jackfruit seed starch · Composite · Controlled release · Drug delivery

1 Introduction

Development of controlled releasing dosage forms using biocompatible and biodegradable polymeric excipients is a popular trend in the drug delivery research, wherein polymers are being thoughtfully chosen to encapsulate a variety of drugs and also to monitor the releasing of encapsulated drug materials in a controlled manner

A. K. Nayak
Department of Pharmaceutics, Seemanta Institute of Pharmaceutical Sciences, 757086 Mayurbhanj, Odisha, India

S. Alkahtani
Department of Zoology, College of Science, King Saud University, 2455, 11451 Riyadh, Saudi Arabia

M. S. Hasnain (✉)
Department of Pharmacy, Palamau Institute of Pharmacy, Chianki, 822102 Daltonganj, Jharkhand, India

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over a longer period [1–3]. In addition, these systems usually exhibited several benefits over the conventional immediate release dosage systems, such as like reduction of drug concentration fluctuations within the therapeutic range, decreased risk of dose-dumping chances, reduced dosing frequencies, more predictable gastric emptying, minimization of side effect incidence, and enhanced patient compliances. [5–7]. Among various controlled drug delivery systems, multiple-unit oral dosage forms have been researched during last few decades [7–9]. The uses of such multiple-unit oral dosage forms are advantageous than the single-unit-controlled releasing oral dosage forms as these (*i.e.*, multiple units) are capable to distribute within a larger area of the gastrointestinal tract, releasing of drugs in a predictable as well as controllable way to bypass the vagaries of gastric emptying and to decrease the possibilities of dose dumping to reduce localized mucosal damage of gastrointestinal tract [10–12]. In recent years, various multiple-unit oral-controlled drug-releasing systems like composite beads and microparticles containing various plant polysaccharides are being investigated by several research groups [13–35].

In recent years, trends toward the exploration and exploitation of plant source originated materials in various important biomedical applications have been recognized as the most emerging field for research and development in the current years [36–39]. These materials are abundantly available from plant resources, and therefore, renewability is the prime advantage for the uses of these materials [40–43]. Plant polysaccharides are currently recognized as a popular biopolymeric material group with a variety of important physicochemical characteristics like high swelling capability in the aqueous environment, degradation into physiological metabolites, excellent stability in the wider pH alterations, etc. [44–47]. Most of the plant polysaccharides are extracted from leaves, pods, fruits, seeds, rhizomes, roots, exudates, etc. [43, 47]. Therefore, these materials are recognized as low cost materials because of very low production cost and larger availability options for the natural resources [48–51]. Moreover, in the biomedical area, the biodegradation of these plant polysaccharides into physiological metabolites support these to be as effectual biopolymeric candidates for various biomedical uses like drug-delivering systems, wound managements, tissue regeneration applications, etc. [51–54]. Recently, a considerable volume of research attempts have been performed in the drug delivery research field for their utilization as various pharmaceutical excipients, such as emulsifier, suspending agents, disintegrating agents, binding agents, granulating agents, matrix formers, release retardants, enteric coats, film formers, and mucoadhesive agents. [43, 47, 55–70]. Among various plant polysaccharides, starches are being widely studied as pharmaceutical excipients in the formulation of several kinds of pharmaceutical dosage forms [71–82]. Even natural starches are being employed to design many controlled release composite beads/microparticles, when processed with other biocompatible polymers [82–87]. Plant-derived starches researched for this purpose are potato starch [88], rice starch [89–91], jackfruit seed starch (JSS) [92–95], tapioca starch [96], etc. In recent years, JSS has been utilized to prepare controlled drug-releasing composite beads, when processed with various anionic polysaccharides, such as like sodium alginate [92, 93], gellan gum [95], and low methoxy pectin [94]. The current chapter presents a useful discussion on already reported JSS-based composite beads for controlling sustained release of drugs. First part of the current chapter comprises extraction characterization, properties, and pharmaceutical uses of JSS. The final potion includes discussions about various already reported JSS-based composite beads for controlled release of drugs.

2 Jackfruit Seed Starch (JSS)

The jackfruit (*A. heterophyllus* Lam.; family: Moraceae) is a common tropical fruit candidate, generally occurred in India, Malaysia, Bangladesh, China, Vietnam, Indonesia, Philippines, Thailand, and Brazil [97, 98]. By tradition, the *Artocarpus* species is being employed as one of the folk medicinal material in treatment of malarial fevers, worm infections, diarrhea, diabetes, and other disorders [98]. The ripe jackfruit contains mature seeds, which are found to as yellow-colored edible bulbs. Generally, a ripe and mature jackfruit contains 100–500 seeds, which are about 3–5 mm thick. These ripe and mature seeds are separated from horny endocarpus coated within subgelatinous exocarpus—a thin membrane of whitish color. These are of oval and oblong or round shaped [98]. These seeds are consumed in boiled, steamed as well as roasted forms as food materials by the many communities as these contains almost similar compositions as that of food grains [97]. In addition, jackfruit seed flour is being utilized in the preparation of biscuits, breads, sweets, etc. [99]. Jackfruit seeds are nutritious and rich in carbohydrates. It also contains lignans, isoflavones, and some mineral components (like potassium, magnesium, manganese, etc.) [97–100]. The carbohydrates present in the jackfruit seeds mainly possess high amounts of starches [101].

JSS is being extracted from mature and ripe jackfruit seeds and purified. Nayak et al. (2015) reported extraction of JSS from raw jackfruit seeds [45]. In this extraction procedure, 250 g of raw jackfruit seeds were taken. For the removal of superficial cohesive layer, these seeds were cleaned using distilled water. Arils of the superficial coat of these seeds were eliminated by hand, and then, thinner brownish spermoderms were peeled using a solution of 0.6 M potassium hydroxide for a period of 3 h at the room temperature. The pale-white cotyledons were exposed by the alkali treatment and washing of these several times by distilled water to eliminate the alkali traces present on the seed surfaces, completely. Then, these alkali-treated seeds were reduced to small pieces using cutter. These small pieces were wet-grounded by pestle and mortar using distilled water, where seed to water ration was maintained 1:3 to prepare slurry and followed by centrifugation at 3000 rpm for 20 min in room temperature. Centrifuged residue was collected through scraping and resuspended in the solution of 0.5 M sodium thiosulfate maintaining the residue to solution ration of 1:1 for 24 h with a stirring accommodation at regular break for the elimination of protein fractions. Collected filtrated portion was then centrifuged for 5 min with 2000 rpm of centrifugation speed and a brownish layer appeared at the upper portion of the whitish residue, which was scrapped off very cautiously. The whitish residue was made neutralized using 0.1 M hydrochloric acid solution. Subsequently, it is rinsed using plenty of distilled water. The obtained material was further rinsed for two times using 50% of ethanol. The collected extracted substance was then dried for overnight at a temperature of 40 °C. The dried JSS cakes were grounded and then passed through 0.15 mm mesh-size sieve. The extracted JSS was stored within an airtight desiccator. The extracted JSS was subjected to measure various physicochemical characteristics: color, odor, taste, aqueous solubility, pH, and viscosity of aqueous JSS solution. The extracted JSS was found whitish colored, odorless, and tasteless powders. The pH of 1% solution of extracted JSS was determined as 6.22 ± 0.15 at 37 ºC temperature. Extracted JSS was found to produce more aqueous solubility in hot water. However, it exhibited less aqueous solubility in cold water. The viscosity of 1% solution of extracted JSS at 37 ºC temperature and 100 rpm spindle speed was determined as 45.18 ± 1.37 cps. The extracted JSS was subjected to various important phytochemical identification tests, which indicated the presence of carbohydrates. Currently, JSS is recognized as an alternative potential contender material to the conventionally and commercially utilized starches. In recent times, it is being used in various industrial applications including foods and pharmaceuticals [102, 103].

Approximately, 25–35% of amylose content is present in JSS and is also comparable with that of potato starch [104]. JSS displays some important characters relating to physicochemical properties, such as granule size and shape, crystallinity, solubility, viscosity, swellability, and acid resistance, as compared to other conventional starches [103, 104]. Its gelatinization temperature is higher as compared to other conventional starches [105]. Rengsutthi and Charoenrein (2011) studied the morphological structures of extracted JSS granules by using scanning electron microscopy (SEM) [102]. SEM images indicated a range of round-shaped to bell-shaped granules. Average diameter of JSS granules was measured as 10 μm. On the other hand, Bobbio et al., (1978) reported rounded and bell-shaped granules of JSS ranging of average diameter, 7–11 μm [106].

During past few years, JSS is being utilized in the formulations of various pharmaceutical dosage forms as pharmaceutical excipients [105, 107]. Already, it was explored as binding agent in pharmaceutical tablets [108]. Due to highly viscous properties, JSS is also investigated for its suitability as emulsifier and suspending agent in emulsions as well as suspensions, respectively [107]. As mucoadhesive agent, JSS was also utilized to develop biomucoadhesive dosage forms [109]. The cross-linked carboxymethyl JSS was investigated as tablet disintegrants [105]. Recently, it has been utilized as polymer blends with other biocompatible polymers like sodium alginate [92, 93], gellan gum [95], and low methoxy pectin [94] to prepare controlled drug-releasing composite beads.

3 Alginate-JSS Composite Beads for Controlled Drug Release

The usefulness of JSS was tested as polymer blends with alginate to formulate controlled drug-releasing composite beads [92, 93]. Alginates are a group alginic acid salts derived from marine sources, in particular, from few brown marine algae [110, 111]. Alginates are mainly anionic copolymer of α-L-guluronic acid (G) and β-D-mannuronic acid (M) units arranged in the asymmetrical pattern of varying ratios of G-G, M-M, and M-G units linked with 1, 4-glycosidic linkages [112, 113]. Alginates are biocompatible and biodegradable polysaccharide group, which are extensively being utilized in many food applications as emulsifier, stabilizer, thickener, etc. [114–116]. US FDA already has recommended these as one of the GRAS materials [117, 118]. These are also widely employed as biopolymer in several biomedical applications including drug delivery [119–122]. The sodium salt of alginic acid–sodium alginate–has been extensively researched and employed as aqueous soluble biopolymer in numerous formulas of drug delivery dosage forms because of its considerable viscosity in aqueous solutions [123–126]. The important most property of sodium alginate is the ability of ionotropic gelation by the influence of metal cations, such as Ca^{2+}, Ba^{2+}, Zn^{2+}, and Al^{3+} [127–130]. The divalent and/or trivalent metal cations are thought to be incorporated with the electronegative cavities of alginate structure similar to eggs in the so-called "Egg Box model" structuring ionotropically cross-linked alginate hydrogels as a result of the intermolecular interactions in-between-COO^{-} groups positioned in the alginate structure and metal (divalent and/or trivalent) cations [131–133]. Over the decades, numerous ionotropically cross-linked alginate hydrogels have been explored as carrier matrices for drug-releasing applications [4, 55, 128, 133–135]. Nevertheless, the drug-releasing capability of these ionotropically cross-linked alginate hydrogels is linked with some drawbacks like lower drug encapsulation efficiencies due to longer curing time and burst releasing of the encapsulated drugs as a result of speedy degradation in the alkaline intestinal pH [9, 128, 133, 136]. To manage these potential drawbacks, numerous modifications of ionotropically cross-linked alginate hydrogels have been researched by different research groups [4, 137, 138]. Among these modifications, uses of other biopolymers as blends with sodium alginate are being explored to develop various alginate-based composite hydrogel beads for maximizing encapsulations of drugs and sustained-controlled releasing of encapsulated drugs [3, 4, 9, 18, 139–142].

Recently, alginate-JSS composite beads for controlled release of metformin HCl (an oral hypoglycfemic agent, usually given in the management of Type-II or non-insulin-dependent diabetes mellitus) were developed employing polymer-blends solutions of sodium alginate-JSS and calcium chloride (as an ionotropic cross-linking gelation agent) [92]. These alginate-JSS beads of metformin HCl were developed and optimized by using a statistical optimization method based on the 3^2 factorial design (comprising two independent factors with three different levels) and response surface methodology to analyze the influence of sodium alginate-to-JSS ratio in the bead formula and concentrations of calcium chloride on the drug encapsulations and releases. It was noticed that increased drug encapsulation efficiency of these composite beads and reduced cumulative in vitro releases of metformin HCl after 10 h were found with lowering of sodium alginate-to-JSS ratio (increasing JSS contents in polymer-blends) and increasing calcium chloride (ionotropic cross-linking agent) concentrations. From the numerical optimization analyses, the optimal formulation variable settings were selected as sodium alginate-to-JSS ratio of 1.03 and calcium chloride concentration of 10.39%. Employing these optimal formulation variable settings, optimized alginate-JSS composite beads of metformin HCl were formulated. The optimized composite bead formulation exhibited 97.48 ± 3.92% of drug encapsulation efficiency and cumulative in vitro release of metformin HCl of 65.70 ± 2.22% after 10 h of drug-releasing study.

Drug encapsulation efficiencies of alginate-JSS composite beads of metformin HCl were calculated within the range, 69.94 ± 2.18% to 97.48 ± 3.92%. Average diameter of alginate-JSS composite beads of metformin HCl was calculated within a range of 0.89 ± 0.07–1.30 ± 0.11 mm, whereas the optimized composite beads of metformin HCl showed the average diameter of 1.16 ± 0.11 mm. Surface morphology of optimized alginate-JSS composite beads of metformin HCl was examined using scanning electron microscopy (SEM). The photomicrograph of composite beads demonstrated irregular shaped discrete beads (Fig. 1). These beads were free from

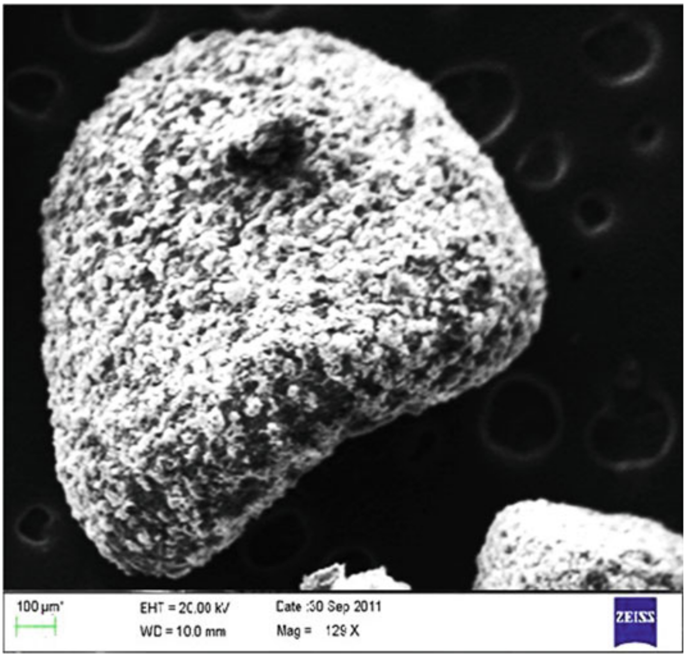

Fig. 1 Photomicrograph of optimized alginate-JSS composite beads of metformin HCl demonstrating irregular shaped discrete beads [92]. (Copyright © 2013 Elsevier B.V.)

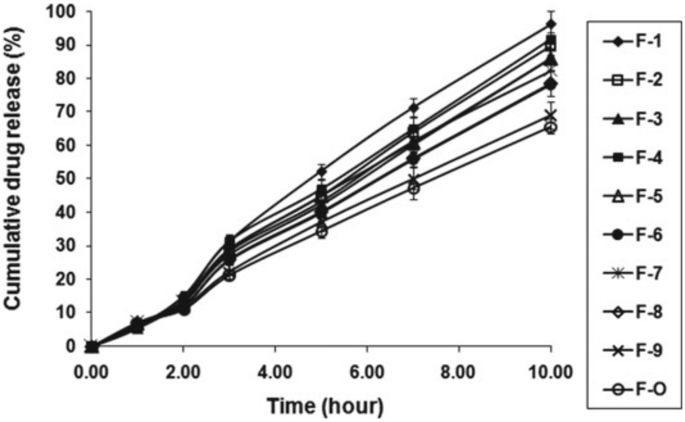

Fig. 2 In vitro releases of metformin HCl from various alginate-JSS composite beads of metformin HCl [Mean ± S.D., $n = 3$] [92]. (Copyright © 2013 Elsevier B.V.)

agglomeration and possessing a rough surface with typical wrinkles and cracks. These beads exhibited significant characters of metformin HCl after encapsulation without any types of drug-excipients interactions, examined in Fourier transform infrared (FTIR) spectroscopic analyses.

In vitro metformin HCl releasing from various alginate-JSS composite beads in gastric pH (1.2) for initial 2 h and next in intestinal pH (7.4) for the remaining release periods revealed a prolonged sustained metformin HCl releasing pattern over 10 h (Fig. 2). Initial metformin HCl releasing in the gastric pH was found not more than 15% after 2 h. In vitro metformin HCl releasing from these composite beads was followed zero-order kinetics pattern along with super case-II transport mechanism. These results can be attributed by the phenomena of matrix-dissolution through the enlargement or relaxation of the polymeric chains. In vitro swelling index of optimized alginate-JSS composite beads of metformin HCl was found primarily lower in gastric pH (1.2) in comparison with that of intestinal pH (7.4) (Fig. 3). In the intestinal pH, an utmost swelling profile of composite beads was detected at 2–3 h. After which, erosion as well as dissolution of the ionotropically gelled alginate-JSS composite bead matrices occurred.

Ex vivo wash off assessments of optimized alginate-JSS composite beads of metformin HCl using goat intestinal mucosal tissue were revealed comparatively quicker in the intestinal pH (alkaline) than that in the gastric pH (acidic). The percentage of composite beads adhered to the mucosal membrane in the acidic pH (1.2) was observed 62.52 ± 4.46% over 8 h, whereas this was found 37.60 ± 3.43% in the intestinal pH (7.4) (Fig. 4). The results of ex vivo wash off of these optimized alginate-JSS composite beads of metformin HCl suggested good mucoadhesivity with the mucosal membrane in the gastric as well as intestinal pHs.

In vivo levels of blood glucose as well as the percentage of average decline of blood glucose after oral administrations of pure metformin HCl and optimized alginate-JSS

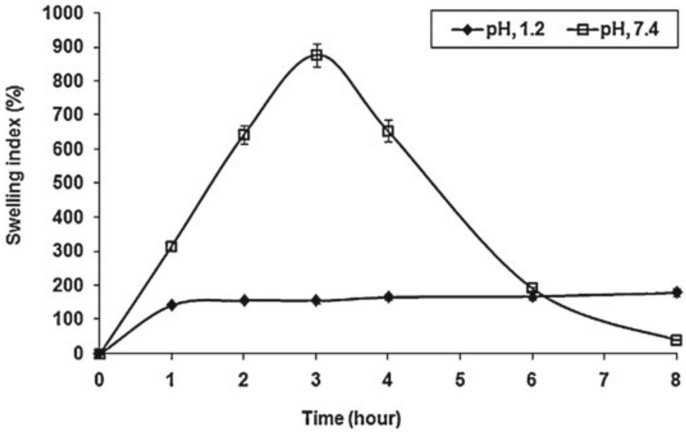

Fig. 3 In vitro swelling behavior of optimized j alginate-JSS composite beads of metformin HCl in gastric pH (1.2) and intestinal pH (7.4) [mean ± S.D., $n = 3$] [92]. (Copyright © 2013 Elsevier B.V.)

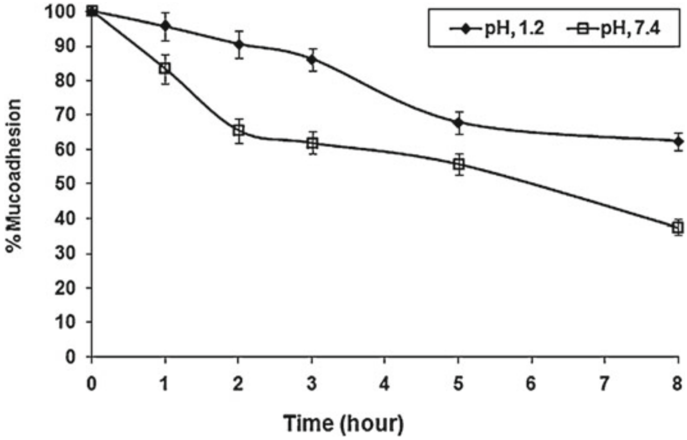

Fig. 4 Results of ex vivo wash-off test to assess mucoadhesive properties of optimized alginate-JSS composite beads of metformin HCl in gastric pH (1.2) and intestinal pH (7.4) [mean ± S.D., $n = 3$] [92]. (Copyright © 2013 Elsevier B.V.)

composite mucoadhesive beads of metformin HCl to alloxan-induced diabetic Albino rats were measured and analyzed (Fig. 5). The alloxan-induced diabetic rats treated with pure metformin HCl showed a speedy decline in levels of blood glucose within 3 h of oral administration, and after that, it was recovered speedily toward the regular level of blood glucose. On the other hand, alloxan-induced diabetic rats treated with optimized alginate-JSS composite mucoadhesive beads of metformin HCl exhibited a decline in levels of blood glucose, which was comparatively slower than that of pure metformin HCl up to 3 h. However, the decline in the levels of blood glucose was

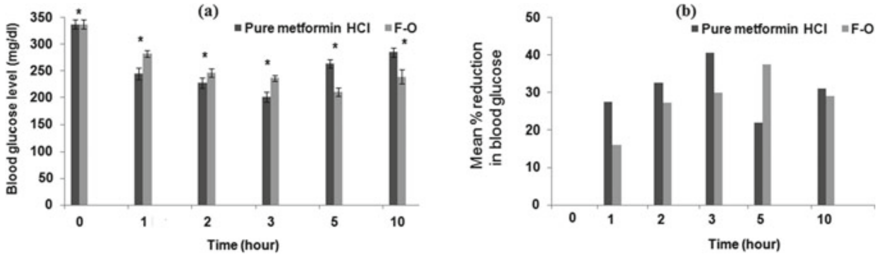

Fig. 5 **a** Comparative in vivo levels of blood glucose and **b** comparative in vivo mean percentage reduction in levels of blood glucose in alloxan-induced diabetic rats after oral administration of pure metformin HCl and optimized alginate-JSS composite beads of metformin HCl (F-O) The data were analyzed for significant differences (*$p < 0.05$) by paired samples t-test [92]. (Copyright © 2013 Elsevier B.V.)

increased progressively with the increment of time and was found to be sustained over a period of 10 h. These in vivo pharmacodynamic results recommended a significant ($p < 0.05$) antidiabetic action by the oral administration of optimized alginate-JSS composite mucoadhesive beads *in the* diabetic rats *over a longer period*.

In another study, alginate-JSS composite beads containing piogliazone (an oral anti-diabetic drug having short biological half-life of 3–5 h) were developed via ionotropic gelation technique using calcium chloride as ionotropic cross-linking agent and evaluated for the use in the treatment of non-insulin-dependent diabetes mellitus (Type–II) [93]. For the formulation optimization, a computer-aided statistical optimization method based on 3^2 factorial design along with response surface methodology was employed to check and analyze the influences of sodium alginate-to-JSS ratio and concentrations of calcium chloride on the drug encapsulation efficiencies and cumulative percentage drug releases. From the analyses of three-dimensional response surface plots and corresponding two-dimensional contour plots, influences of the tested factors (independent variables, such as sodium alginate-to-JSS ratio and concentrations of calcium chloride) on the responses measured (dependent variables such as drug encapsulation efficiency and cumulative drug release after 10 h) were verified and analyzed. An increased pioglitazone encapsulation efficiency and reduced of cumulative in vitro pioglitazone release after 10 h were found with the decreasing of sodium alginate-to-JSS ratio (increasing JSS contents in the polymer-blends employed) and the increasing concentration of calcium chloride as the ionotropic cross-linking agent. The optimized alginate-JSS composite beads of pioglitazone exhibited 94.07 ± 3.82% of drug encapsulation efficiency and 64.87 ± 1.83% of cumulative in vitro drug release after 10 h.

Pioglitazone encapsulation efficiencies of various alginate-JSS composite beads of pioglitazone were ranged between 64.80 ± 1.92 and 94.07 ± 3.82% [93]. Average bead sizes of these various alginate-JSS composite beads of pioglitazone were ranged between 0.77 ± 0.04 and 1.24 ± 0.09 mm. An average bead size of the optimized composite beads of pioglitazone as 0.85 ± 0.05 mm was calculated by optical microscopy. SEM image of the optimized alginate-JSS composite beads of pioglitazone demonstrated bead surface morphology of irregular shaping with very rough surface structure, where some typical cracks as well as wrinkles were seen. FTIR spectroscopic analyses suggested the drug-excipients compatibility with the nonexistence of drug-excipient interaction between pioglitazone and sodium alginate-JSS polymer blends employed in the preparation of these composite beads through ionotropic gelation by calcium chloride.

In the gastric pH (acidic) medium, in vitro pioglitazone releases from these composite beads were found very slow (< 16% after 2 h) by reason of pH-sensitive contraction characteristics of the alginate gel in the acidic pH. In intestinal (alkaline) pH, a comparative faster in vitro releasing pattern of encapsulated pioglitazone was noticed as a result of higher swelling of the ionotropically gelled alginate matrices in the alkaline pH medium (7.4). After 10 h of in vitro release study, the cumulative pioglitazone released from these alginate-JSS composite beads was ranged, 64.87 $\pm$ 1.83–92.66 $\pm$ 4.54%. In vitro pioglitazone HCl releasing from these composite beads was followed zero-order kinetics pattern along with super case-II transport mechanism. In vitro swelling performances of various alginate-JSS composite beads of pioglitazone were found to be controlled by pH and the compositions of the swelling mediums. In vitro swelling pattern of these composite beads of pioglitazone was found lower in gastric pH as compared to that of intestinal pH. This reduced swelling was occurred as a result of shrinkage of alginate-based at the acidic pH (1.2). Maximum swelling pattern of these composite beads of pioglitazone was detected at 2–3 h in the intestinal pH, and then, the erosion and dissolution of these composite matrices took place.

In vivo pharmacodynamic efficiencies of optimized alginate-JSS composite beads of pioglitazone were carried out in the alloxan-induced diabetic Albino rats. Comparative levels of blood glucose and the mean percentage reduction of blood-glucose levels in these diabetic rats after oral administration of pure pioglitazone and optimized alginate-JSS composite beads of pioglitazone were measured and compared. These optimized composite beads of pioglitazone demonstrated a significant ($p <$ 0.05) hypoglycemic action in the treated alloxan-induced diabetic Albino rats *over prolonged period* of 10 h after administration through oral route.

3.1 Gellan Gum-JSS Composite Beads for Controlled Release Drug Delivery

Gellan gum is an anionic extracellular natural polysaccharide obtained from microbial sources (in particular from *Pseudomonus eloda*) [143, 144]. In recent years, it is extensively utilized in several food and pharmaceutical applications [27, 30]. Gellan gum consists of a tetrasaccharidic structure with a repeating sugar units of glucose, glucuronic acid, and rhamnose (2: 2: 1 of molar ratio) [145]. Deacetylated gellan gum has been investigated for its capacity to produce ionotropically gelled gellan gum-based matrices by the influence of some divalent and trivalent metal cations (in particular, Ca^{2+} and Al^{3+} ions) [22, 144, 146]. The gelation mechanism of deacetylated gellan gum involves the development of double helical junctional zones subsequently the aggregation of double helical segments to the three-dimensional (3D) network structures through the ionotropic cross-linking interaction with divalent and/or trivalent metal cations and also through the hydrogen bonding [39]. The ionotropic cross-linking gelation of deacetylated gellan gum by divalent and/or trivalent metal cations takes place through the ionotropic cross-linking interaction in-between two carboxylic groups of glucuronic acid residues (present in the gellan gum structural backbone) and divalent and/or trivalent metal cations [144]. On the basis of the ionotropic cross-linking gelation nature of deacetylated gellan gum, different drugs are being encapsulated for the sustained-controlled releases of these [22, 146]. The features of these ionotropically cross-linked gellan gum-based systems were found dependant on various factors, such as gellan gum types, gellan gum concentrations, cross-linker types, cross-linker concentrations, pH of the medium, and curing time. [27, 30, 39]. These ionotropically gelled gellan gum beads are stable enough in lower pH range and unstable in higher pH range because of their rapid swelling rate in higher pH as compared to that of lower pH. This leads more rapidly and premature releasing of encapsulated drugs from a variety of ionotropically gelled gellan gum-based beads in the alkaline intestinal pH [68]. To conquer these inconveniences, various types of modifications of ionotropically gelled gellan gum-based matrices are being researched [22, 27, 95, 145]. Among these modifications, uses of other biopolymers as blends with gellan gum are being explored to develop various ionotropically gelled gellan gum-based composite hydrogel matrices to maximize drug encapsulation efficiency and sustained-controlled drug release [22, 27, 68].

Effectiveness of JSS was evaluated as a sustained drug-releasing matrix formers and mucoadhesive agent in the formulation of gellan gum-JSS composite beads of metformin HCl by means of employing calcium chloride as ionotropic cross-linking agent and gellan gum-JSS composite as polymer blends [95]. To optimize the formula of ionotropically gelled gellan gum-JSS composite beads of metformin HCl, a computer-aided statistical optimization method based on 3^2 factorial design using response surface methodology was employed. From the numerical optimization analyses, the optimal formulation variable settings were selected as gellan gum-to-JSS ratio of 1.38 and calcium chloride concentration of 1.58%. Employing these optimal formulation variable settings, optimized gellan gum-JSS composite beads of metformin HCl were formulated, which exhibited drug encapsulation efficiency of 92.67 ± 4.46% and cumulative in vitro release of metformin HCl of 61.30 ± 2.37% after 10 h of drug-releasing study.

Drug encapsulation efficiencies of all these gellan gum-JSS composite beads of metformin HCl were calculated within the range, 67.72 ± 3.20–92.98 ± 4.13%. Average bead sizes of all these gellan gum-JSS composite beads of metformin HCl were calculated within the range, 1.21 ± 0.11–1.67 ± 0.27 mm. Average diameter of optimized gellan gum-JSS composite beads of metformin HCl was measured as 1.67 ± 0.27 mm. SEM photomicrograph of the composite beads demonstrated a few typical wrinkles as well as cracks on the bead surface with a few pores (Fig. 6). On the basis of FTIR spectroscopy analysis, it was observed that after encapsulation by

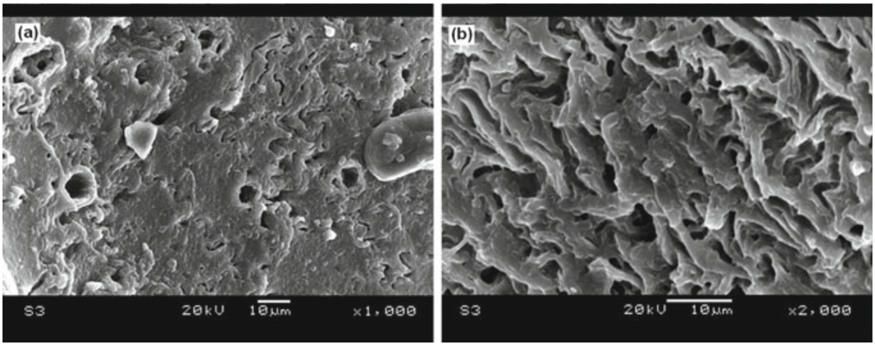

Fig. 6 SEM photograph of optimized gellan gum-JSS composite beads of metformin HCl at **a** × 1000 and **b** × 2000 magnification [95]. (Copyright © 2014 Elsevier B.V.)

ionotropic gelation, the optimized gellan gum-JSS composite beads of metformin HCl contained considerable characters of metformin HCl devoid of any types of interactions between metformin HCl and polymer blends (gellan gum-JSS) used.

In vitro metformin HCl releases from gellan gum-JSS composite beads of metformin HCl in the acidic medium of gastric pH (1.2) was very slow (*i.e.,* < 15.30% after initial 2 h) (Fig. 7). However, it was found faster in the alkaline medium of intestinal pH (7.4), which might be attributable to the fact of rapid swelling of these composite matrices. The higher and faster swelling power of gellan gum

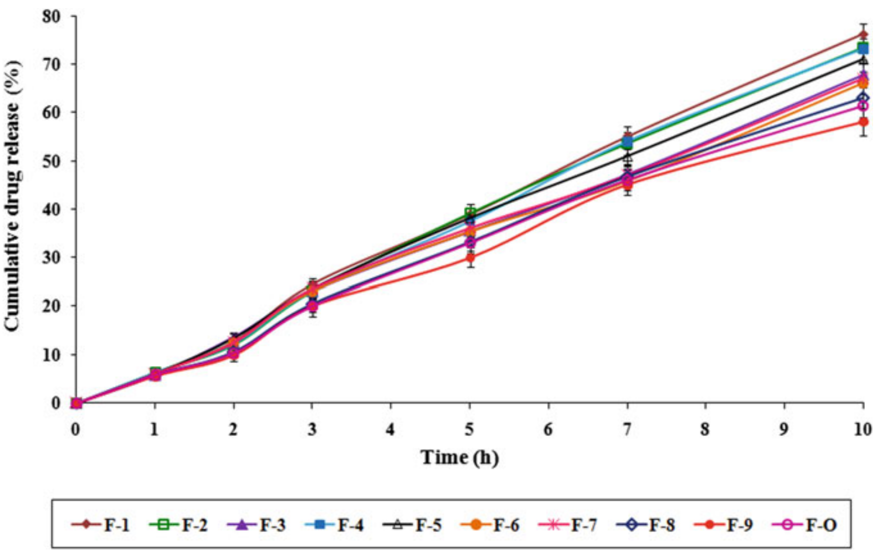

Fig. 7 In vitro drug release from various gellan gum-JSS composite beads of metformin HCl [Mean ± S.D., $n = 3$] [95]. (Copyright © 2014 Elsevier B.V.)

matrices in the alkaline medium might be caused due to the electrostatic repulsion among the ionized –COO^- groups of the gellan gum backbone. In vitro cumulative metformin HCl releases from these ionotropically gelled gellan gum-JSS composite beads of metformin HCl were found in the range, 58.19 ± 1.47–76.27 ± 3.20%. It was also found that the in vitro releasing of metformin HCl from gellan gum-JSS composite beads followed a controlled releasing pattern (zero-order kinetics) along with super case-II transport mechanism controlled by swelling and relaxation of drug releasing matrices. In vitro swelling of optimized gellan gum-JSS composite beads of metformin HCl was found to be dependent on the pH of the swelling media. Initially, these gellan gum-JSS composite beads demonstrated lower swelling pattern in the acidic media of gastric pH (1.2) with respect to that in the alkaline medium of intestinal pH (7.4) (Fig. 8). These composite beads swelled speedily in the alkaline pH (7.4) within 2-3 h following erosion and dissolution of the swelled matrices.

Ex vivo wash off assessments of optimized gellan gum-JSS composite beads of metformin HCl was tested using goat intestinal mucosal tissue in the intestinal pH (alkaline) and gastric pH (acidic). At the intestinal pH, a comparative quicker ex vivo

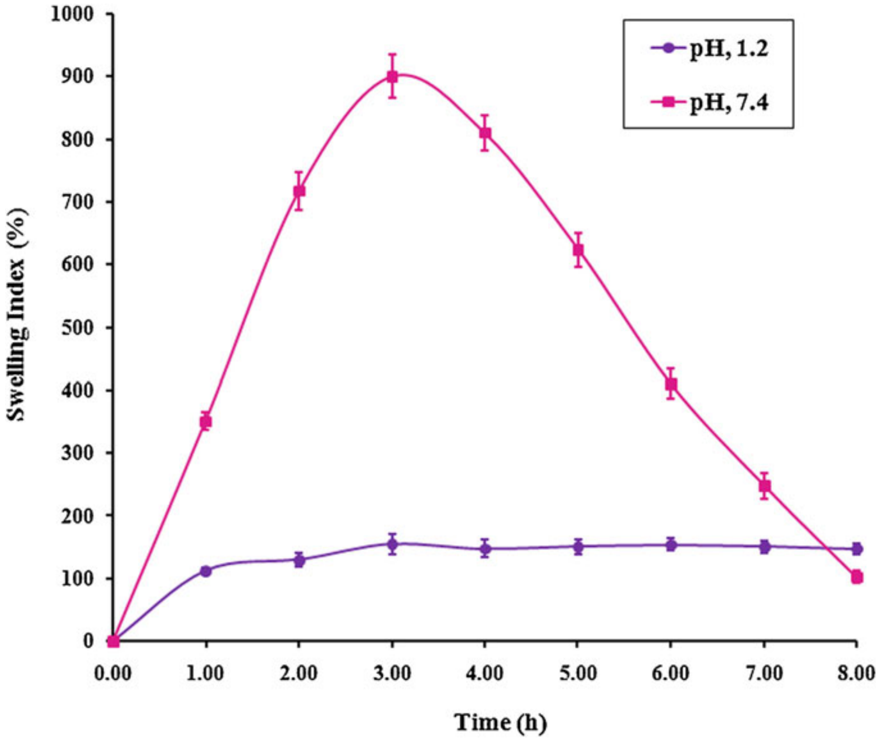

Fig. 8 In vitro swelling behavior of optimized gellan gum-JSS composite beads of metformin HCl in gastric pH (1.2) and intestinal pH (7.4) [Mean ± S.D., $n = 3$] [95]. (Copyright © 2014 Elsevier B.V.)

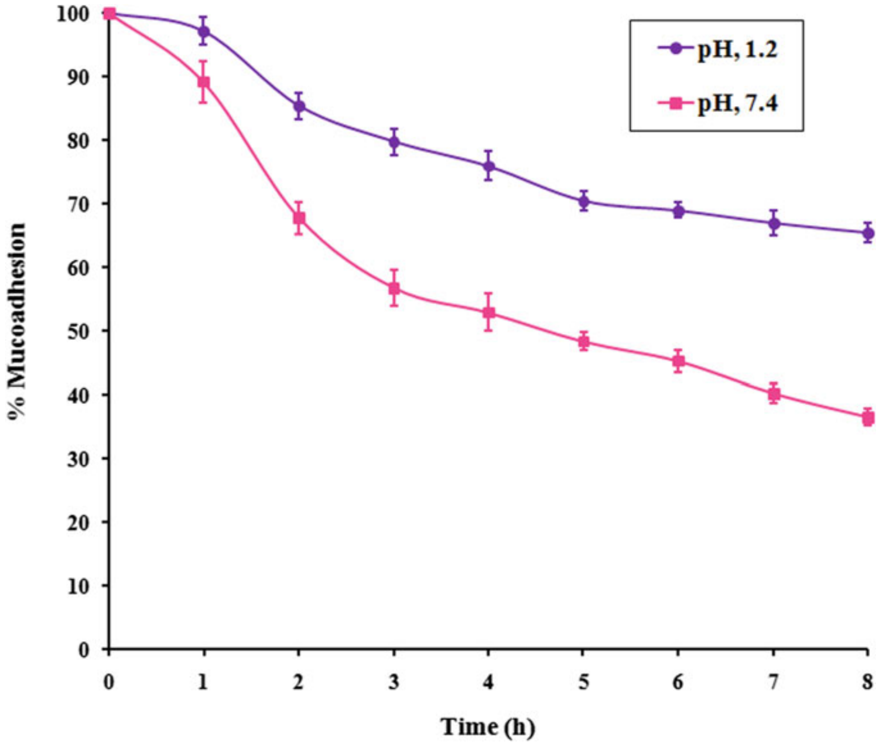

Fig. 9 Ex vivo mucoadhesivity of optimized gellan gum-JSS composite beads of metformin HCl in gastric pH (1.2) and intestinal pH (7.4) [Mean ± S.D., $n = 3$] [95]. (Copyright © 2014 Elsevier B.V.)

wash off was reported than that in acidic pH (Fig. 9). Thus, the results of ex vivo wash off showed a good mucoadhesive potential of these composite beads for the used in mucoadhesive gastroretentive drug delivery to achieve improved bioavailability of the encapsulated drugs.

. The comparative in vivo levels of blood glucose as well as the percentage of average decline in the in vivo levels of blood glucose after administration of pure metformin HCl and optimized gellan gum-JSS composite mucoadhesive beads of metformin HCl through the oral route in the alloxan-induced diabetic Albino rats were measured and analyzed (Fig. 10). The results of the pharmacodynamic evaluation suggested a significant ($p < 0.05$) antidiabetic action *over a prolonged period in the* treated diabetic rats. Thus, for the attainment of the tight blood-glucose level, the efficacy of these formulated ionotropically gelled optimized gellan gum-JSS composite mucoadhesive beads of metformin HCl was found beneficial, and improved patient compliance in the non-insulin-dependent diabetes mellitus (Type–II) may also be produced via controlling, prolonging as well as enhancing the systemic absorption of metformin HCl.

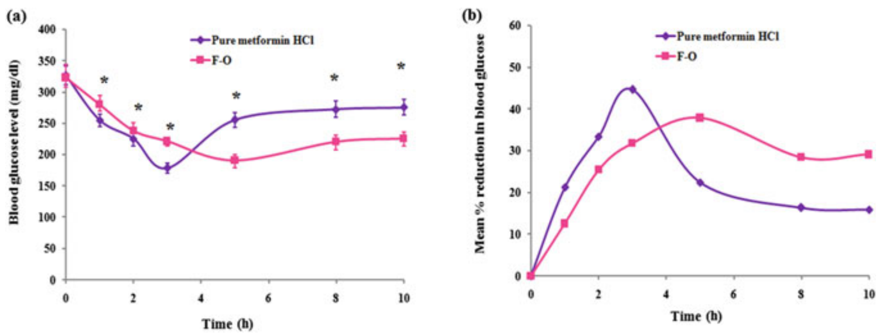

Fig. 10 **a** Comparative in vivo levels of blood glucose and **b** comparative in vivo mean percentage reduction in levels of blood glucose in alloxan-induced diabetic rats after oral administration of pure metformin HCl and optimized gellan gum-JSS composite beads of metformin HCl (F-O) The data were analyzed for significant differences (*$p < 0.05$) by paired samples t-test [95]. (Copyright © 2014 Elsevier B.V.)

3.2 Pectinate-JSS Composite Beads for Controlled Release Drug Delivery

Pectin is a naturally derived water soluble, non-toxic, anionic polysaccharide. Industrially, it is extracted from citrus peels, banana peels, sugar beet roots, apple pomace, pumpkin pulp, etc. [147]. In various pharmaceutical and food applications, it has been utilized as additives, thickeners, emulsifying agents, gelling agents, matrix formers, etc. [2, 24, 39, 147]. Because of its excellent biocompatibility, high biodegradability, appropriate mechanical property, and acid stability, it is extensively utilized as biopolymer in a variety of biomedical applications including drug delivery [26, 28]. The backbone of pectin molecules comprise predominantly a long sequences of partially methyl esterified and linearly linked α-(1-4) D-galacturonic acid residues interrupted with α-(1–2) connected α-L-rhamnopyranose units at regular intervals [39]. According to the degree of methoxylation, pectins are grouped as high methoxyl pectins (50–80% degree of methoxylation) and low methoxyl pectins (25–50% degree of methoxylation) [24]. The degree of methoxylation controls a variety of essential attributes of pectins, such as aqueous solubility and gel-forming potential. In general, low methoxy pectins has shown the potential of ionotropic cross-linking by various divalent metal cations like Ca^{2+}, Zn^{2+}, etc., to produce rigid ionotropically gelled pectinate beads by reason of ionotropic electrostatic interaction in-between $–COO^{-}$ groups of pectin-backbone and divalent metal cations [147]. The ionotropic gelation of $–COO^{-}$ groups of low methoxy pectin with divalent metal cations as cross-linker stimulates the arrangement of the so-called “Egg Box” structure [2]. This “Egg Box” structure of low methoxy pectin is to some extent unlike than that of the ionotropic gelation of sodium alginate [39]. In recent years, because of the biodegradability, non-toxicity, acid stable characteristics, economic, and environmental friendly preparation methodology, numerous ionotropically gelled pectinate matrices composed of low methoxy pectin have been exploited as sustained drug-releasing carriers for oral drug delivery [147–150]. The characteristics (such as morphology, drug encapsulation, swelling, and drug release) of these pectinate matrices are generally found dependent on the pectin contents, degree of methylation, formulation methodologies, concentrations of cross-linkers, cross-linking time, pH, etc. [149]. These ionotropically gelled pectinate matrices has been experienced lesser encapsulations of drugs [24, 26, 28]. Moreover, the higher degrees of solubility as well as swellability of these pectinate matrices cause early and premature releases of the encapsulated drugs when exposed to the environment of alkaline pH [2, 149]. To curtail these above-said weaknesses, modifications of ionotropically gelled pectinate matrices have already been researched [40, 148]. Recently, polymer blends of low methoxy pectin with other biocompatible polymers have also been researched to formulate ionotropically gelled pectinate-based composite matrices to improve drug encapsulation, controlled drug-releasing patterns, mucoadhesion, swelling, stability, pharmacodynamic suitability, etc. [24, 26, 28].

In a study, JSS was blended with anionic low methoxy pectin to prepare ionotropically gelled pectinate-JSS composite beads to control the release of metformin HCl by using calcium chloride as an ionotropic cross-linking agent [94]. To optimize the formulations of pectinate-JSS composite beads containing metformin HCl, a 3^2 factorial statistical optimization design based on the response surface methodology was employed, where the influences of low methoxy pectin and JSS contents on the drug encapsulation efficiencies and cumulative percentage drug releases after 10 h were analyzed and optimized. It was noticed that both the efficiency of drug encapsulation was found to be enhanced, and the cumulative percentage drug releases after 10 h was found to be decreased with the increment of low methoxy pectin and JSS contents in the composite bead formula. From the numerical analysis, the selected optimal process variable setting used for the preparation of optimized pectinate-JSS composite beads containing metformin HCl was low methoxy pectin content of 715.38 mg and JSS content of 349.87 mg. The optimized composite beads containing metformin HCl exhibited 94.11 $\pm$ 3.92% of drug encapsulation efficiency and 48.88 $\pm$ 2.02% of cumulative in vitro drug release.

Drug encapsulation efficiencies of these ionotropically gelled pectinate-JSS composite beads of metformin HCl were found within the range, 66.65 $\pm$ 2.47–94.11 $\pm$ 3.92%, and the average bead sizes were found within a range, 1.52 $\pm$ 0.15–2.06 $\pm$ 0.20 mm. The optimized pectinate-JSS composite beads of metformin HCl demonstrated average bead diameters of 2.06 $\pm$ 0.20 mm. The morphological analysis of optimized pectinate-JSS composite beads of metformin HCl was done by SEM observation. SEM photograph showed particles of spherical shaped without any kinds of agglomeration (Fig. 11). On the basis of FTIR spectroscopy analyses, it was observed that after encapsulation by ionotropic gelation, the optimized pectinate-JSS composite beads of metformin HCl contain considerable characters of metformin HCl devoid of any types of interactions between these metformin HCl and polymer blends (composed of pectinate-JSS) used.

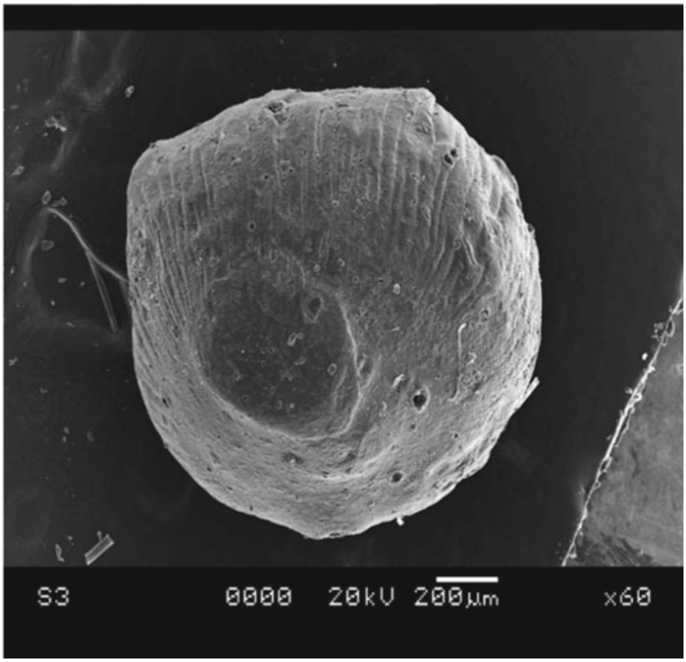

Fig. 11 SEM image of optimized pectinate-JSS composite beads of metformin HCl [94]. (Copyright © 2013 Elsevier B.V.)

In vitro release of metformin HCl from various formulated pectinate-JSS composite beads showed prolonged sustained release over 10 h of drug release study (Fig. 12). The release of metformin HCl in acidic medium (gastric pH, 1.2) was found very slow, which was measured as less than 15% after 2 h due to contraction of ionotropically gelled pectinate-based matrices in acidic pH. Because of higher swelling of the ionotropically gelled pectinate-based matrices in the phosphate buffer (alkaline pH, 7.4), faster in vitro release of metformin HCl from these composite beads were observed. The cumulative drug releases of various formulated pectinate-JSS composite beads of metformin HCl were found in a range, 48.88 ± 2.02–89.72 ± 4.03% after 10 h. In vitro metformin HCl releases from pectinate-JSS composite beads followed controlled releasing pattern (zero-order kinetics was measured in curve fitting) along with super case-II transport mechanism controlled by swelling and relaxation. In vitro swelling of optimized pectinate-JSS composite beads of metformin HCl was found to be controlled by pH and compositions of swelling mediums. The swelling patterns of optimized pectinate-JSS composite beads of metformin HCl were found much lower in acidic gastric pH (1.2) as compared with that in alkaline intestinal pH (7.4) (Fig. 13). Less swelling of these pectinate-JSS composite beads in acidic pH (1.2) was observed. Maximum swelling pattern of these composite beads of metformin HCl was detected at 2–3 h in intestinal pH, and then, erosion and dissolution of these composite matrices took place.

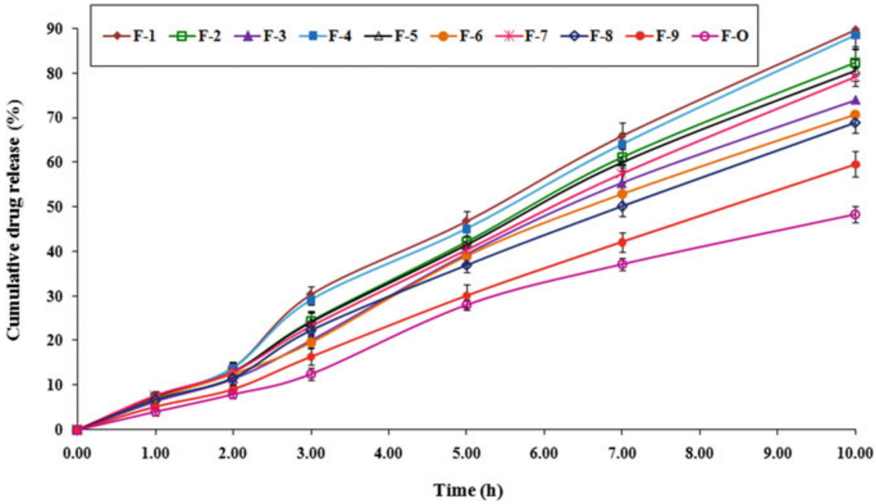

Fig. 12 In vitro drug release from various pectinate-JSS composite beads of metformin HCl [Mean ± S.D., $n = 3$] [94]. (Copyright © 2013 Elsevier B.V.)

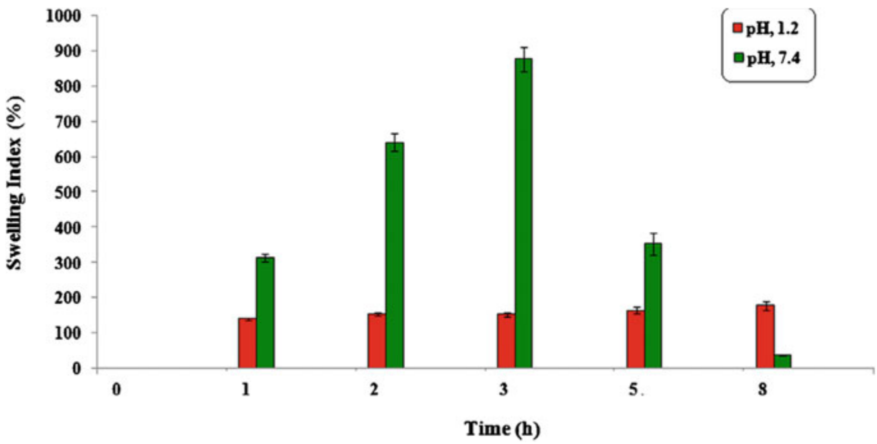

Fig. 13 In vitro swelling behavior of optimized pectinate-JSS composite beads of metformin HCl in gastric pH (1.2) and intestinal pH (7.4) [mean ± SD, $n = 3$] [94]. (Copyright © 2013 Elsevier B.V.)

Ex vivo wash off study of optimized pectinate-JSS composite beads of metformin HCl was performed using goat intestinal mucosal tissues. Ex vivo wash off of these composite beads in alkaline intestinal pH (7.4) was found faster as compared to that in acidic gastric pH (1.2). The percentage of these beads adhered to the intestinal mucosal tissue in gastric pH (1.2) was reported as 70.05 ± 4.22% over 8 h of wash off; while in the intestinal pH (7.4), this was found 36.64 ± 3.45% (Fig. 14).

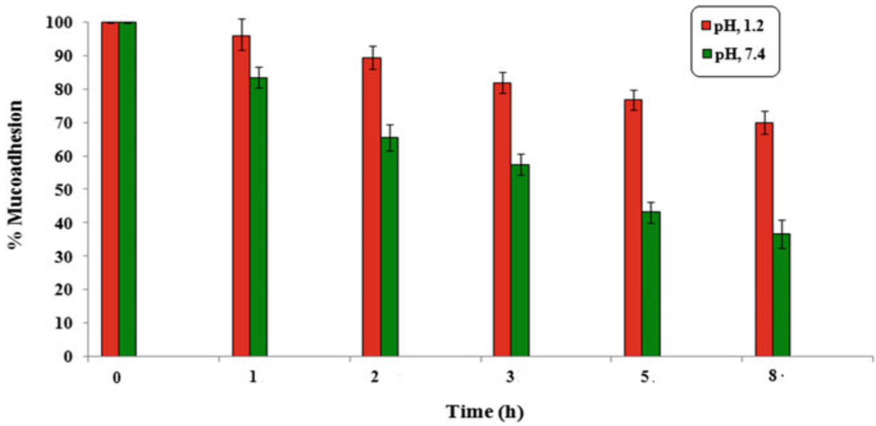

Fig. 14 Ex vivo mucoadhesivity of optimized pectinate-JSS composite beads of metformin HCl in gastric pH (1.2) and intestinal pH (7.4) [mean ± SD, $n = 3$] [94]. (Copyright © 2013 Elsevier B.V.)

In alloxan-induced diabetic Albino rats, in vivo pharmacodynamic study of optimized pectinate-JSS composite mucoadhesive beads of metformin HCl after oral administration was performed through determining the blood-glucose levels. The relative in vivo blood-glucose level and the average percentage reduction in blood-glucose level demonstrated a significant ($p < 0.05$) antidiabetic action *over a prolonged period in the* alloxan-induced diabetic rats (Fig. 15). These pectinate-JSS composite mucoadhesive beads of metformin HCl *may possibly be lucrative in terms of* prolonged systemic absorption of metformin HCl controlling tight blood-glucose level with higher patient compliances.

4 Conclusion

JSS extracted from jackfruit seeds is one of the natural derived starch materials, which is being used in various industrial applications including foods and pharmaceuticals. JSS is reported as potential pharmaceutical excipients in the formulations of various pharmaceutical dosage forms, such as binding agent and disintegrants in pharmaceutical tablets, emulsifier in emulsions, suspending agent in suspensions, and biomucoadhesive agent in biomucoadhesive dosage forms. Recently, JSS has been utilized as polymer blends with other biocompatible polymers like sodium alginate, gellan gum, and low methoxy pectin to prepare controlled drug-releasing composite beads. All these JSS-based composite beads were prepared through ionotropic-gelation technique. These JSS-based composite beads were found suitable to encapsulate various types of drugs and to release encapsulated drugs in sustained manner

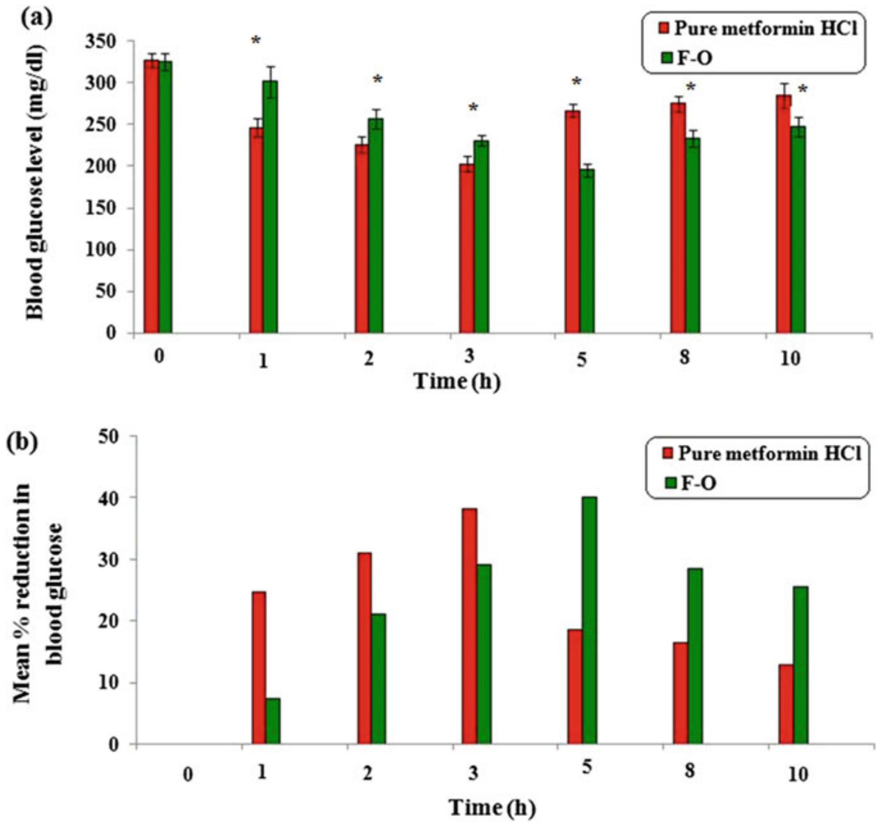

Fig. 15 **a** Comparative in vivo levels of blood glucose and **b** comparative in vivo mean percentage reduction in levels of blood glucose in alloxan-induced diabetic rats after oral administration of pure metformin HCl and pectinate-JSS composite beads of metformin HCl (F-O) The data were analyzed for significant differences (*$p < 0.05$) by paired samples t-test [94]. (Copyright © 2013 Elsevier B.V.)

over prolonged period. All these JSS-based composite beads demonstrated significant hypoglycemic actions in the treated alloxan-induced diabetic Albino rats *over prolonged period* after administration through oral route.

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Polymeric Nanocomposites for Cancer-Targeted Drug Delivery

Luiza Steffens Reinhardt, Mabilly Cox Holanda de Barros Dias, Jussania Gnoatto, Anna Wawruszak, Marta Hałasa, Pablo Ricardo Arantes, Neil J. Rowan, and Dinara Jaqueline Moura

Abbreviations

α-CD	A-cyclodextrin
ADCs	Antibody-drug conjugates
Ag	Silver
ALK	Anaplastic lymphoma kinase
AuNR	Gold nanorod
BBB	Blood brain barrier
BD	Brownian dynamics
BRAF	V-raf murine sarcoma viral oncogene homolog B1
BRCA	Breast cancer gene
BRD4	Bromodomain containing 4
BTB	Brain tumor barrier
CDK4/6	Cyclin-dependent kinase
CDK7	Cyclin-dependent kinase 7

L. S. Reinhardt (✉) · D. J. Moura
Laboratory of Genetic Toxicology, Federal University of Health Sciences of Porto Alegre, Porto Alegre, Rio Grande do Sul, Brazil
e-mail: luizasteffens@live.com

M. C. H. de Barros Dias · N. J. Rowan
Biosciences Research Institute, Athlone Institute of Technology, Athlone, Co. Westmeath, Ireland

J. Gnoatto
Chemistry Institute, Federal University of Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil

A. Wawruszak · M. Hałasa
Department of Biochemistry and Molecular Biology, Medical University of Lublin, Lublin, Poland

P. R. Arantes
Department of Bioengineering, University of California, Riverside, USA

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CS	Chitosan
CSCs	Cancer stem cells
CT	Chemotherapy
CTS	Chondroitin sulphate
DDFT	Dynamic density functional theory
DOX	Doxorubicin
DPD	Dissipative particle dynamics
EGFR	Epidermal growth factor receptor
FEM	Finite element method
FDM	Finite difference method
FVM	Finite volume method
GO	Graphene-oxide
HATs	Histone acetyltransferases
HDACs	Histone deacetylases
HDIs	Histone deacetylase inhibitors
HER2	Human epidermal growth factor receptor 2
IGF-1	Insulin-like growth factor-1
IONPs	Iron oxide NPs
LB	Lattice Boltzmann
LOI	Loss of imprinting
mTOR	Mammalian target of rapamycin
MET	Hepatocyte growth factor receptor
MD	Molecular dynamics
MC	Monte Carlo
MTX-PEG	Methotrexate-PEG
NIR	Near-infrared radiation
NK	Natural killer
NP	Nanoparticle
NSCLC	Non-small cell lung carcinoma
NTRK	Neurotrophic receptor tyrosine kinase
PAA	Poly (acrylic acid)
PARP	Poly (ADP-ribose) polymerase
PCL	Polycaprolactone
PD-1	Programmed cell death protein 1
PD-L1	Programmed cell death protein 1-ligand
PDMS	Poly(N-isopropylacrylamide)-metal NPs
PEG	Poly (ethyleneglycol)
PEI	Poly(ethylene imine)
PGA	Poly (glutamic acid)
PI3K	Phosphatidylinositol 3-kinase
PLGA	Poly (lactic-co-glycolic acid)
PLLA	Poly-l-lactic acid
PMMA	Poly (methyl methacrylate),
pNIPAM	Poly(N-isopropylacrylamide)
PS	Polystyrene

PTT	Photothermal therapy
PTX	Paclitaxel
PU	Polyurethane
PVA	Polyvinyl alcohol
PVDF	Polyvinylidene fluoride
QM	Quantum mechanics
RGD peptide	Arginylglycylaspartic
ROS	Reactive oxygen species
RT	Radiotherapy
SEs	Super-enhancers
SHH	Sonic hedgehog signalling molecule
TDGL	Time-dependent Ginzburg–Landau
VEGF	Vascular endothelial growth factor
WS_2-NT-CM-PEI	Tungsten disulphide nanotubes-ceric ammonium nitrate-PEI

1 Introduction

Cancer represents a multifaceted group of diseases that present unregulated cellular death and growth processes. Cancer is developed as a consequence of DNA mutations that can be triggered by the environment or/and DNA replication errors or can be hereditary [1]. This process is comprised by three stages, initiation, promotion and progression, and it is known as the carcinogenesis multistep model [2].

The conventional cancer management involves several different treatment options depending on different parameters and characteristics such as the tumour type and the grade, nevertheless, surgical resection, systemic chemotherapy (CT), radiotherapy (RT) and immunotherapy are the most common therapies. CT drugs are not specific, thus, they can affect healthy cells developing numerous side effects [3]. As an alternative to the CT conventional treatment, nanotechnological approaches that comprise targeted and controlled drug delivery systems can be used to improve the therapy outcomes including patients' quality of life and survival by enhancing drugs distribution, pharmacokinetics and stability [3–5].

Cancer-targeted drug delivery refers, quantitatively and selectively, to drug accumulation within the tumour site [6, 7]. Preferably, for CT formulations to be effective in cancer therapy, they must be biofunctionalized with suitable size and surface charge and must target specific biomolecules.

In the nanosystems field, polymeric nanocomposites have been shown promising features for cancer-targeted drug delivery [8–12], and thus, this chapter will discuss what is known about the use of nanocomposites for cancer management, what is been produced and tested, how these systems can bypass CT drawbacks and how multiscale molecular simulation for nanostructured polymer systems can guide the development of nanocomposites.

2 Cancer Overview

Cancer is currently recognized as a major disease estimating over 18 million of new cases, and resulting over 9,5 million deaths in 2018 worldwide [13]. The most common cancer types for both sexes and all ages in 2018 are lung, breast and colorectal cancer, which account for over 30 percentage of all cancer cases [13]. Taking into account the gender, the top five most common types of cancer are lung, prostate, colorectal, stomach and liver for men, and breast, colorectal, lung, cervix uteri and thyroid for women in 2018 [13].

Cancer is a collective term for diseases characterized by abnormal and uncontrolled cell growth affecting nearby tissues. Cancer cells are able to spread to other body parts (metastasis) using blood and lymph systems [14]. The mechanism of cancer formation is multifactorial and is initiated by environment as well as genetics, highlighting the combination of external factors and internal genetic modifications, which lead to cancer disease [15]. Several risk factors have been identified as crucial in tumorigenesis. These include lifestyle factors such as smoking, alcohol consumption, diet, obesity, cancer-causing substances and other external factors including age, radiation, infectious organisms, environmental pollution and sunlight [16, 17].

Among all risk factors, smoking is well documented [18, 19]. It has to be emphasized that not only regularly smokers belong to the group of increased risk of cancer, but also second-hand smokers are affected [18]. There are growing evidence that obesity is strongly associated with cancer risk and progression [20–22]. The excess of energy resulting from obesity is responsible for changes in insulin, insulin-like growth factor-1 (IGF-1), leptin and steroid hormones levels, what in turn leads to altering nutritional environment, and is able to support tumour initiation and progression [21]. Alcohol consumption is classified as carcinogenic due to its first product of metabolism: acetaldehyde. Acetaldehyde produces free radicals, promoting cancer development [23]. Additionally, ethanol is associated with reduction of inflammatory mediators increasing tumour formation [24]. Lifestyle factors required to be paid attention, as they are potentially changeable, and people are able to reduce or completely eliminate the impact of these factors on their life.

Next to environmental factors, landscape of cancer is created by genetic alterations as well as epigenetic abnormalities. Balance between tumour suppressor genes and oncogenes allows unrestricted cell growth leading to cancer progression. Activation of oncogenes and/or inactivation of tumour suppressors enhances division or inhibits cell apoptosis, as protein products of these genes regulate different cellular pathways that control cell proliferation, migration and apoptosis [25]. The development of malignant tumour occurs as a result of stepwise accumulation of alterations in both proto-oncogenes and tumour suppressor genes, which both are referred to as critical genes [25]. Genetic alteration includes genomic instability as well as genetic mutations affecting changes within nucleotide sequence. In turn, epigenetic modifications do not alter DNA sequence, wherein they are associated with histone modifications, DNA methylation, loss of imprinting (LOI) and regulation by miRNA [25, 26].

The initiation of the carcinogenesis process occurs as a result of either spontaneous mutations or mutations induced by chemical, physical and biological carcinogens.

Tumour suppressor genes encode molecules responsible for the regulation of many pathways and mechanisms involved in programmed cell death, cell division, differentiation and migration, repair of DNA damage, cell cycle checkpoints as well as inhibition of tumour metastasis [27]. Because the main function of tumour suppressors is to suppress the development of tumour, the appearance of mutations within these genes results in the onset of cancer development. By contrast, proto-oncogenes are normal genes that encode proteins involved in cell growth and cell differentiation, nonetheless, as a result of the mutation, the specific protein involved in the regulation is overexpressed and an oncogene is formed. Among the proto-oncogenes it should be distinguished: genes encoding growth factors, transcription factors, receptor and cytoplasmic tyrosine kinases as well as serine/threonine kinases [28, 29].

The traditional cancer treatment strategies include surgery, RT, CT and immunotherapy. Nowadays, innovative cancer therapies such as targeted therapies are being widely studied [30]. CT targets both, rapidly dividing cancer cells as well as normal cells, and is commonly used in combination with surgery or RT depending on type and stage of cancer [31]. The main limitation of CT is high number of serious side effects [32]. CT has short-term and long-term side effects. Short-time side effects are correlated with high toxicity of used drugs and occur during cancer treatment. In turn, long-term side effects include further complications proceeding after adjuvant CT. The side effects depend on the agents' specificity, dosage and duration of treatment [33]. According to The American Cancer Society, the most common CT side effects are: fatigue, loss of hair, infections, anaemia, vomiting, changes in appetite, obstipation, diarrhoea, pain with swallowing, destroying of nerve system as well as skin, nails, urine and bladder changes, and weight and sexual function changes [34]. Additionally, patients treated with CT may develop resistance to chemotherapeutics, leading to cancer recurrence and progression [35].

More accurate and advanced genetic and molecular characterization of cancer landscape significantly contributed to development of efficient immunotherapies. Immunotherapy supports the recognition of cancer cells as foreign to the host immune system and stimulates the immune system [36]. The main goal of this therapy is to improve antitumour response and decrease side effects commonly occurred during CT treatment. The mechanism of action is based on activation (or activation enhancement) of immune system to attack and destroy cancer cells using natural mechanism, which are often avoided in cancer progression [37]. There are growing number of immuno-drugs approved by US Food and Drug Administration [37], and many of immuno-drugs are currently under clinical trials [38]. Although the immunotherapy seems to be burdened with less side effects than CT, the serious adverse effects such as autoimmunity and non-specific inflammation have been observed [37].

Recently, the changes in genetic profile causing mutations and modifications of oncoproteins as well as tumour suppressor proteins have become promising targets in anticancer treatment [39]. These molecular targets are crucial for designing new drugs, which are able to directly affect the targets. In targeted therapy small molecules are used to enter inside the cell, and attack a specific protein or gene [40], as well as monoclonal antibodies, which in turn are dedicated for targets placed outside the cells [41], and cancer vaccines as well [39]. The main limitation for molecular targeted therapy is its effectiveness only in cells that express a particular target. Moreover, as in CT, development of drug resistance can be expected [39].

Although the growing number of preclinical and clinical studies show satisfactory and promising results for patients at different types and stages of cancers [42–45] leading to decrease of overall cancer morbidity and mortality, the new strategies for cancer treatment are highly needed to reduce side effects and improve patient prognosis. Molecular biology combined with nanotechnology seems to be the key direction in searching for new cancer strategy assuring low-cost, non-invasive and more personalized oncological care [46].

3 Understanding Targeted Therapy

Targeted therapy is one category of cancer treatment that uses CT drugs as well as other compounds to precisely recognize and assault strictly defined types of cancer cells. Targeted therapy has an anticancer effect through numerous mechanisms, including: induction of apoptosis, inhibition of proliferation, suppression of metastasis, regulation of immune function as well as multidrug resistance reversal [47]. Trastuzumab, a recombinant monoclonal antibody, which can recognize the extracellular domain of human epidermal growth factor receptor 2 (HER2) transmembrane protein, was one of the first target-specific drugs that have been registered for clinical use of cancer. Recently, designing targeted drugs and incorporating them into treatment has become the therapeutic standard [48]. Modern targeted therapies include: monoclonal antibodies [48], antibody-drug conjugates [49], nanobodies [50], antiangiogenic agents [51], signal transduction inhibitors [52], immunotherapeutic agents [53], cancer stem cells targeted drugs [54], miRNAs [55] and complexes of super-enhancers targeted agents [56].

3.1 Antibody–Drug Conjugates (ADCs)

Antibody–drug conjugates (ADCs) are a category of innovative and promising therapeutic strategy for cancer therapy. ADCs are able to target highly expressed antigens on the surface of carcinoma cells and then selectively deliver cytotoxic active agents. The goal of this therapy is to reduce systemic cytotoxicity in comparison with classic CT drugs and optimizing tumour targeting. Researchers aim to improve the effectiveness of ADCs by using a cleavable linker, which allows the delivery of the toxic payload to surrounding cells that do not expressed the target protein. Thus, ADCs act not only on the heterogeneous tumour, but also on its microenvironment consisting of various cell populations [49].

3.2 Nanobodies

The development of targeted therapy significantly expanded the treatment options for oncological patients and sets new directions of research in the field of anti-cancer therapies. Monoclonal antibodies have become a treatment standard in recent years. Despite the popularity of this therapy, traditional monoclonal antibodies have a number of limitations related to their use, such as limited ability to penetrate the tumour, high ability to develop therapeutic resistance and high production costs. Recently discovered nanobodies are an alternative to monoclonal antibodies. By combining the therapeutic advantages of standard antibodies and the targeting potential of nanoscale delivery, nanobodies approach allows high translational potential in preclinical and clinical studies [50].

3.3 Antiangiogenic Agents

The vascular endothelial growth factor (VEGF) pathway is the key mediator of angiogenesis in cancer. Creating new blood vessels on the basis of an existing one is the key process for supplying nutrients and oxygen to proliferating cancer cells, which promotes tumour growth and formation of distant metastases. Therefore, many types of therapies, including tyrosine kinase inhibitors or monoclonal antibodies target this axis [51].

3.4 Immunotherapeutic Agents

Targeting innate checkpoint molecules on macrophages and natural killer (NK) cells has appeared as a new rational approach against tumours, which are resistant to T cell-mediated immunity. Because different monoclonal antibodies against carcinoma surface proteins have been clinically approved in haematological disorders, innate checkpoint blockade can play a pivotal role in augmenting phagocytosis and antibody-mediated cellular cytotoxicity [57]. Targeting the programmed cell death protein 1 (PD-1) and programmed cell death protein 1 - ligand (PD-L1) interactions is a relatively novel cancer therapeutic strategy. Inhibitors of PD-1/PD-L1 include small-molecule chemical compounds, peptides and antibodies [53].

3.5 Cancer Stem Cells (CSCs)-Targeted Therapy

Cancer stem cells (CSCs) are a population of cancer cells, which is responsible for tumour initiation, metastasis and relapse as well as drug and radiation resistance.

Therefore, targeting CSCs is considered a novel potential anticancer-targeted therapeutic strategy. CSCs play a key role in immune evasion, immunomodulation and effector immunity, which changes immune system balance. NOTCH, mammalian target of rapamycin (mTOR), sonic hedgehog signalling molecule (SHH) and Wnt/β-catenin are associated in CSCs targeted therapies due to the fact that they are involved in regulation the CSCs colonies progression and drug resistance. Understanding the signalling pathways regulating progression of CSCs and drug resistance is crucial in conducting effective targeted therapies [54].

3.6 MiRNAs-Targeted Therapy

MicroRNAs are able to regulate activity both oncogenes and tumour suppressor genes. Therefore, alteration in the expression of microRNAs can lead to tumorigenesis. Expression profiling of microRNAs has increased the possibilities of application of microRNAs as potential biomarkers and targeted therapeutic targets in cancers [55].

3.7 Complexes of Super-Enhancers Targeted Therapy

The overexpression and hyper activation of oncogenes commonly occur in many types of cancers. Latterly, the increased activation of oncogenes by super-enhancers (SEs) has attracted significant attention. Numerous studies indicate that the SEs and their associated complexes play an important role in the development of different types of malignant tumours. Clinical trials have demonstrated that small-molecule inhibitors, like bromodomain containing 4 (BRD4) and cyclin-dependent kinase 7 (CDK7) inhibitors are able to target the SEs resulting in considerable positive effect on cancer treatment [56].

3.8 Nanotechnology-Based Histone Deacetylase Inhibitors

Epigenetic reprogramming, including DNA histone modification and DNA methylation, regulates the expression of genes involved in immune checkpoints, cellular proliferation and the response to antineoplastic drugs [58]. Histone acetylation and deacetylation catalyzed by histone acetyltransferases (HATs) and histone deacetylases (HDACs) are the posttranslational epigenetic mechanisms of gene expression regulation. These epigenetic modifications of DNA structure affect the action of transcription factors, which can repress or induce gene transcription. Mutations and changes of the expression of HDAC genes can cause the aberrant transcription of key genes, which regulate many pivotal cancer pathways, such as cell-cycle regulation, cell proliferation or apoptosis [59]. Histone deacetylase inhibitors (HDIs) have been accomplished therapeutic success in haematological diseases. Unfortunately, their application in solid tumours is hampered by the low treatment efficacy and confronts big challenges. Medicine with the use of nanotechnology could prolong the circulation half-life, improve drug stability and increase intratumoral drug accumulation. Hence, nanomedicine seems to be a promising approach to enhance HDIs therapy efficacy [60].

Targeted drugs used in the most common types of cancer (breast, colorectal, lung, prostate, skin) are summarized in Table 1.

Hence, since targeted medicine or targeted therapy means precise drug efficiency combine with minor side effects and interaction, at the molecular level, between a biomolecule and a drug, multifunctional drug delivery systems such as polymeric nanocomposites can be designed as biofunctionalized carriers that not only transport drugs but also, when combine to biomolecules such as membrane receptors, nucleic acids, antibodies and enzymes, are able to efficiently target cancerous cells.

4 Nanocomposites

Polymers demonstrate several advantages when it comes to develop drug delivery systems due to their ability to maintain a suitable stability and enhance mechanical and physical properties of compounds, however, depending on the polymer chose, if synthetic or natural, it can present some limitations for specific types of tumours and therefore, restrict its application. Combinations and composites of polymers can bypass these drawbacks and improve the quality of these systems, moreover, by combining nanosized materials while producing nanocomposites the drug delivery system can have multifaceted uses and purposes and it might be able to reach challenging areas such as the brain.

Amid the benefits of a nanocomposite system, this approach can avoid nanoparticle (NP) agglomeration by using a polymeric matrix where the NP can be dispersed [67], besides, the nanocomposite biodegradability increases after producing a composite with nanosized systems [68]. By rule, a nanocomposite is a two-phase system, where, at least one constituent must present a nanosized dimension up to 100 nm [69]. An important characteristic of nanocomposites is a large surface area, which results in higher interaction between its nanocomponents with the polymeric matrix [70].

Likewise, nanocomposite drug delivery systems theoretically are able to achieve requirements to deliver an effective cancer treatment owing to the following features:

a. Nanocomposites enhance drug pharmacodynamics and pharmacokinetics profiles.
b. Nanocomposites can selectively eradicate tumour cells without affecting healthy cells.
c. Nanocomposites prolong and control the release of drugs.

Table 1 Targeted drugs used in the most common types of cancer based on https://www.cancer.org

Type of the cancer	Subtype of the cancer	Type of targeted therapy	Drug	Reference
Breast cancer	Targeted therapy for HER2-positive breast cancer	Monoclonal antibodies	Trastuzumab–pertuzumab Hyaluronidase	[61]
		Antibody–drug conjugates	Ado-trastuzumab emtansine Fam-trastuzumab deruxtecan	
		Kinase inhibitors	Lapatinib, Neratinib Tucatinib	
	Targeted therapy for hormone receptor-positive breast cancer	CDK4/6 inhibitors	Palbociclib Ribociclib Abemaciclib	
		mTOR inhibitor	Everolimus	
		PI3K inhibitor	Alpelisib	
	Targeted therapy for women with *BRCA* mutations	PARP inhibitors	Olaparib Talazoparib	
	Targeted therapy for triple-negative breast cancer	Antibody–drug conjugate	Sacituzumab govitecan	
Colorectal cancer	Targeted therapy for colorectal cancer	Drugs that target blood vessel formation (VEGF)	Bevacizumab Ramucirumab Ziv-aflibercept	[62]
		Drugs that target cells with *EGFR* mutations	Cetuximab Panitumumab	
		Kinase inhibitor	Regorafenib	
Lung cancer	Targeted drug therapy for non-small cell lung cancer	Angiogenesis inhibitors	Bevacizumab Ramucirumab	[63]
		EGFR inhibitors used in NSCLC with *EGFR* mutations	Erlotinib Afatinib Gefitinib Osimertinib Dacomitinib	
		EGFR inhibitors that target cells with the *T790M* mutation	Osimertinib	
		EGFR inhibitors used for squamous cell NSCLC	Necitumumab	

(continued)

Table 1 (continued)

Type of the cancer	Subtype of the cancer	Type of targeted therapy	Drug	Reference
		Drugs that target cells with *ALK* mutations	Crizotinib Ceritinib Alectinib Brigatinib Lorlatinib	
		Drugs that target cells with *BRAF* changes	Dabrafenib Trametinib	
		RET inhibitors	Selpercatinib	
		MET inhibitors	Capmatinib	
		Drugs that target cells with *NTRK* mutations	Larotrectinib Entrectinib	
Prostate cancer	Targeted therapy for prostate cancer	PARP inhibitors	Rucaparib Olaparib	[64]
Skin cancer	Targeted therapy for basal and squamous cell skin cancers	Hedgehog pathway inhibitors	Vismodegib Sonidegib	[65]
		EGFR inhibitors	Cetuximab	
	Targeted therapy drugs for melanoma skin cancer	BRAF inhibitors	Vemurafenib Dabrafenib Encorafenib	[66]
		MEK inhibitors	Trametinib Cobimetinib Binimetinib	
		Drugs that target cells with *C-KIT* changes	Imatinib Nilotinib	

Abbreviations: ALK - anaplastic lymphoma kinase, BRAF—v-raf murine sarcoma viral oncogene homolog B1, BRCA—breast cancer gene, CDK4/6—cyclin-dependent kinase, EGFR—epidermal growth factor receptor, HER-2—human epidermal growth factor receptor 2, MET—hepatocyte growth factor receptor, mTOR—mammalian target of rapamycin, NSCLC—non-small-cell lung carcinoma, NTRK—neurotrophic receptor tyrosine kinase, PARP—poly (ADP-ribose) polymerase, PI3K—phosphatidylinositol 3-kinase, VEGF—vascular endothelial growth factor

d. Nanocomposites improve the cellular uptake of delivered drugs by a targeted approach.
e. Nanocomposites can diminish drugs dose decreasing its side effects [8, 11, 12, 71, 72].

5 Polymeric Nanocomposites

Recent advances in the biological, chemical and physical fields combined with the challenges and possibilities in nanomedicine have led to new developments in polymer-based nanocomposites for diverse biological applications.

Polymeric nanocomposites are very attractive structures with a dual assembly: one phase is called reinforcing (strong and low-density materials) and is embedded in the matrix phase (tough or ductile materials) [73] and consists of nanomaterials and polymers (synthetic or natural) that form a multiphase solid material [73, 74]. These complex materials generate an adjustable platform with different properties and functionalities, improving the overall features of the component materials used for their synthesis.

Polymeric nanocomposites present several advantages including the retaining, protecting and releasing of biological compounds such as drugs, genes, enzymes and fluorophores for treatment, imaging and diagnostics [75]. Nanocomposites also present advantages as enhanced chemical, electrical, thermal, magnetic, optical, catalytic and mechanical properties [76]. Therefore, polymeric nanocomposites promote enhanced solubility in aqueous medium, high stability in biological systems and increased biocompatibility [74, 75, 77]. Consequently, this multifaceted matrix has shown great potential in drug and gene delivery as suitable drug carriers due to improved features compared to pure NP and polymers.

NP addition into polymeric matrix changes the characteristics of polymers as drug carriers such as: decreases the burst release leading to slower and sustained release, improves drug stability, allows the encapsulation with two or more compounds and facilitates active targeting by functionalization with specific receptors [78]. The drug delivery behavior by polymeric nanocomposites has been evaluated in several studies due to unique features. Both organic and inorganic particles are silica, gold, carbon nanotubes, quantum dots, graphene, liposomes, dendrimers and with diverse forms are core-shell, tubes, sheets, spherical, cylindrical, bring great potential for polymeric nanocomposites on the biomedical field [79–81]. The NP have many advantages to the drug delivery system because of their adjustable particle size, charges and surface [82].

5.1 *Types of Polymers Used for Nanocomposites Synthesis*

Several polymers can be applied for biological purposes such as natural polymers including polysaccharides or proteins and synthetic polymers. The most common polymers used for nanocomposites synthesis are listed in Table 2.

The natural polymers present advantages as biological recognition, remarkable interactions with cells to promote proliferation, adhesion, non-immune response, and biodegradability [97]. However, they demonstrate poor mechanical strength, high speed of degradation and limited supply [98].

Table 2 Types of polymers (natural and synthetic) used for nanocomposites synthesis

Polymers (synthetic and natural)	Biomedical applications/ characteristics	Source	Reference
Polycaprolactone (PCL)	Drug carrier; implantable material	Synthetic	[83]
Poly (methyl methacrylate) (PMMA)	Drug carrier (high drug permeability); biocompatible	Synthetic	[84]
Poly (L-lactic acid) (PLLA)	Drug carrier; scaffolds for tissue regeneration	Synthetic	[85]
Poly (lactic-co-glycolic) acid (PLGA)	Biocompatible; tailorable degradation rate; ease modifying the surface	Synthetic	[86]
Poly (ethylene glycol) (PEG)	Biocompatible; soluble in water; drug carrier	Synthetic	[87]
Polystyrene (PS)	Biocompatible; drug delivery	Synthetic	[88]
Polyvinylidene fluoride (PVDF)	Thermal stability; stimulus-responsive; tissue regeneration	Synthetic	[89]
Polyvinylalcohol (PVA)	Easy degradable; biocompatible decompose necrotic masses	Synthetic	[90]
Poly (glutamic acid) (PGA)	Biodegradable, biocompatible; water-soluble	Synthetic	[91]
Poly (acrylic acid) (PAA)	Biocide properties; biocompatible	Synthetic	[92]
Polyethyleneimine (PEI)	Drug delivery; attachment promoter	Synthetic	[93]
Alginate	Drug delivery; cell transplantation; biocompatible	Natural	[94]
Collagen	Cell attachment ability; biodegradation	Natural	[95]
Chitosan	Antibacterial activity; hydrophilicity; bone regeneration	Natural	[96]
Cellulose	Hydrophilicity; biofunctionality; biocompatible	Natural	[94]
Hyaluronic acid	Swelling capability; non-immunogenic	Natural	[95]
Starch	Biodegradable; biocompatible	Natural	[94]
Gellan gum	Bioadhesive; biocompatible	Natural	[94]

(continued)

Table 2 (continued)

Polymers (synthetic and natural)	Biomedical applications/ characteristics	Source	Reference
Chondroitin sulphate (CTS)	Biodegradable; water adsorbent	Natural	[95]

Abbreviations: CTS—Chondroitin sulphate,PAA—Poly (acrylic acid), PCL—Polycaprolactone, PEG—Poly (ethylene glycol), PGA—Poly (glutamic acid), PLGA—Poly (lactic-co-glycolic) acid, PLLA—Poly (L-lactic acid), PMMA—Poly (methyl methacrylate), PS—Polystyrene, PVA—Polyvinylalcohol, PVDF—Polyvinylidene Fluoride

In contrast, synthetic polymers present good mechanical properties, controllable degradability, adequate supply, and they are cheaper. However, some synthetic polymers can show uncontrollable shrinkage and possible local toxicity [99].

Nanocomposites are very interesting structures to overcome these disadvantages and expand the potential of polymers by the connection between them and NP, improving the overall characteristics of materials.

5.2 *Polymeric Nanocomposites and the Advantages for Cancer-Targeted Therapy*

Material science has been an important tool bringing innovations to the treatment, diagnosis, imaging, contrast agent, photothermal ablation agents and magnetic resonance imaging (MRI) in cancer through the materials composition [100]. Due to their complex structure with NP and specific matrix carriers, polymeric nanocomposites improve a set of factors increasing the drug effectiveness in the biological system [101, 102]. CT drugs can affect the healthy tissues and not just the tumor; therefore, these nanomaterials have shown great potential for target-specificity drugs reducing the side effects and increasing the treatment effectiveness [103, 104].

Polymeric nanocomposites achieve many advantages on their use for cancer treatment, such as:

a. Increases the lifetime of chemotherapeutics;
b. Improves the solubility of hydrophobic drugs;
c. Allows the controlled and sustained drug delivery;
d. Enhances the bioavailability due to accumulation of nanosystems in the tumor tissues;
e. Protects the drugs against degradation mechanism of the body;
f. Avoids immunological recognition by surface functionalization;
g. Permits site-specific active targeting through the use of ligands as antibodies, peptides, growth factors;
h. Avoids multiple-drug resistance due to passive targeting;
i. Allows multimodal system acting, concerning different therapeutic approaches (such as hyperthermia) and diagnosis (such as bioimaging).

These advantages make polymeric nanocomposites a potential system for improved treatment if compared to traditional therapies [93, 105–107]. Different types of nanosystems-based nanocomposites with their formulation techniques are summarized in Table 3.

Polymeric nanocomposites have wide applications in controlled, sustained and targeted drug delivery. As shown in the table above, these systems present a set of advantages for cancer therapy as pH-dependant behavior, infrared-light sensitivity, multitargeting and specific targeting as well. The multifunctional features of nanocomposites bring new alternatives in many fields, such as molecular medicine, mostly cancer diagnostics, therapeutics, theranostics and imaging.

5.3 Localized Treatment of Solid Tumors with Polymeric Nanocomposite Systems

Worldwide, large efforts have been made in order to develop nanocomposites systems for localized treatment of tumors, owing to the systemic side effects associated with CT-based approaches. Another important fact is the major percentage of patients with cancer suffer from metastasis [131, 132]. Moreover, some drugs, biomolecules or nanocarriers cannot penetrate the body barriers including membranes, the brain blood barrier (BBB) and the brain tumor barrier (BTB), which suggests the necessity of targeted drug delivery systems for solid tumors therapy.

5.3.1 Injectable Hydrogels

The use of composite nanosystems also could be beneficial for the treatment of solid tumors prior or after surgical procedures guaranteeing suitable drug concentrations in the tumor site and affected regions. Localized treatment can be achieved mainly by using two platforms: intratumoral injection or direct implantation into the tumor site. Even though surgical resection is the standard procedure to treat solid tumors, the complete resection is often impossible and the tumor recurrence incidence is a still a challenge [133]. Thus, with the benefits that the mechanical properties of polymers provide, polymeric hydrogels can be injected into tumors greatly improving the stability of common used drugs.

Recently, Cacicedo et al. produced a nanocomposite by combining cellulose hydrogel with DOX-loaded lipid nanocarriers. It was observed that its intratumor administration in vivo in an orthotropic breast cancer mouse model significantly reduced tumor size, metastasis incidence and side effects associated with DOX application [134], suggesting that is possible to achieve better responses with lower doses of CT drugs. Another possibility for injectable polymeric nanocomposites is the delivery of photothermal therapeutic agents which provide an in situ thermal effect and the drug release can be controlled by local light-radiation heating. Hence, an

Table 3 Polymeric nanocomposites and their formulation techniques, which have conferred targeting ability and efficacy for anticancer therapeutics

Polymeric nanocomposites	Fabrication technique	Therapy and experimental model used and outcome	Reference
PMMA-Si–Gd NPs functionalized with folic acid	Self-assembly	DOX; MCF-7 (breast cancer cell line); pH-responsive; prevent drug leakage, targeting effect in breast cancer cells	[108]
Tungsten disulphide nanotubes-ceric ammonium nitrate-PEI (WS_2-NT-CM-PEI)	Focused ultrasound in an emulsion solvent diffusion	PTT; MCF-7 and HeLa (cervical cancer cell line) cells; improved PTT activity in the functionalized group	[109]
Poly(N-isopropylacrylamide)-metal nanoparticles (PDMS)	Soft lithography	MDA-MB-231 (Breast cancer cell line); device multimodal implantable	[9]
Gold nanorod-attached PEGylated graphene-oxide (AuNR-PEG-GO)	Self-assembly	PTT; A431(epidermoid carcinoma cell line) and xenograft mice were used for testing; improved PTT activity	[110]
PLGA polymeric vesicles-Quantum dots	Emulsion evaporation	Busulfan; J774A (macrophage) cells and rats were used for testing; enhanced drug delivery and improved imaging	[111]
PLGA-PEG-nanoparticles	Double emulsion method	Curcumin; MCF-7 cells; enhanced cytotoxic effects	[112]
PEG-phospholipids-graphene oxide	Hydration method	Resveratrol; Mice; chemo-PTT; eradicated xenografted tumor	[113]
PEG-PCL micelles functionalized with cyclic RGD peptide	Core-crosslinked; Solvent exchange	DOX; U87-MG (glioblastoma) cells; specific target	[114]
PLGA-Silver (Ag) nanofibres	Electrospinning	Hep-G2 (Liver carcinoma cell line); enhanced antitumor activity	[115]
PVA-graphene oxide-organoclay (PVA-ODA-MMT)	Electrospinning;	Osteocarcinoma cells; enhanced antitumor activity	[116]
PCL-polyurethane-Au nanoparticles (PCL-Diol-PU/Au)	Sol–gel	Temozolomide; U87-MG cells; lower burst release; gold coating enhanced the cytotoxicity	[117]

(continued)

Table 3 (continued)

Polymeric nanocomposites	Fabrication technique	Therapy and experimental model used and outcome	Reference
Polyethylene glycolated methotrexate-PEG-chitosan-iron oxide nanoparticles (MTX-PEG-CS-IONPs)	Electrospinning	Prodrug: MTX-PEG; Cy5.5; HeLa cells and xenograft mice; improved anticancer activity	[118]
Organic frameworks-cyanine-Cis-aconityl-DOX (COF-IR783-CAD)	Non-solvent-aided coacervation followed by a chemical crosslinking	DOX; 4T1 (breast cancer cell line) and xenograft mice; chemo-PTT; significant tumor ablation	[119]
Sodium alginate-Poly dopamine-Polyvinylpyrrolidone (ALG/PDA-PVP)	Reversible condensation	DOX; HT29 (human colon cancer cell line); enhanced therapeutic effect	[120]
Polypyrrole/chitosan shell Ag (AgCl/PPC)	One-step electrostatic spraying	3-amino-2-phenyl-4(3H)-quinazolinone; Ehrlich ascites carcinoma cells (EAC); increased cell death in group treated compared to empty formulation and pH-dependant release	[121]
Iron oxide-polyethylene-glycol NPs (Fe3O4-PEG-NPS)	Magnetic stirring and dialysis	DOX or paclitaxel; A2780 and OVCAR-3 (human epithelial ovarian cancer cell lines); apoptosis activation through NF-kB and BAX overexpression and Bcl-2/survivin inhibition; DOX-loaded was more efficient than paclitaxel-loaded in vivo	[122]
PMAA-grafted Chitosan NPs with Fe3O4 (DOX@Fe3O4@CS/MAA)	Coprecipitation	DOX; MDA-MB-231 and MCF-7 (breast cancer cell lines); cell viability reduction and DNA fragmentation; pH-dependant release	[123]
Gold nanorods dopamine and PEG-coated (AuNR-PDA)	Graft-polymerization by crosslinking	DOX or Methylene blue; HeLa cells and xenograft mice; ROS generation under laser irradiation and apoptosis induction; PTT combined therapy led to improved tumor reduction	[124]

(continued)

Table 3 (continued)

Polymeric nanocomposites	Fabrication technique	Therapy and experimental model used and outcome	Reference
N-naphthyl-O-dimethymaleoyl chitosan micelles with nanocrystals of iron(III) and manganese(II) (NCS-DMNPs)	Pegylation and self-polymerization	DOX; NIH3T6.7 (fibrobasts) cells; bioimaging (diagnosis) and drug delivery	[125]
Fe_3O_4 PLGA-PVP-coated nanoparticles anchored with Herceptin and tamoxifen	Reductive amination and dialysis	Tamoxifen; MCF-7 and HeLa cells; hemocompatible and pH-dependant release; apoptosis induction and selective uptake by MCF-7 (HER2 +); PTT combined therapy with tumor reduction	[126]
N-isopropylacrylamide (NIPAm) and methacrylated β-cyclodextrin-based macromer (MβCD) with incorporated Au-NRs and AD-DOX (NIPAm- MβCD-AuNRs-AD-DOX)	Precipitation and dialysis	DOX and PTT; MCF-7, HeLa cells and S180 (murine sarcoma) cells; slow 1-month release from implant; PTT combined therapy with drug release rate increase; biocompatible and pH-dependant system	[127]
Pluronic F127 system with iron oxide (PF127-Fe_3O_4-DOX)	Self-crosslinking copolymerization	DOX; BE–2–M17 (neuroblastoma cell line) cells; pH-dependant behavior; effective in vitro	[128]
Lanthanide upconverted NPs co-doped with YB + 3 and TM + 3, mesoporous silica coated with a folate-conjugate and copolymerized MAPEG (MUCNPs@C18@PSMN-FA)	Emulsion/solvent evaporation technique	DOX; Human KB cell lines with folic acid receptor, A549 (human alveolar adenocarcinoma cell line) without folic acid receptor and Beas2B (Human bronchial epithelial cells); NIR mediating release and folate-receptor targeting; bioimaging	[129]
Dopamine loaded poly(N-isopropylacrylamide)-propylacrylamide copolymer (pNIPAm-co-pAm/DP)	Free radical polymerization and self-assembly	PTT and Bortezomib or DOX; MC3T3-E1 (osteoblast precursor cell line) and CT26 (colon cancer cell line) cells; PTT combined therapy with apoptosis and irreversible changes in cell morphology; pH-dependant and NIR-dependant release	[130]

Abbreviations:Ag—Silver, AuNR—Gold nanorod, CS—chitosan, DOX—doxorubicin, DP—dopamine, GO—graphene oxide, UONPs—iron oxide nanoparticles, MAPEG—Poly (ethylene glycol) methacrylate, MTX-PEG—methotrexate-PEG, PCL—Polycaprolactone, pAM: propylacrylamide, pNIPAm—poly(N-isopropylacrylamide), PDNH—poly(DBAM-co-NASco–HEMA),PDMS—Poly(N-isopropylacrylamide), PEG—Poly (ethylene glycol), PLGA—Poly (lactic-co-glycolic) acid, PMAA—Poly(methacrylic acid), PMMA—Poly (methyl methacrylate), PTT—Photothermal therapy,PU—polyurethane, PVA—Polyvinylalcohol, RGD peptide—Arginylglycylaspartic acid, WS2-NT-CM-PEI—Tungsten disulphide nanotubes-ceric ammonium nitrate-PEI, NIPAM—N-isopropylacrylamide, NIR—Near-infrared irradiation, PTT—Photothermal therapy and ROS—reactive oxygen species ideal system must be multifaceted and biocompatible at the same time, and it should control the release of sufficient CT drugs and other agents such as photothermal agents for extended periods and preferentially target only cancerous cells. In view of this, Liu et al. designed and produced a very elegant injectable nanocomposite hydrogel by using methoxy poly(ethylene glycol)-b-poly(ε-caprolactone-co-1,4,8-trioxa[4.6]spiro-9-undecanone) (mPECT) diblock copolymer with gold nanorods (AuNR-PECT) combined with paclitaxel-loaded mPECT NP (PTX/mPECT NP) and α-cyclodextrin (α-CD) for local chemo-photothermal synergetic cancer therapy by applying near-infrared radiation (NIR). The authors tested this system in breast cancer models (in vitro and in vivo) and observed that it was capable of delivering a synergetic treatment and inhibiting tumor growth and recurrence [133]. What makes this system incredibly promising is the fact that it can be applied for different combined and multidrug approaches for targeting different types of tumors by changing the target ligands.

In this context, Xu et al. synthesized a core-shell nanocomposite with PEGylated (polyethylene glycol modified) magnetic Prussian blue (PB) NP for the controlled release of DOX, targeted photothermal ablation and pH-triggered CT of tumor cells. The authors observed a significant growth inhibition in vitro in HeLa cells [135]. However, more experiments are necessary to prove these effects. Similarly, Fan et al. produced cyclo (Arg-Gly-Asp-d-Phe-Cys) [c(RGD)] conjugated DOX-loaded PEG Fe3O4@polydopamine (PDA) NP to achieve an integrated tumor diagnosis and treatment [136]. It was shown that this system was able to target tumor cells and it presented a suitable stability, moreover, by using xenograft tumor nude mouse injected with HCT-116 cells it was possible to detect clear contrast signals therefore, this nanocomposite demonstrated effective CT-photothermal therapy under NIR irradiation.

Magnetic NP have been considerably studied to treat cancer cells and more recently, they attracted attention to be used as a multimodal therapy owing to the possibility to deliver drugs and heat locally [137–144]. From this perspective, Hervault et al. developed magnetic nanocomposites by combining a thermo and pH responsive polymeric shell (PEG methyl ether methacrylate—PEGMA, di(ethylene glycol) methyl ether methacrylate—DEGMA, 3-(trimethoxysilyl)propyl methacrylate—TMSPMA and 3-vinylbenzaldehyde) with an iron oxide core [145] for the delivery of DOX. This system demonstrated suitable physical and chemical properties and showed potential for in vitro and in vivo testing. Taken together, these studies provide a in situ strategy for the clinical application of nanocomposites in cancer CT-phototherapy.

6 Future Challenges in Cancer Therapy

One of the biggest challenges related to the applicability of polymeric nanocomposites is the translation of an in vitro study to an in vivo study. It is known that most frequent actions for in vitro release studies embrace: cumulative release that occurs when a compound is released into the same amount of media volume and non-cumulative release that takes place when there is a continuous replacement of the media mimicking a living organism where the drug concentration drops.

These methods are commonly used in in vitro biological experiments where cytotoxic analysis is performed by using cancer cell lines. Although experimental conditions of in vitro evaluations mimic those of living organisms, for complex drug delivery system as nanocomposites to be recognized as appropriate and safe for antitumor treatment, in vivo studies are still indispensable. Unfortunately, only a few studies tested nanocomposites systems in vivo, therefore their applicability is still uncertain.

In all assurance, to reach a successful therapy without harming healthy tissues has been the key effort of targeting approaches. It is estimated the enhancement of drug delivery to cancer cells with an improved well-developed nanoproduct [6, 7], however, even with intense research developing in the nanocomposites field, no significant clinical results have been described. It is a challenge to bypass drug delivery issues such as early drug degradation, bloodstream circulation time, tissue membranes and systemic toxicity, therefore, for some cancers, the main promising approach to achieve suitable drug delivery is a biofunctionalized nanocarrier. In fact, several nanocarriers have an initial burst release that can cause acute toxicity or release the total amount of a therapeutic compound before reaching the tumor cells, fortunately, both of these issues can be addressed by using targeted drug delivery with polymeric nanocomposites.

7 Multiscale Molecular Simulation for Nanostructured Polymer Systems

Nanostructured polymer systems present particular features which range from the angstrom level of an individual bond between atoms, to nanometres of the polymer chain, micrometres, millimetres, larger in solutions and polymeric nanostructured (Fig. 1).

The different time scales for each material properties may range from femtoseconds to seconds or even hours. On the literature, there are many examples of multiscale nature of nanostructured polymer systems [147–155]. Because of that, several computational methods were developed in order to address these issues [156–164]. These new methods present novel options to design, optimize and predict polymeric structures and properties.

Until the present moment, no computational method is able to cover different size scales of polymers [165] systems. Therefore, the multiscale simulation framework is considered one of the best choices to deal with this issue. The multiscale approach combines various methods and, in the computational chemistry field, it is considered one of the key topics. In order to perform a multiscale simulation, different theories

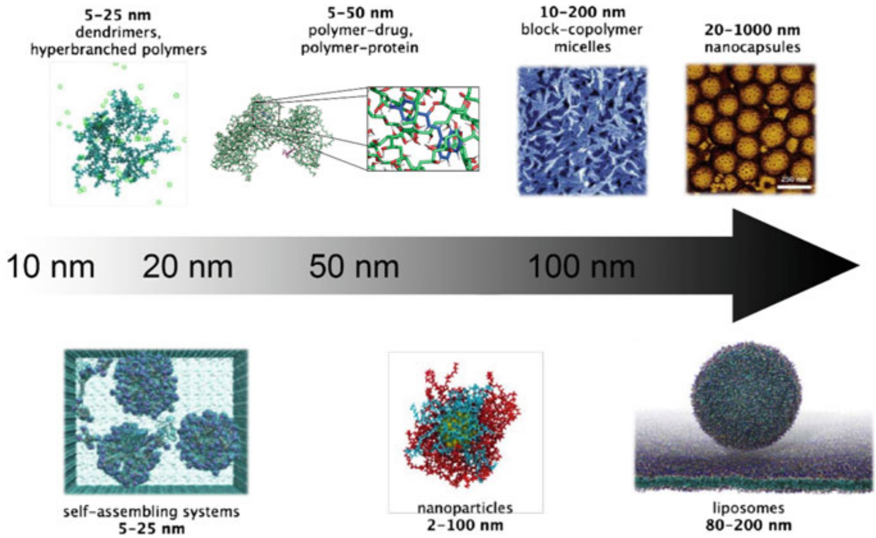

Fig. 1 Nanostructured polymer systems of potential interest for biomedical applications. Increasing structural complexity the dimensions of polymeric systems change from few nanometres to hundreds of nanometres Adapted from Laurini et al. [146]

and models from four characteristics length and time scales are combined. These features can be divided into the following scales:

I. The quantum scale: In this method, the nuclei and electrons are part of the calculation, and quantum mechanics (QM) calculations are used to model their state. This approach allows to investigate several phenomena related to chemical reaction, such as rupture and formation of chemical bonds between atoms, the transitions in electrons configurations and other important phenomena on polymers material, that need to be modelled at quantum scale.

II. The atomistic scale: In the atomistic calculations, all atoms are explicitly represented and treated by a single spheres. The force field, typical interactions in the system, is responsible for the potential energy of the system. These interactions include the bonded interactions that are the bond length, the bond angle and the dihedral angle potentials between atoms. In addition to the bonded interactions, force fields also comprise non-bonded interactions. Non-bonded interactions act between atoms in the same molecule and those in other molecules. Force fields typically distribute non-bonded interactions into two: electrostatic interactions and van der Waals interactions. Molecular dynamics (MD) and Monte Carlo (MC) simulations are examples used at this level to model molecular processes comprising a larger group of atoms, such as proteins, membranes and nucleic acids.

III. The mesoscopic scale: At this scale, a molecule is defined as a microscopic particle, identified as a bead. In this method, some details of the system are presented indirectly which offers the opportunity to simulate the phenomena on time scales barely accessible by atomistic simulations. An interesting example is coarse-grained model, where the particles are accumulated in beads. During the calculation, interactions between the beads are used to characterize the system. There are a several methods that were developed to investigate the mesoscopic structures of polymers, including: Brownian dynamics (BD), lattice Boltzmann (LB), dissipative particle dynamics (DPD), dynamic density functional theory (DDFT) and time-dependent Ginzburg–Landau (TDGL).

IV. The macroscale: In this methodology, the characteristics of atoms and molecules are disregarded, and the system is considered as a continuous. The constitutive laws are responsible for the behaviour of the system. These laws are associated with conservation laws to simulate several phenomena. The main functions, such as velocity and stress components, are continuous. On the other hand, a finite amount of locations which separate continuity regions is considered discontinuous on these calculations. The central supposition at this scale is in substituting a heterogeneous material with a corresponding homogeneous model. To perform molecular simulations at this scale, there are used the following methods: finite volume method (FVM), finite element method (FEM) and finite difference method (FDM).

The development of nanostructured polymer systems requires a comprehensive knowledge of the phenomena at different time and length scales. Because of that, the theoretical and computational methods present great progress, allowing the study of these systems. Finally, given the peculiarities of the polymeric systems, no single method can be used for their simulation. Consequently, it is advantageous to rely on a multiscale molecular modelling approach that has been presented in this chapter. The methodology discussed is an overall design approach for complex nanostructured systems to be effectively interpreted during the experiments and for the design of active nanocomposites and nanosystems.

Final Considerations

Polymeric nanocomposites have been shown suitable mechanical and physicochemical properties to be use as drug delivery systems for cancer therapy. These properties including increased thermal stability, enhanced tensile strength and decreased side effects can be improved by using ligands to target cancer cells. Cancer-drug delivery obtained by targeted approaches has the potential to overcome CT limitations by increasing drug concentrations in the tumor cells and at the same time diminishing the side effects to the normal cells.

Although polymeric nanocomposites are able to provide an efficient drug delivery, it has been observed a lack of in vivo testing, which makes difficult to verify their efficacy. Thus, it is essential, for polymeric nanocomposites research, a collaboration among researchers, regulatory agencies and industry to ensure that these innovative approaches are effective, non-toxic and can be used in the treatment of patients. Further development in nanocomposite technology may prove potent, safe and efficient cancer-targeted drug delivery approaches.

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Biopolymeric-Inorganic Composites for Drug Delivery Applications

Shaimaa A. Khalid, Ahmed S. Abo Dena, and Ibrahim M. El-Sherbiny

Abstract The everyday progress in the development of new nanomaterials has grasped the attention of drug delivery researchers in recent years. The combination of more than one material to form composites in order to gain new interesting properties and enhance the performance of one another has shown a noticeable progress in the synthesis and applications of drug delivery systems. For instance, biocompatible and biodegradable materials such as biopolymers and inorganic materials are widely used in this aspect. This chapter focuses on the use of biopolymeric–inorganic composites in the preparation of drug delivery systems. The types of biopolymeric and inorganic materials that can be combined into composite materials and their characteristics are summarized herein. The given materials are just examples for the composite materials of interest, and many other composites can be synthesized from different types of inorganic and biopolymeric materials.

1 Introduction

The research of drug delivery and development field regulating the drug sustained release remains a major challenge with advances in technology to overcome the drawbacks of conventional drug therapies attributed to lack of selectivity, unfavorable pharmacodynamics, side effects, short circulating time, and limited drug solubility [1–3]. In addition, translational medicine presents a major obstacle for

S. A. Khalid · A. S. Abo Dena · I. M. El-Sherbiny (✉)
Nanomedicine Laboratory, Center for Materials Science, Zewail City of Science and Technology, 6th of October City 12578, Giza, Egypt
e-mail: ielsherbiny@zewailcity.edu.eg

S. A. Khalid
Food Hygiene Department, Animal Health Research Institute (AHRI), Agricultural Research Center, Dokki, Giza, Egypt

A. S. Abo Dena
Pharmaceutical Chemistry Department, National Organization for Drug Control and Research (NODCAR), Giza, Egypt

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the use of intelligent materials to control the distribution and release of different agents. The new successful methodologies used for drug delivery are of practical importance to prevent the unnecessary changes in drug stability and properties. In this context, drug delivery aims to (i) decrease the side effects of drugs by targeting the desired organ precisely and (ii) to control the release of the drug in order to avoid the classical overdose or underdose [4]. Furthermore, drug delivery systems (DDSs) protect the drugs of interest from rapid degradation or clearance and increases their concentration in the target tissues [5]. Sustained delivery of drugs poses essential advantages such as reducing the number of doses, limiting the side effects, lowering fluctuations in concentration in the blood stream, reducing dose-dumping risks, enhancing bioavailability, site specificity, and allowing better patient compliance [6, 7]. Numerous formulations for sustained drug release were developed in the form of microparticles [8, 9], nanoparticles [10, 11], tablets [12–14], beads [15–17], buccal patches [18], capsules [19–21], implants [22, 23] , etc. These drug releasing systems are commonly formulated using various excipients such as natural/synthetic/semisynthetic biopolymers, inorganic materials (such as metal powders), and bioceramics. Recently, various biopolymers–inorganic composites were developed in the form of nano- or micron-scale particles or fibers to sustain the release of certain drug substances over as long period of time as possible. The synergy of biopolymers and inorganic materials resulted in improved mechanical properties, better drug release sustainability and enhanced encapsulation/loading of many pharmaceutically active substances [24, 25].

Recently, modern science uses materials from natural sources for the treatment of a wide variety of diseases. The use of such natural materials in biomedical and clinical applications is increasing because of their biocompatibility, non-immunogenicity, good mechanical properties, and biodegradability [26]. Natural biopolymers can be obtained from a wide variety of microbes, plants and animals of both land and aquatic inhabitants. In addition, the functional properties of biopolymers such as rheology or gel-forming ability may be used for the chemical modification of pharmaceutical products or for the encapsulation of medicinal products for sustained delivery [27]. Based on their properties, chemically engineered natural biopolymers are the promising biomaterials for drug delivery systems instead of discovering new synthetic polymers due to above-mentioned fascinating characteristics. Functional groups such as hydroxyl, amino, and carboxylic acid groups have broad applicability and strong potential for cross-linking to induce biological inter action with cell-specific agents. Thus, the functionalization of biopolymers through these functional groups can be accomplished to improve their biocompatibility, facilitate the integration of cells, and provide new DDSs. Biopolymer derivatives from plant or animal sources consist of interstitial spaces and stretched cross-linked structures that assist in shape and volume modification depending on different stimuli such as temperature, pH, solvent composition, magnetic and electric fields. They are usually used for their hydrophilic nature and hydrogel-forming ability that may help in the entrapment of interesting bioactive molecules [28, 29]. They also provide softness, low interfacial tension, selective permeability, and super-absorbance [30]. Different shapes such as slab, cubical, and cylindrical can be tailored due to their hydrogel-forming ability.

Generally, composites are material mixtures consisting of two or more physically/chemically interacting materials in which one of them is the reinforcing phase (sheets, particles or fibers) and the other is the matrix phase (ceramics, polymers or metals) within which each constituent retains its distinct characteristics. Indeed, these materials offer new physico-mechanical properties to the composites [31, 32]. Composites are classified based on the composition of matrix reinforcement: inorganic–inorganic composite (e.g., ceramic–ceramic composites, metal–metal composites, ceramic–metal composites, etc.), organic–inorganic composites (e.g., polymer–metal composites, polymer–ceramic composites, etc.) and organic–organic composites [31]. Recently, various composites were developed for different biomedical applications such as the delivery of drugs, antimicrobial agents, cancer therapy, wound dressing, stem cell therapy, and biosensors [33–35].

2 Drug Delivery Systems

Drug delivery is a strategy for the delivery of drugs to specific body areas and the release of them in the desired rate. Locally releasing drugs can help reduce the side effects of systemic the rapies while, at the same time, maintaining more optimal concentration of drugs. Also, DDSs could enhance the drug bioavailability [36]. DDSs deal with the encapsulation and loading of drugs through drug carriers such as microparticles, nanoparticles, microcapsules, nanocapsules, hydrogel, implants, films spheroids, gels, dendrimers, beads, biocomposites, and scaffolds [37–39]. Hence, the main objective of using biopolymer carriers is to protect the drug against degradation or damage and to facilitate the delivery of the drug to the site of action precisely [40]. Accordingly, drug delivery may be steady, controlled, or targeted drug delivery. Common DDSs that can be integrated with drugs can be in the form of bulk, micro- or nanoforms.

3 Use of Biopolymers in Drug Delivery

Biopolymers are polymeric biomolecules composed of covalently bonded monomeric units derived from living organisms such as animals, plants, and microorganisms [39, 41]. Recently, a large number of biopolymers were classified as biodegradable biomaterials which can be gradually degraded in the body. Therefore, they are excellent candidates to be used in DDSs [41]. The most common biopolymers that found a wide implementation in DDSs are summarized (Fig. 1).

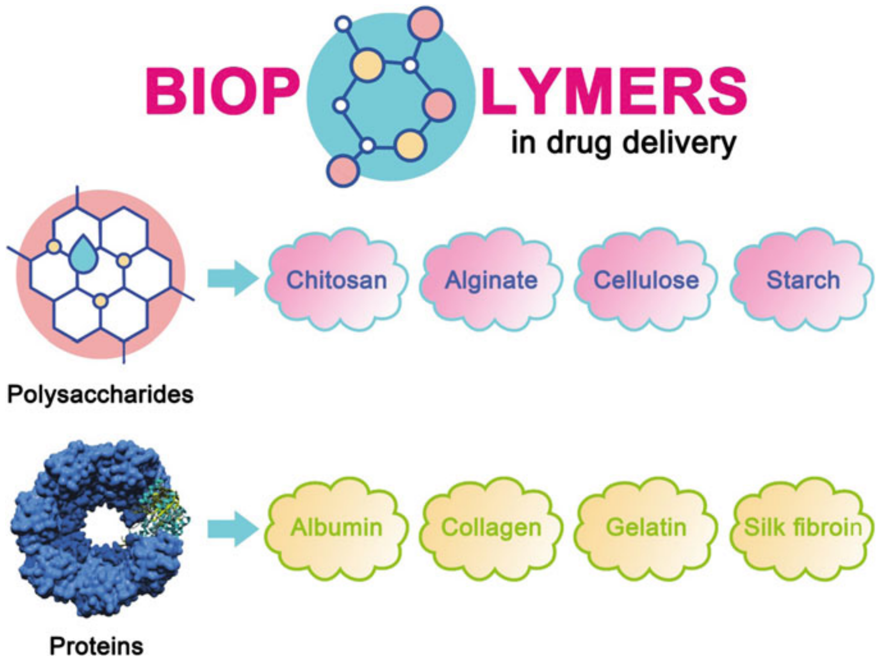

Fig. 1 Some of the common biopolymers used in drug delivery applications

4 Polysaccharides

Polysaccharides are composed of repeating units of monosaccharides. They can be used in DDSs after structural modifications [42]. Chitosan, cellulose, alginate, and starch are the most common polysaccharides used in drug delivery [29]. These common polysaccharides are summarized below.

4.1 Chitosan

Chitosan is a polycationic, non-toxic, highly biodegradable and biocompatible polymer derived from the shells of crustaceans, such as shrimps, prawns and crabs, in the form of chitin.Chitin is then modified by alkaline deacetylation to form chitosan consisting of β(1→4) glucosamine and N-acetyl glucosamine [29]. Chitosan has many advantages in developing micro/nanoparticles: (i) ability to control the release of active agents, (ii) avoids the use of hazardous organic solvents, and (iii) provides particles soluble in aqueous acid solutions. Chitosan has a great potential for pharmaceutical applications with high charge density, drug loading ability, and mucoadhesion [43]. As a biopolymer, it can be used for enhancing the bioavailability and release of phytochemicals in DDSs through the interactions of its positive amino groups. For example, polyphenols of strawberry extract were encapsulated in chitosan tripolyphosphate resulting in a sustained release profile of polyphenols at the physiological pH [44]. With the presence of tripolyphosphate (TPP) and polyglutamic acid (γ-PGA), chitosan can form hydrogel structures by ionotropic gelation. It can also form insoluble matrix by polyelectrolyte complexation with polyanions (such as alginate) or by precipitation at pH above its pKa value (i.e. around pH 6.5). In addition, in the presence of glutaraldehyde, it forms hydrogels by covalent cross-linking [45]. The polycationic chitosan was found to enhance intestinal permeation, via the adherence to the anionic intestinal wall, and promote the opening of tight junctions among epithelial cells [46, 47].

Coating of magnetic nanoparticles with chitosan protected them from oxidation reduced their toxicity and aggregation, increase the storage life with increasing in stability. Moreover, chitosan provides suitable functional groups that enable it to bind many drug molecules. In addition, the presence of chelating functional groups enhances the adsorption performance of magnetic chitosan [48]. Chitosan has proven its efficiency to intercalate, exfoliate, and disperse two-dimensional sheets including typically montmorillonite clay and graphene oxide layers. Owing to the presence of ammonium groups in chitosan, stable colloidal chitosan–clay and chitosan–graphene oxide solutions can be obtained [49].

Chitosan has a notable effect on fat metabolism and improves the aqueous solubility of poorly soluble drugs. Chitosan produces gel with molecular counterions such as polyphosphates and sulfates, allowing a wide range of applications including biochemical gel trapping of objects such as plant embryos, whole cells, and algae, pharmaceutical surface coating and food products [50]. Vanillin–chitosan–calcium ferrite hybrid nanoparticles were synthesized by the ionic gelation method, and their release of curcumin was studied at different pH and variable magnetic fields [51].

The chemical properties of chitosan allow its application in various biopolymer–inorganic composites. These composites have attractive characteristics allowing their application in a large number of areas. The good reducing properties of chitosan allowed its use as a capping/reducing agent in the preparation of gold–chitosan hybrid nanoparticles [52]. This chitosan coating enhanced the antimicrobial as well as the physicochemical properties of gold nanoparticles. Other studies reported the use of chitosan for the synthesis of silver nanoparticles [53].

4.2 Alginate

Alginate is a natural anionic polysaccharide with wide biomedical applications due to its low toxicity, low cost, biocompatibility, etc. [54]. Under mild conditions, alginate can form a hydrogel by ionotropic gelation in the presence of cross linkers such as calcium ions, and also by polyelectrolyte complexation with an oppositely charged polymer [28]. The polymeric chain of alginate is composed of (1→4) linked β-D-mannuronic (M) and α-L-guluronic acid (G) residues and is polyanionic in nature with the presence of carboxyl functional groups in the polymeric chain resulting in mucoadhesive properties with the hydrophobic mucosal layer of the intestinal wall which are enhanced by hydrogen bonding. Such interaction increases the residence time of the polymeric vesicles within the intestinal wall with an improvement in absorption efficiency [45].

Slow and sustained release of anti-HIV drug zidovudine was achieved by the encapsulation of the drug into alginate amide derivative prepared by coupling of glutamic acid. A slow release mechanism was observed in phosphate buffer saline of pH 7.4 [55].

Alginate aerogel was cross-linked with Fe(III) and loaded with ibuprofen with adsorptive deposition from superficial CO_2. Release of ibuprofen from the composite was faster at pH 7.4 than at pH 2.0 due to higher swelling properties and faster dissolution of alginate in PBS [56]. Yin et al. [57] showed the prospective sustain release in gastric fluid (pH 2.1) and intestinal fluid (pH 7.4) of indomethacin from agar–alginate hydrogel with Ca^{+2} composite.

4.3 Cellulose

Cellulose-based nanoparticles have many surface properties that help colloidal stability. Moreover, cellulose nanofibers have the ability to enhance the aqueous stability of drugs and increase the sustain release ability [58]. Bacterial cellulose/graphene oxide composite was found to be effective DDSs as a nanocomposite drug carrier [59]. The presence of abundant surface hydroxyl groups makes cellulose an attractive heterogeneous support with transformable oxygenated sites for chemical modification and/or anchoring different types of species (complexes, clusters, nanoparticles, etc.) [60]. Typical examples include the deposition of porous zeolite on various vegetal fibers and the replication of the cellulose surface by titanium dioxide using surface-reactive sol–gel chemistry. Cellulose surface modification allows for introducing more efficient chelating ligands on the surface, e.g., carboxymethylcellulose that can be used for controlling the growth of nanoparticles. This chemistry accounts for broadening the versatility of cellulose as a seed for nanoparticle synthesis and stabilization and consequently extends the advanced application range of cellulose-based biopolymeric–inorganic composites. Several excellent review articles summarized the use of cellulose as a support for molecular complexes, metal nanoparticles, metal oxides, and metal sulfides and their applications in catalysis, sensing and separation [61].

4.4 Starch

Starch, a common edible polysaccharide, is isolated from different natural sources such as wheat, corn, rice and potato. Starch has wide biomedical and pharmaceutical applications as it can be used as a carrier for controlled drug release and in other applications like bone replacement and repair. Morphological, rheological and mechanical properties, enzymatic digestibility, swelling and solubility are key factors behind the selection of starch for these applications [62]. Therefore, starch has been identified as a suitable biopolymer candidate for the synthesis of biopolymeric–inorganic composites for drug delivery applications.

For instance, corn starch with weight of $(162.5)_n$ was combined with multi-walled carbon nanotubes (MWCNTs) by ultrasonic-assisted method for zolpidem delivery [63]. Ampholytic starch excipients [62], acetylated starch nanocrystals [64], and dialdehyde starch derivatives [65] were widely used in DDSs as these materials have core–shell gel structures that display sponge-like viscoelastic attributes when suspended in an aqueous medium. Starch hydrogels were successfully used in oral drug delivery of probiotic bacteria *Lactobacillus plantarum*, and the loaded bacteria was resistant to bile salt solution and abnormal conditions of the gastrointestinal tract in comparison to the non-capsulated bacteria [66].

5 Proteins

Proteins are common biological polymers of amino acids with high molecular weights. Proteins such as collagen, gelatin, albumin, and fibroin were used for the synthesis of many DDSs [67]. Below, the most common types of proteins used for drug delivery are summarized.

5.1 Albumin

Albumin is the most ample protein in human blood plasma (35–50 g/L human serum) [68]. Due to its non-toxicity, biodegradability, and non-immunogenicity, albumin was proven to be appropriate for the drug delivery for many antibiotics and chemopreventive agents [69, 70]. Wunder et al. studied the intake of albumin by synovial fibroblasts of rheumatoid arthritis patients and the potency of methotrexate (MTX) which was covalently coupled to human serum albumin (HSA). The developments showed that inflamed paws in arthritic mice were accumulated with significantly higher amounts of albumin. Liver and kidneys showed significantly lower amounts of albumin. The protein was metabolized by human synovial fibroblasts in vitro and in vivo and the results suggested that MTX-HSA could be more beneficial in the suppression of the onset of arthritis in mice [71].

5.2 Collagen

Collagen is an extracellular fibrous protein that is mainly present in connective tissue of mammals. Collagen has a wide range of applications in wound healing due to its ability to enhance ingrowth of intrinsic tissues [72]. It was used in DDSs in the form of films, fibers, foams, nanoparticles, hydrogels, and minirods [73, 74]. Collagen can be utilized in delivery of platelet-derived growth factors [75], chondrocyte transplantation [76], antibiotics [77], and glucocorticoids [78]. Collagen was coupled with porous titanium dioxide as a drug delivery system for ibuprofen [79].

5.3 Gelatin

Gelatin is a biopolymer resulting from partial hydrolysis of collagen [80]. Gelatin microparticles can be utilized to deliver large molecules as they contain high surface-loading capacity allowing drug molecules to be absorbed into the gelatin hydrogel [81] . Due to its ability to form cross-linked structures, gelatin was used as a tissue adhesive for wound healing and pain management [82]. The introduction of gelatin microspheres into calcium phosphate cement (CPC), a potential material for its biocompatibility and osteoconductivity revealed that the delayed degradation of gelatin microspheres inside the CPC-gelatin scaffolds rendered good handling properties [83].

5.4 Silk Fibroin

Silk fibroin proteins are natural fibers and promising materials for controlled drug delivery because of their biocompatibility, mechanical hardness, self-assembly, and processing malleability [84]. One of the most common uses of silk fibroin is that it can be used in the form of elecrospun fibers, films, three-dimensional scaffolds, and hydrogels.

6 Inorganic Materials in Biopolymer Composites

Many inorganic materials show interesting properties such as high antibacterial activity, high electrical and thermal conductivity, excellent magnetic properties, easy surface functionalization chemical inertness, and biocompatibility. The incorporation of inorganic magnetic nanomaterials in biopolymeric matrices provides excellent characteristics resulting from their combined properties that are suitable for a wide range of applications such as drug delivery, therapeutics, diagnostics,

Fig. 2 Examples of inorganic materials that can be combined with biopolymers to form composites with high potentiality in drug delivery applications

and other biomedical applications. The following sections summarize some of the common types of inorganic materials used in biopolymer composites for drug delivery applications (Fig. 2).

7 Metals

Generally speaking, the incorporation of metals into a biopolymeric matrix demonstrated a very wide range of applications. For instance, biopolymeric–metal nanocomposites play a vital role in the regeneration and repair of skin wounds. Moreover, calcium is important for proliferation and differentiation of keratinocytes and for the hemostasis of mammalian skin. Silver nanoparticles (AgNPs) control the proliferation of bacteria which in turn decreases the inflammations. Zinc is also an important factor for protein synthesis, and development of T cells [85].

Silver has unique properties with excellent electrical and thermal conductivity among all metals and silver-based nanocomposites have a wide range of applications in conductive inks, antimicrobials, electronic devices, and catalysis [86, 87]. Silver-based materials exhibit good antimicrobial effects against pathological infections [88]. The development of biopolymer matrices with silver-based materials achieves better treatment strategies. The ability of blood clotting in wound dressing improves with AgNPs/chitin composites synthesized by Kumar et al. [85]. In addition, biopolymers can be used as stabilizers for nanoparticles suspensions. For example, the biopolymer pectin was used to stabilize polyaniline nanoparticles and form silver–polyaniline (PANi) nanocomposites. Biocompatibility studies of these Ag/PANi NPs in vitro with MTT assay showed an increase in cell viability. In addition, *Escherichia coli* adhesion was increased with the use of higher amounts of pectin [89]. The pectin-stabilized polyaniline nanoparticles were further used for the development of glucose biosensor [90] and for the detection of bacteria [91].

AgNPs were impregnated in collagen-based dressings to be used in wound healing with high antimicrobial activity. The nanocomposites are easy to synthesize via the electrospinning process. AgNPs in the collagen–AgNPs composite are responsible for controlling the deposition of collagen which leads to an improvement of the fibril alignments in wound healing processes [92, 93]. Furthermore, Song et al. [94] developed a new nanocomposite material by covalent cross-linking from AgNPs, histidine, and collagen and the scaffold showed promising antibacterial activity with good mechanical properties. The in vivo study showed regeneration of infected full-thickness burnt skin.

Recently, a wound scaffold was engineered from AgNPs with curcumin and plumbagin. In this study, collagen was used for cross-linking and the resulting fabrications offer higher activity for wound healing [95, 96].

Gelatin-based composites with AgNPs were fabricated for oral wound dressing and the nanocomposites showed antimicrobial properties against bacteria that caused periodontal disease [97]. Moreover, a novel wound dressing of AgNPs with silk fibroin was tested in rabbit model and the results showed that in a short period of time the defects of skin were covered and the wound surface was flattened [98]. Indeed, different nanocomposite materials based on AgNPs were fabricated with various biopolymers such as carboxymethyl chitosan and silk fibroin [99], keratin [100–102], chitosan [103–107], sodium alginate [108–110], and cellulose [111, 112]. Verma et al. [113] revealed accelerated wound healing and antimicrobial activity of sericin and chitosan-capped AgNPs in a rat wound model. The results showed that the combined effect of AgNPs and chitosan in the composite material increased the production of collagen, and re-epithelialization occurred in open wounds which eventually led to rapid wound healing. Anisha et al. [114] reported the use of starch as a stabilizing agent in the preparation of AgNPs-loaded chitosan and hyaluronic acid-based nanocomposite wound dressing sponges. Starch was also used as a reducing agent for the synthesis of colloidal AgNPs [115].

Several diethanolamine-modified olive oil incorporated high methoxyl containing pectin (DMP)-GG hydrogel nanobiocomposites were prepared using zinc acetate crosslinker for the intragastric controlled metformin HCl (MFM) delivery. Upon changing the GG:DMP mass ratios, nanofiller type (neusilin, bentonite, or fluorite) as well as oil addition, the nanocomposites demonstrated different drug encapsulation efficiencies of 50–85% and prolonged drug release of 69–94% in 8 h at pH 4.5 (acetate buffer). The optimal oil-entrapped nanocomposites released the MFM through case-II transport mechanism with the drug release kinetics following the zero-order model. The optimum system established outstanding gastro retentive features and significant hypoglycemic influence in streptozotocin-induced diabetic rats confirming suitability for the treatment of type-2 diabetes [116].

Polysaccharide-based hydrogel in combination with carbon nanotubes, gold nanowires, and gold nanoparticles (AuNPs) were studied to improve the electrical coupling between adjacent cardiac cells in vitro. As with all critically sized constructs, vascularization is paramount to the success of a cardiac construct. One approach is to add proangiogenic growth factors to the matrix to improve host infiltration [117]. Chitosan–AuNPs composites were used for electrochemical study and immobilization of leukemia cells k562 [118]. In addition, an ionic liquid-based enzymatic biosensor was reported by Brondani et al. [119] where AuNPs dispersed in ionic liquid (1-butyl-3-methyl imidazolium hexafluorophosphate) were supported in a chitin matrix cross-linked with glyoxal and epichlorohydrin. Peroxidase enzyme was entrapped in this ionic liquid matrix, and the biosensor electrode was constructed via mixing the resulting mixture with graphite powder and Nujol. The biosensor was successfully applied for the determination of rosmarinic acid content in pharmaceutical samples using square-wave voltammetry. Daneshpour et al. [120] used Fe_3O_4/trimethyl chitosan/Au nanocomposite as a tag to label DNA probe and polythiophene as an immobilization platform for quantitative evaluation of methylation in tumor suppressor gene RASSF1A. In this case, trimethyl chitosan could form nanocomplex with anionic compounds such as Fe_3O_4 and AuNPs via electrostatic interaction. Differential pulse voltammetry-based studies helped to analyze the methylation in the studied tumor suppressor gene with high specificity and low detection limit.

Calcium silicate is a low-density highly porous material that was proven to be used in tissue engineering and drug delivery [121–123]. Wu et al. [122] synthesized a core–shell composite of alginate and calcium silicate for the delivery of bovine serum albumin by in situ one-step method and the entrapment efficiency was found to be 75%. The composite structure exhibited a good sustain release pattern in PBS at pH 3.4 and 7.4. The core–shell structure loaded with bovine serum albumin showed the best performance in apatite-mineralization in simulated body fluids. A composite of calcium silicate–alginate was also developed by reinforcing of calcium silicate into alginate matrix [121]. The entrapment efficiency of metronidazole was found to be 61.70–93.10%. In vitro release study of metronidazole from the as-prepared beads proved the pattern of controlled release in the pH of the gastric medium and the beads were floating which is suitable for helicobacter pylori eradication.Nanocomposites of carboxymethyl guar gum (CMG) and different amounts of nanosilica were fabricated and used for the transdermal delivery of diclofenac sodium [124]. It was indicated that the nanocomposite containing 1 wt% of nanosilica was the optimal formulation. The nanocomposite hydrogel with 1wt% of nanosilica had the slowest drug release among all nanocomposites. Hasnain et al. [25] formulated novel kinds of ionotropically gelled calcium alginate–polyvinylpyrolidone (PVP)–nanohydroxyapatite composite-based bead matrices as carriers for diclofenac sodium. The mean sizing of these composite beads ranged from 0.98 ± 0.07 mm to 1.23 ± 0.15 mm, and the encapsulation efficiency of thebeads was from 65.82 ± 1.88 to 94.45 ± 3.72%. The anticancer drug DOX loaded in alginate/$CaCO_3$ composite was assessed in vitro with an elevated durability in aqueous solution. The nanoparticles were prepared by carbonate modified co-precipitation technique [125].

Microencapsulation of pancreatic islets in calcium alginate/poly-L-lysine succeeded in producing insulin in response to blood glucose levels when implanted in rats [126]. Chen et al. [127] reported an enzyme-based approach to in situ cell trapping within a biopolymeric hydrogel matrix. They used calcium-independent microbial transglutaminase, known for crosslink proteins, to catalyze gel formation from a pre-gel solution of gelatin and *E. coli* cells.

Hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] is a bioceramic material composed of 70% inorganic compounds of apatite calcium phosphate and 30% of organic materials such as collagen and bone marrow cells. It can be extracted from bones of animals or chemically synthesized. It is widely used in the delivery of drugs and other biomedical applications [128]. Owing to its features such as biocompatibility, osteoconductivity and ability to absorb a range of chemicals, it is used in scaffolds of bone tissue regeneration and bone implantable drug release [129]. Moreover, Hydroxyapatite was integrated with biopolymers to improve the osteoconductivity, mechanical behavior, and drug adsorption [130]. Hydroxyapatite–alginate nanocomposite was prepared for controlling the release of ofloxacin encapsulated within the matrix [131]. In another work, hydroxyapatite–alginate nanocomposite beads were designed for the delivery of diclofenac sodium [24] and the release of diclofenac sodium from the nanocomposite showed a pH-responsiveness indicating the compound's sustained release. Recently, core–shell *Gum Acacia*–hydroxyapatite nanocomposite was synthesized with a hydroxyapatite core and *Gum Acacia* shell and naringenin drug was encapsulated into the hydroxyapatite core via pellet press technique [132]. The *Gum Acacia*–hydroxyapatite crystallite size was diminished from 89 to 63 nm by increasing the *Gum Acacia* concentration from 0 to 10%. The pellet samples were dipped into the simulated body fluid in order to examine their bioactivities by scanning electron microscopy. The antimicrobial, hemolytic capacity, and biocompatibility of the drug-containing core–shell composites were also assessed.

Chitosan-based nanogel of CdSe quantum dots was used for drug delivery and imaging of mouse melanoma B16F10 cells. The nanogel showed a good cell viability of the melanoma cells as evident from the in vitro cytotoxicity results [133]. Scaffolds of polymer/ceramic composite have been designed with improved bioactivity, flexibility, good mechanical properties and osteoconductivity that are suitable for use in tissue engineering of bone [134].

Oxidized starch–CuO nanocomposite hydrogels were produced in situ through the synthesis of CuO NPs (with diameters of 39–50 nm) in swollen oxidized starch hydrogels so that the number of CuO NPs was enhanced by increasing the Cu^{2+} concentration. The swelling of the nanocomposite hydrogels examined at two pH values (2.1 and 7.4) proved that they had pH-sensitive swellings relative to the neat oxidized starch hydrogel. Moreover, a controlled drug release was detected for the CuO NPs incorporating oxidized starch [135].

In addition to the above-mentioned metals, titanium (Ti) and its alloys are biocompatible materials with good mechanical properties and a wide range of applications in human bone tissues due to its ability to resist corrosion [136].

8 Metal Oxides and Hydroxides

8.1 *Titanium Dioxide (TiO_2)*

The recent rise in scientific interest of titanium dioxide (a.k.a. titania or titanium (IV) oxide) is attributed to its photoactivity [137]. UV illumination of TiO_2 in aqueous media results in the formation of an array of reactive oxygen species (ROS) which have the ability to induce cell death in a wide range of diseases ranging from psoriasis to cancer. The incorporation of TiO_2 and other metal oxides in polymeric matrices has found a great interest in the last few years due to their potential use in novel medical therapies.

Hybrid composites of IBU/MC/TiO_2 (titanium tetra *n*-butoxide) have reported a slower release in gastric pH, thus minimizing the side effects of the loaded drug via minimizing the interactions between the drug and the gastric juice [138]. Archana et al. synthesized a nanocomposite formed of TiO_2, chitosan and poly(N-vinylpyrrolidone) and used it to prepare antimicrobial dressing [137]. The dressing demonstrated a good biocompatibility and excellent wound healing induction of open excision type wounds in albino rats.

Radmansouri et al. reported the preparation of chitosan/cobalt ferrite/TiO_2 nanofibers for the delivery of doxorubicin hydrochloride [139]. The formulation was used for hyperthermic treatment of tumor cells via controlled drug release. The results proved the possibility of use of the synthesized nanofiber composite in localized cancer therapy via the simultaneous effect of chemotherapy and hyperthermia.

8.2 *Zinc Oxide (ZnO)*

ZnO composites with biopolymers showed promising medicinal and biomedical activities in recent years. For instance, chitosan/zinc complexes can enhance wound healing and demonstrate good antimicrobial properties [140]. During the preparation of physically cross-linked chitosan hydrogel beads (in the presence of sodium tripolyphosphate crosslinker), ZnO nanoparticles can be synthesized in situ for application as drug delivery platforms. The diameter of the resulting particles lies in the range 10–25 nm [141]. These nanocomposites exhibit high swelling ratio in diverse aqueous solutions, pH sensitivity, and controlled release of ZnO NPs.

Carboxymethyl chitosan (CMCS) and ZnO NPs coated with sodium alginate hydrogel beads were used to control the release of diclofenac sodium and avoid its sensitivity to gastrointestinal environment and minimize their irritating action on the stomach [142]. In another study, pH-sensitive fluorinated CMCS NPs were reported as DDSs using N-(3-aminopropyl)-imidazole pre-grafted to the CMCS to fabricate the pH-sensitive NPs whose surface was then modified with perfluorobutyric anhydride to obtain the fluorinated NPs [143]. The cellular uptake tests confirmed that the surface-fluorinated NPs enhanced the cellular uptake and improved the cytotoxicity in diverse tumor cells without recognition between ligands and host.

Some ZnO-based hydrogel beads were prepared to improve the release of curcumin and to prevent the burst release observed in pure hydrogels. In addition, these hydrogel beads were found to decrease the fast-physiological clearance of curcumin and its sensitivity to ultraviolet light and alkaline solutions [144]. In order to solve the disadvantages of CMCS drug carriers (such as poor mechanical properties and burst drug release), ZnONPs were added to the CMCS beads that were coated with CS via self-assembly to obtain core–shell polyelectrolyte complexes. The 5-fluorouracyl (5-FU) anticancer drug was loaded onto the ZnO/CMC/CS nanobiocomposite hydrogel beads. The in vitro 5-FU release and swelling tests were carried out in simulated gastrointestinal condition, and the pH sensitivity of the nanocomposite beads was examined. The beads displayed a sustained drug release profile based on the ratios of CS, CMC, and ZnO NPs [145]. Antibacterial chitosan–GEL/zinc oxide (CS–GEL/*n*ZnO) nanocomposite hydrogels were achieved by in situ synthesis of *n*ZnO and employed as naproxen drug carriers [146].

Antibacterial CS–GEL/*n*ZnO nanocomposite hydrogels were prepared by in situ synthesis of *n*ZnO and employed as naproxen drug carriers. The scaffolds demonstrated good antibacterial, cytocompatibility, swelling, cell attachment, and biodegradation characteristics. Moreover, the scaffolds exhibited high porosity (pore sizes were in the range 50–400 μm) and *n*ZnO was suitably dispersed into the CS–GEL matrix without agglomeration.

8.3 Magnetic Materials

Magnetic materials (especially metallic ferrous nanoparticles) are of great importance in many applications such as therapeutics, diagnostics, real-time imaging and drug delivery. Superparamagnetic materials include three main iron oxides, namely hematite (α-Fe_2O_3), maghemite (γ-Fe_2O_3), and magnetite (Fe_3O_4). In addition, transition metals (e.g., Cu, Co, Mn and Ni) demonstrate superparamagnetic properties when mixed with iron oxides. The magnetic properties of these materials rely on their size and crystallinity [147]. One of the disadvantages of applying these magnetic nanoparticles in drug delivery is that they can be easily transported and distributed to the body organs by the blood stream. In some cases, this leads to exerting undesired side effects. This problem can be overcome by adding a capping/coating biomaterial such as natural/synthetic biopolymers that in turn prevent the undesired interactions with the body tissues. Moreover, these biopolymer coatings are usually used to achieve a certain surface functionalization of these magnetic materials, thus allowing their use in the desired specific application.

In one of the previous studies, magnetic Fe_3O_4 nanoparticles were functionalized with (3-amino propyl) triethoxy silane, coated with CS and tragacanth gum (TG), and were used for encapsulation of the curcumin drug [148]. The in vitro study of curcumin release was tested at pH 7.4 and 3.4 and at temperatures of 37 and 40 °C. The results indicated a higher swelling of the nanocomposite at pH 3.4 and 40 °C.

Anti-colon cancer therapy was developed by Fe_3O_4@cellulose nanocrystals (MCNCs) containing curcumin where the entrapment efficiency of curcumin was about 99.35% [149]. The exposure of the nanocomposite to an external field of 0.7 T led to the release of 53.30% of curcumin after 4 days. The proposed composite successfully inhibited the growth of human colon cancer cells by 18%.

A self-healing chitosan–alginate hydrogel was coated with magnetic gelatin microspheres (MGMs) to prepare an anticancer DDS. The hydrogel was formed through crosslinking of carboxyethyl chitosan as well as oxidized alginate by means of Schiff-base reaction [88]. The MGMs incorporated with 5-FU anticancer drug was achieved via emulsion crosslinking process to enhance the biological and mechanical properties of the hydrogel. It was found that adding MGMs with 30 mg/mL concentration to the composite hydrogel caused an appropriate performance and revealed exceptional self-healing capability under physiological conditions [150].

8.4 Aluminosilicates

Silica gels are popular class of bioencapsulation materials owing to their ability to maintain biological activity of the entrapped biomolecules such as enzymes or cells. Silica-based materials have a broad range of dimensions, chemical compositions and forms that can be prepared by sol–gel chemistry. In addition, sol–gel matrices provide chemical inertia, physical rigidity and negligible swelling in aqueous and organic solutions [151]. To achieve long-term stability, hybrids from silica-based materials with biopolymers are often utilized. Kaushik et al. [152] used silica nanoparticles–chitosan composite for the detection of ochratoxin-A by rabbit-immunoglobulin antibodies (IgGs). Through a physical adsorption method, bovine serum albumin and antibodies were integrated into the electrode of chitosan–silica composite.

A composite system of gelatin with mesoporous silica nanoparticles was used as a pH-responsive DDS for the delivery of doxorubicin, allowing its delivery in acidic conditions [81]. Another successful doxorubicin nanocomposite DDS was designed from silk fibroin and sericin with silica and demonstrated a high loading capacity and sustained release of DOX. This complex was very effective in the cancer therapy of human cervical carcinoma (HeLa) cells [153].

9 Carbon Materials–Biopolymers Composites

In the past decade, the development of novel carbonaceous nanomaterials has inspired scientists with new ideas in diagnostics and therapeutics sp^2 carbonaceous nanomaterials, notably zero-dimensional (0D) carbon dots and fullerenes, one-dimensional (1D) carbon nanotubes (CNTs), and two-dimensional (2D) graphene, have grasped the attention of the researchers from various fields including drug delivery [154]. The integration of biopolymers with the carbon nanomaterials provides them with more desired properties such as good mechanical stability, active functional groups, and better drug encapsulation ability.

A composite of CNTs and alginate (CNT-ALG) was fabricated by filling CNT in Ca-alginate to increase the mechanical stability. Thus, the CNT-ALG composite microspheres having loose internal structural features were formulated via decreasing the concentration of ALG sol. In addition, a triblock copolymer of PEO137-b-PPO44-b-PEO137 was also introduced within the CNT-ALG composite matrices to enhance the dispersion capability of CNTs in aqueous solution. In this investigation, theophylline was loaded as a model drug candidate within these CNT-ALG composite microspheres [155]. Starch was also used to prepare a nanocomposite with CNTs as a DDS. Hollow efficient nanoparticles and better loading capacity were obtained from core–shell which was used to deliver DOX in liver hepatocellular cells [156].

Single-walled carbon nanotubes (SWNTs) have high electrical and thermal conductivities and high strength; so it represents a potential for polymer matrix composites reinforcement [157]. The nanotubes can enhance the mechanical and electrical characteristics of polymer matrices [158]. SWNTs were integrated into polymer composites to be used after myocardial infarction for repairing of the left ventricles. This device was implanted in rats for in-vivo experimentation resulting in improved ejection fraction and fractional shortening in comparison to untreated hearts due to enhancement of intercellular adhesive junctions and electrochemical junctions [159].

Because of their chemical stability, homogeneity and porosity, carbon nanospheres are another attractive class of carbonaceous nanomaterials. The spherical structure is the result of pairing of pentagonal and heptagonal carbon rings to form waving flakes that follow the sphere's curvature [160]. Different techniques can be used for the preparation of carbon nanospheres such as hydrothermal, pressure carbonization, and thermal/catalytic decomposition but the hydrothermal technique provides a mild, green, and cheap route for the synthesis of homogeneous nanospheres with functional surface groups [161]. When the nanospheres are used for enzyme immobilization, composite the incorporation of a hydrogel-forming biopolymer such as alginate can provide excellent stability. Han et al. [161] reported a highly sensitive amperometric biosensor for the detection of glucose using immobilized glucose oxidase.

Graphene oxide (GO) is a carbon-based material with unique two-dimensional structure which can give extraordinary chemical and physical properties including excellent mechanical properties, large surface area, high carrier mobility, fast electron transportation, good biocompatibility, and good thermal conductivity. GO is composed of a single layer of sp^2 carbon atoms with a two-dimensional honeycomb lattice structure [162, 163]. GO has many applications in drug delivery, biological imaging, tissue engineering, electrochemical biosensors and bioassays [164, 165]. Schauer et al. [166] found that GO sheets increase the proliferation of stem cells and mechanical strength of CS where new functional groups can be introduced by chemical crosslinking to produce composites with good water dispersion, aqueous stability, and biocompatability and antimicrobial properties. By self-assembly, GO and CS can be integrated to create a biopolymer composite, in which GO is distributed uniformly in CS. This composite has good mechanical properties and can be used for electrochemical and biomedical applications [167, 168].

DOX as anticancer drug was loaded onto a CMC-GO composite as a pH-responsive DOX DDS which exhibited a higher release rate at pH 5 (i.e., in the tumor microenvironment) than at pH 7.4.

10 Metal–Organic Frameworks (MOFs)

Metal–organic frameworks (MOFs) are inorganic–organic solid structures built of the connection of inorganic subunits to each other through polytopic organic ligands that define cavities of various shapes and sizes [169]. Due to their unique adsorption capacity, large surface area, tunable pores and controlled porosity, MOFs resulted in great progresses in a variety of applications [170]. The highest molecular diversity of MOFs finds its root in the possibility of combining an unlimited number of organic linkers with metallic nodes intimately [7–9]. This gives MOFs the ability to recognize molecular properties through surface functionalization and molecular sieving by regulating pore sizes [171]. Hence, MOFs have many wide applications such as catalysis [172], drug delivery [173], photocatalysis [174], sensing [175], photonics, molecular imaging [176], electronic and optoelectronic devices, energy storage [177], and proton conductivity [178]. The incorporation of biomolecules within MOFs is a step toward more innocuous materials. Thus, the successful achievements in biomimetic and bioinspired functional materials triggered a confrontation between nano-sized MOFs and bio-based building blocks, resulting in a new generation of crystalline materials known as bio-MOFs, metal–biomolecule framework or MOF biocomposites [179, 180].

The performance of polymer@MOF membranes depends massively on the particle size of the MOF crystals and their uniform coating on the material surface. These two parameters need to be balanced to prevent defect when the crystals are bigger and pore clogging of the support if the crystals are too small. The use of CS as an interfacial compatibilizer or a binding agent circumvents some of these drawbacks and accounts for the successful formation of ternary membrane-based composites. Zhu et al. [181] reported the use of CS for enhancing interfacial interactions between hollow ceramic fibers of α-Al_2O_3 and the carboxylic groups of the BTC ligands. The use of CS was pivotal for controlling growth of MOF crystals and serves also as intermediate linker to stretch MOFs to the support. Consequently, MOF crystals of ~300 nm were fabricated, which preclude their inclusion in the pore structure of the hollow ceramic fibers (pore size of around 200 nm) and thus avoid their blockage. The resulting membrane was evaluated for hydrogen separation from a mixture of H_2, CO_2, N_2, and CH_4 [181].

11 Clay Minerals-Biopolymers Composite

Clay minerals are appropriate for modified drug delivery because of their crystalline structures, high surface area-to-volume ratio, high porosity, chemical inertness, swelling properties, good adsorption properties, colloidal particle size, and high exchange capacity of cations [182, 183]. Different types of polymers can be used as carriers for clay minerals in order to sustain their release and control their diffusion [184]. Clays are inexpensive materials, can be modified by impregnation of metal/metal complex, ion exchange, acid, and pillaring treatment to improve the desired catalysis functionality [185]. They develop a colloidal suspension of elementary platelets with negative charges upon delamination with water that can be used to make composites with cationic polymers such as CS [183].

Layered double hydroxides (LDHs), also known as anionic clays, are lamellar ionic solids carrying a layer of positive charges with two types of metallic cations and weakly bound exchangeable anions. They are used for enzyme immobilization in many areas including biosensor applications [186]. However, the inorganic clay matrix based on LDHs tends to develop cracks on dry storage, thereby limiting their reusability. Ding et al. developed a biopolymer composite from negatively charged alginate and positively charged LDH that aggregate together forming a gel resulting in a biocompatible matrix with good adhesion properties [186].

Montmorillonite (MMT) is a natural soft phyllosilicate inorganic clay material formed of aluminum magnesium silicate hydroxide of sodium and calcium with high surface area and ability of exchanging cations [187, 188]. MMT was recognized as safe by USFDA, and it is recommended to be utilized in drug delivery applications [189–191]. Many researchers investigated MMT–alginate composites as the negative charges on the COO^- group of alginate molecules improve the electrostatic interactions with the positive charge of MMT molecules [192]. So, the alginate–MMT composite formed by the reinforcement/incorporation of MMT into alginate matrix can improve the sustainability of drug release due to the enhancement of drug adsorption capacity [190]. Irinotecan, an anticancer drug, was encapsulated into alginate–MMT by the crosslinking gelation method [189]. Kevadiya et al. [189] fabricated alginate–MMT composite for the control of diclofenac sodium release in which Na-alginate and diclofenac sodium were intercalated into MMT by the ionic gelation method. The efficiency of diclofenac sodium encapsulation was increased with the addition of MMT in the bead formulation. Another work by Kaygusuz and Erim [191] reported the fabrication of a pH-responsive alginate–MMT composite with bovine serum albumin as a model protein to investigate the intestinal drug release from the composites. The presence of MMT reinforcement in the composite helps in increasing the loading efficiency of bovine serum albumin to 78% compared to 40% in case of calcium alginate beads.

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Natural Polymeric-Based Composites for Delivery of Growth Factors

M. D. Figueroa-Pizano and E. Carvajal-Millan

In the complexity of the human body, individual cells need to be connected and communicated continuously between them to realize an ordered and efficient work, regardless of whether or not they are performing the same function within an organ. Around the cells, the extracellular matrix (ECM) creates support and a specific environment where thousands of active molecules are released by the same cells to establish these communication and signalization processes [1, 2]. The active molecules are recognized by specific receptors at the cellular membrane and they can trigger many internal biochemical mechanisms related to cell survival [3]. Thus, cells are stimulated in a synchronic manner to meet with the specific actions that they need and, at the same time, complement the other ones.

In this context, growth factors (GFs) are an extended group of naturally released signaling molecules that have critical roles determining the fate of the cell. They are small and soluble glycoproteins responsible for conveying information from cell to cell to control and regulate their functions, such as proliferation, differentiation, morphogenesis, and migration [4, 5]. They also actively participate in mechanisms as homeostasis, inflammation, wound healing, and antiviral response [6, 7]. Structurally, GFs are formed by polypeptide dimers, which vary in number and type of amino acid residues [8, 9]. GFs are secreted by different kinds of cells (stem, neurons, epithelial, hepatic, muscular, blood, etc.) generally in the same organ or tissue where they go to act [7]. A single GF can be produced in many types of cells in different stages of maturation; however, it can evoke different effects depending on the delivery concentration, the target cell, and the maturation phase of the target cell [7, 10]. All mature forms of GFs reside in the ECM in association with molecules as collagen,

M. D. Figueroa-Pizano (✉) · E. Carvajal-Millan
Biopolymers-CTAOA, Research Center for Food and Development (CIAD), Hermosillo, Sonora, Mexico

E. Carvajal-Millan
e-mail: ecarvajal@ciad.mx

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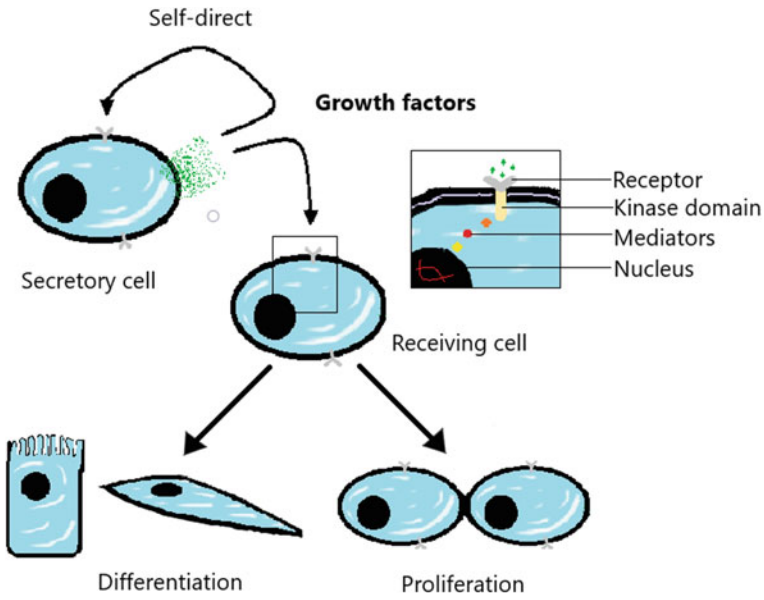

Fig. 1 Schematic representation of different functions regulated by GFs

elastin, glycosaminoglycan, and other components. Those associations protect them from enzymatic degradation and regulate the binding with their receptors, therefore also regulate their activities [11].

GFs can diffuse through the ECM and execute rapid communication between nearby cells, including those from a different population, and act in autocrine or paracrine forms [12, 13]. Some of them also can perform an endocrine action. Besides, the GFs precursors, generally presented as immature protein on the cellular membrane, may be able to stimulate neighboring cells in a juxtacrine way. The carried message by GFs is sending through their binding with a transmembrane receptor, in canonical or non-canonical signaling pathways, into target cells [14, 15]. The receptors are proteins conformed by an extracellular or binding domain, a transmembrane domain, and an intracellular tyrosine kinase domain. After a GF (ligand)binds its receptor by extracellular domain, conformational changes start the transduction process, and the cytoplasmic domain turns into a tyrosine kinase enzyme. In this way, some intrinsic signalization pathways are activated and end in a modification of gene expression, which is translated as the cell behavior [6, 7]. Figure 1 shows a schematic representation of the ways GFs action.

The large number of GFs detected has been grouped into several families considering standard features as their structure, activities, and target cells. The most studied and comprehended families of GFs are presented below:

1. *Fibroblast growth factor* (*FGF*) *family* is considered as one of the most varied collections of GFs due to its members are similar in structure, but they can

act in different ways [5]. Around 22 proteins belong to this set of GFs and have been separated into six subfamilies, which are classified into two main classes: paracrine-action FGFs and endocrine-action FGFs [16–18]. In fact, the FGF family encompasses proteins with autocrine, juxtacrine, paracrine, and endocrine action form [19]. Along the embryonic stage, they are produced in all tissues to regulate processes as differentiation, proliferation, apoptosis, and migration of mesenchymal cells [18, 20]. In the mature stage, the paracrine-action FGFs continue these functions to maintain and remodel tissues, while the endocrine-action FGFs regulate some metabolic routes (lipids, carbohydrates, and bile acids) [5, 17]. Generally, paracrine-action of FGFs depends on their union with heparan-sulfate proteoglycan in the ECM. This union gives them stabilization and protection to canonically binding with their receptors [16, 18, 19]. Several members act in blood vessels, skin, bone, nerves, and muscles, and they are considered potent angiogenic factors due to their high regulation of proliferation and migration of endothelial cells.

2. *Platelet-derived growth factor* (*PDGF*) *and Vascular endothelial growth factor* (*VEGF*) *family* enclosed these two main subfamilies of GFs, which share structural and functional characteristics [21]. Structurally, both PDGF and VEGF present a growth factor core domain, which is distinguished by a conserved cysteine-knot fold with eight resides [22, 23]. These subfamilies play the most important roles during blood vessel formation and they have been recognized as possible regulators of neurogenesis. They also perform essential functions in hematopoietic development and neuroprotection process [24].

    (a) *Platelet-derived growth factor* (*PDGF*) *subfamily* owes its name due to some members are produced and released from platelets in the blood clotting process [25]. The cysteine-knot fold presented in PDGF proteins is composed of eight residues that maintain the 3D structure [22]. PDGFs regulate the proliferation and migration of mesenchymal cells and guide the organogenesis throughout the embryonic stage [26]. The action of these factors depends on the specific receptor which they binding. In the early stage, they seem to influence skin, lungs, kidney, and intestine formation, by linking their alpha-receptor (PDGFRα) [22, 24]. In contrast, PDGFs union with their beta-receptor (PDGFRβ) regulates the angiogenesis (creation of blood vessels from already formed) and the formation of the connective tissues. The primary stages of hematopoiesis are also controlled by the union between PDGF and PDGFRβ [25]. Besides, they can act as chemotactic factor because they attract and stimulate neutrophil and macrophage cells in injury zones [21, 27]. The PDGFs bio-availability in the ECM is limited by their interactions with α_2-macroglobulin and PDGF-associated protein (PAP). Also, their binding with glycosaminoglycan and collagen affects their bioactivity [21, 25, 28].
    (b) *Vascular endothelial growth factor* (*VEGF*) *subfamily* encompasses the most specialized and potent GFs able to stimulate the endothelial cells

leading to the production and maintenance of vascular and lymphatic systems [29]. This GFs family actively regulates *de novo* vascularization in all organs (including bone and pulmonary epithelium) during embryonic stage [30–32]. They mainly regulate the angiogenesis process, increase the permeability of blood vessels, and activate the chemotaxis of monocytes [29, 31]. Structurally, they also show a cysteine-knot fold region and high affinity to bind heparan-sulfate proteoglycans, which are necessary for its recognition [23, 29].

3. *Transforming growth factor* (*TGF-β*) *family* is very diverse and contains two main groups: TGF-β and bone morphogenetic proteins (BMPs). Both classes mainly regulate osteoblast differentiation in mesenchymal stem cells (MSC). Although each of these GFs has its participation, it is not necessarily in the same proportion [33]. TGF-β plays an essential role in the signalization via for the synthesis of components that form ECM, stimulate monocytes and lymphocytes activity, and participate in the wound healing process [34]. However, TGF-β also can inhibit the proliferation and differentiation of epithelial and adipocytes cells. TGF-β can encourage or interrupt apoptosis [35]. All TGF-β present a distinctive segment of nine cysteine residues in their structure, whereas BMPs only have seven [36]. The BMPs are a large group compose of around 13–20 proteins that are the primary regulators of bone and cartilage formation [37]. They actively stimulate the MSC differentiation to be derived in osteocytes and chondrocytes cells at the embryonic stage [38, 39]. BMPs participate in dorsal-ventral designing and help in the development and conservation of skeletal [39, 40]. They are heparan-sulfate binding proteins in ECM, which gives them stabilization, diffusion, and let them establish signalization with the receptors [40].
4. *Epidermal growth factor* (*EGF*) *family* is a significant and profoundly studied group composed of 11–13 proteins (only seven are listed in Table 1) structurally and biologically similar [41–43]. They are engaged to induct differentiation, proliferation, or apoptosis in keratinocytes, oocytes, enterocytes, or lung cells [44, 45]. All GFs that belong to this family present standard glycosylated transmembrane-protein precursors containing six equally and conserved cysteine residues [46]. These protein precursors have an extracellular EGF domain able to stimulate other cells in a juxtacrine manner [42, 44]. Besides, the EGF domain can be cleaved by metalloproteinase to release the mature GFs that present an autocrine or paracrine action [46]. The members of this GFs family have similar affinity by the same receptors, being redundant in function, although each of them can realize a specific role in different tissues or organs (from bone, kidney, and neuron cells) [27].
5. *Neurotrophins* (*NTs*) *or Nerve growth factor* (*NGF*) *family* is a group of GFs specially devoted to guiding critical processes of neural cells. These factors are part of neurotrophic factors (NF), together with the glial cell line-derived neurotrophic factor (GDNF) family, and some members of the cytokine family [47–49]. All NTs proteins share a very similar structure (about 50%) because

Table 1 Principal members of the GFs families, their receptors, their primary function and their secretory, and target cells

Name family	Subfamily	Growth factor (ligands)	Receptors	Functions that stimulate	Secretory cell	Target cell
FGF **Heparan-sulfate binding proteins* (cofactor) +*βKloto/αKlotho* (cofactors)	**FGF-1 subfamily*	FGF-1 FGF-2	All FGFR FGFR-1	Proliferation Differentiation Motility Survival Angiogenesis Apoptosis	Keratinocytes Fibroblasts Endothelial Muscle Chondrocytes Mast cells Osteoblast Macrophages	Fibroblast Epithelial Endothelial Mesenchymal Bone Muscle Neurons
	*FGF-4 *subfamily*	FGF-4 FGF-5 FGF-6	FGFR 1-4			
	*FGF-7 *subfamily*	FGF-3 FGF-7 (KGF) FGF-10 (KGF-2) FGF-22	FGFR-1,2 FGFR-2 FGFR-1,2 FGFR-2			
	*FGF-8 *subfamily*	FGF-8 FGF-17 FGF-18	FGFR-3, 2, 1			
	FGF-9 *subfamily*	FGF-9 FGF-16 FGF-20	FGFR-3, 2, 1			
	FGF-11 *subfamily*	FGF-11 FGF-12 FGF-13 FGF-14				
	FGF-15/19 *subfamily*	FGF-15/19 FGF-21 FGF-23	FGFR 1-4 FGFR-1,3 FGFR-1,3,4			

(continued)

Table 1 (continued)

Name family	Subfamily	Growth factor (ligands)	Receptors	Functions that stimulate	Secretory cell	Target cell
PDGF/VEGF **Heparan-sulfate binding proteins*	PDGF *Subfamily*	*PDGF-AA *PDGF-BB *PDGF-AB PDGF-CC PDGF-DD	PDGFR-αα PDGFR-αα, -ββ, -αβ PDGFR-αα, -αβ PDGFR-αα, -αβ PDGFR-ββ, -αβ	Mitotic: • Proliferation • Survival • Migration Chemotaxis Angiogenesis Production of ECM compounds	Platelets Epithelial Fibroblast Endothelial Macrophages Smooth muscle cell	Mesenchymal Fibroblast Glial cells Muscle Hepatocytes
**Heparan-sulfate binding proteins*	VEGF *subfamily*	*VEGF-A *VEGF-B VEGF-C VEGF-D Placental GF (PlGF)	VEGFR-1, VEGFR-2 VEGFR-1 VEGFR-3 VEGFR-3 VEGFR-1	Angiogenesis: • Proliferation Chemotaxis: • Motility	Endothelial Platelets Fibroblasts Macrophages Keratinocytes Smooth muscle cell Neutrophil	Endothelial Macrophages Smooth muscle cell
TGF-β ***Collagen binding proteins* **Heparan-sulfate binding proteins*	TGF-β *subfamily*	**TGF-β1 **TGF-β2 **TGF-β3 Nodals **Activins Some Growth and Differentiation Factors (GDF)	TβR-I, II TβR-II, I TR-I, II TβR-II, ActRII TβRIII (no-binding)	Inhibition of: • Proliferation • Differentiation Promote: • Differentiation Migration Apoptosis Cell adhesion Cytoskeletal organization	Platelets Fibroblasts Macrophages Keratinocytes	Mesenchymal Epithelial Adipocyte Myocytes Osteoblast

(continued)

Table 1 (continued)

Name family	Subfamily	Growth factor (ligands)	Receptors	Functions that stimulate	Secretory cell	Target cell
	*BMPs *subfamily*	BMP-2 BMP-3 BMP-6 BMP-7 BMP-9 Some GDF	Type I (TβR-I) (all BMPs are more affine for this receptor) Type II (TβR-II)	Bone and cartilage formation: • Proliferation • Differentiation • Morphogenesis • Homeostasis	Fibroblasts Macrophages	Mesenchymal Osteoblast
EGF		EGF TGF alfa Heparin-binding Epidermal growth factor (HB-EGF) Neuregulin 1 Neuregulin 2 Neuregulin 3 Neurogulin 4	ERBB1 ERBB1 ERBB2 (no-binding) ERBB1, ERBB4 ERBB3, ERBB4 ERBB3, ERBB4 ERBB4 ERBB4	Growth Proliferation Migration Differentiation Apoptosis	Epithelial Platelets Macrophages Hepatocytes Keratinocytes	Epithelial Epidermic
Neurotrophins		NGF Brain-derived neurotrophic factor (BDNF) Neurotrophin-3 (NT-3) NT-4/5	TrkA, p75NGFR TrkB, p75NGFR TrkC TrkC	Survival Plasticity Apoptosis Axon growth Dendrite pruning Expression of: Neurotransmitters Ion channels	Schwann cells Fibroblasts Mast cells	Neurons
IGF ****IGF binding proteins*		***IGF1 ***IGF2 Insulin (no GF action)	IGFR-1 IGFR-2	Growth Proliferation Differentiation	Hepatocytes Fibroblasts Macrophages Neutrophils Neurons Enterocytes	Hepatocytes Neurons Many types of cells

(continued)

Table 1 (continued)

Name family	Subfamily	Growth factor (ligands)	Receptors	Functions that stimulate	Secretory cell	Target cell
HGF		HGF HGF-like protein (HLP)	c-Met	Proliferation Morphogenesis Motility Angiogenesis	Fibroblast Mesenchymal Stromal Macrophage	Epithelial Endothelial Melanocytes Hepatocytes

The significance of ‘asterisk’ specifies a characteristic of the FGs depending on the family to which it belongs. The meaning of the asterisk changes for each family and it is specified in the first column on the left below the family name. The GFs marked with an asterisk in the second column have the characteristic of that family they are produced by common genes with identical sequences [48, 50]. Functionally, they regulate the differentiation, survival, migration, and proliferation of neurons since the development of central and peripheral nervous systems [48, 50–52]. In mature neurons, they also control the establishment of synapses and neuron's plasticity. Besides, they help in the regeneration and remyelination of neurons when the inflammatory process occurs as part of neurodegenerative disease [47, 49, 53].

6. *The insulin-like growth factor* (*IGF*) *family* is part of a more complex system of proteins that regulates the cell development throughout the different stages of life and control some physiological aspects [54, 55]. The leading members of this family are positively related to insulin because they share around 50% of structural homology, some receptors, signaling pathways, and in occasions, similar metabolic activities [56–58]. However, IGFs are more focused on the growth-direction, regulating mechanism as proliferation, differentiation, and apoptosis of a great variety of cells [54, 57, 59]. Besides, insulin is produced exclusively in the pancreas and exerts its action freely, while IGFs are secreted mainly by the cells in the liver and other organs, and they bind other specific proteins [60]. IGFs influence the development and restoration of the liver as well as the growth and survival of hepatocyte cells [57, 61]. The production and action of IGFs are in all organs and are stimulated in different grades by growth hormone, as well as depending on the age, lifestyle, and genetic factors [56, 58]. Besides, one of their members (IG-1) acts as a glucose regulator independently of insulin and participates actively in the preparation of injured tissue. While the other (IGF-2) is widely distributed in the brain and central nervous system during the embryonic stage, probably supporting the human development [61]. In adulthood, high levels of IGFs have also found in the nervous system suggesting a necessary action of them [59].

   The bioactivity of IGFs is regulated by their association with one group of six proteins closely related to them, named as IGF binding proteins (IGFBPs) [55, 57]. The IGFs present high affinity by IGFBPs, almost the same as they have for their receptors, so most of the time, they are binding to these proteins [56]. The bounding of IGFs with specific IGFBPs mostly inhibit their action because they can limit their union with their receptor [55]. This binding allows to extend the half-life of IGFs in blood-stream or ECM, protect them from proteolytic degradation, storage them in ECM, carry them to target cells, and modulate their interaction with a specific receptor [54, 56–58, 61]. Usually, free-IGFs have a half-life of 10 min, while bounding with IGFBPs it is extended until 30 min, and even they can form a ternary complex with an acid-labile subunit (associated protein, IGFBP-rPs), which extend their life around 10–13 h [57, 61]. The separation of IGFs from IGFBPs is promoted by decreasing their affinity, for example, phosphorylation of IGFBPs or binding them with other molecules in ECM, as well as by proteolytic action [56].

7. *Hepatocyte growth factor* (*HGF*) *family* is a small group of GFs formed by at least two proteins with great action on the liver. HGF is the principal member that regulates, in a paracrine fashion, the proliferation, morphogenesis, and

motility of epithelial, endothelial, and melanocyte cells [62–64]. It is considered multi-functional GF because during the embryonic development is essential for many organ formation and maturation. At the same time, in adults, it participates in reparation and regeneration processes of the lacerated liver, lungs, gastrointestinal tract, and kidney [62–65]. Recent studies indicate that HGF also plays an essential role in osteogenesis, bone healing, and restoration of the nervous system [65, 66]. Moreover, it exerts a regulative effect on anti-inflammatory, anti-apoptotic, and anti-fibrotic signal pathways in several organs [62, 66, 67]. Even, HGF has been considered as a modulator in glucose metabolism in several cells since their receptor (c-Met) is similar to the insulin receptor [67]. HGF structure is well characterized as 92 kDa heterodimer, which is bounded to heparin-sulfate in ECM, helping their stabilization and interaction with a specific receptor [8].

Other families no less important, are the Wingless (Wnt) proteins and the hematopoietic growth factor, the latter is recognized to encompass many cytokines with the capacity to regulate the production and activities of blood cells [5, 7]. Indeed, groups of transmembrane receptors as Eph and Notch proteins are considered as GFs because of their juxtacrine signaling that drives cell localization, differentiation, proliferation, and homeostasis in different stages of the cell life [68, 69]. Table 1 presents detailed information about more representative families of GFs: ligands, receptors, functions that regulate, secretory cells, and the target cell.

1 Biomedical Applications of GFs

A wide variety of studies have tried to transfer the essential and natural regenerative functions performed by GFs to different medical applications. Over the years, GFs and their receptors have been thoroughly studied, leading to a very well understanding of their regulatory roles on signalization pathways. Endogenous GFs are considered indispensable orchestrators of molecular and cellular responses to rebuild or regenerate damaged organs and tissues [70]. In that sense, many endogenous GFs have been isolated and produced by genetic engineering, as recombinant proteins, and have been used as exogenous therapeutic agents in regenerative medicine [71]. The employment of these exogenous GFs to treat injuries or chronic disease failures is a good strategic to enhance the natural self-renewal capacity of the cells as well as to improve the overall healing results.

The main therapeutic approaches for GFs have been applied in tissue engineering and in wound healing (Table 2). Regarding tissue engineering, exogenous GFs have been supplemented, as therapeutic protein, for restoration and regeneration of bone, cartilage, tendons, cardiac muscle, peripheral muscle, nervous system, the pulp of the immature tooth, and capillary vessels [70, 72–76]. Usually, transplanted tissue grafts employ stem cells, which are stimulated by exogenous GFs to differentiate them in any specialized cells. Hence, the GFs are becoming in a useful tool for the

Table 2 Main GFs used in tissue engineering and regenerative medicine

Tissue/organ/systems	GFs applied	Result
Blood vessels	VEGF	Vascularization and angiogenesis Migration and proliferation of endothelial cells [71, 78, 85]
	PDGF	Increase size and maturation of vessels [86]
	FGF	Maturation of vessels Stimulates endothelial cell migration Matrix deposition [87]
	TGF-β	Vascular morphogenesis [88, 89]
Muscle (myocardium/skeletal)	bFGF	Promotes myoblast proliferation [90]
	VEGF	Migration cardiac progenitor cells Angiogenesis [91]
	PDGF	Pericyte recruitment [92]
	HGF	Migration of cardiac progenitor cells. Normalization of angiogenesis [91, 93]
Nervous system	NGF	Peripheral nerve regeneration Neurite outgrowth [4]
	NT-3	Neuronal survival [76]
	NT-4	Neuronal survival [76]
	FGF-2 (bFGF)	Nerve regeneration: myelin renewal Stimulates Schwann cell survival [4, 76]
Skin	EGF	Stimulates the proliferation of fibroblasts keratinocytes, and vascular endothelial cell Enhances the production of fibronectin [81, 82]
	VEGF	Re-epithelialization process (migration and proliferation of endothelial cells) Angiogenesis [78]

(continued)

Table 2 (continued)

Tissue/organ/systems	GFs applied	Result
	PDGF	Chemotacticfor fibroblasts, neutrophils, monocytes, and smooth muscle cells Activates macrophages to release GFs Promotes fibroblast proliferation Production of extracellular matrix[83]
	FGF	Acts as a mitogen for fibroblasts Induces angiogenesis Stimulates granulation tissue formation Re-epithelialization [83]
	TGF-β-3	Potent chemoattractant for macrophages Stimulates or inhibits proliferation.Granulation tissue formation [83]
	TGF-α	Proliferation of basal cells [71]
	IGF	Promotes re-epithelialization Stimulates fibroblast proliferation[83]
	BMP: BMP-2 and BMP-7	Bone induction: osteoblasts growth and proliferation [13, 73]
Bone	TGF-β	Proliferation periosteal cells Chondrogenic differentiation [73, 74]
	IGF	Osteogenic differentiation [74]
	FGF	Osteogenic differentiation [74] Angiogenesis [13, 74]
	VEGF	Neo-angiogenesis [73] Recruitment of bone-forming cells [13]
	PDGF	Angiogenesis and cell proliferation [74]

*Brain-derived neurotrophic factor

regeneration of tissues or cells with low renewal capacity; for example, HGF has applied to differentiate stem cells into neurons while BMP into tendons [66, 77]. In wound healing management, exogenous GFs have been administrated to treat injuries caused by surgery, trauma, diabetic ulcer, burns, among other types of damages [78–80]. The functional repair of wounds, based on these molecules, is due to the

GFs capacity to generate a microenvironment where proliferation, differentiation and migration processes are stimulating [81, 82]. Besides, GFs can modulate ECM-components production, angiogenesis, inflammatory response, and construction of granulation tissue, which are essential in wound healing [83]. The most recognized GFs involved in wound healing and skin reparation are EGF, KGF, bFGF, PDGF, VEGF, TGF-β, IGF [2, 84].

Beyond the regenerative effects of GFs, they have been harnessed as therapeutic molecules in some diseases or as biomarkers of the disease grade. Because of the pleiotropic effects of HGF, it can serve as treatment of liver, lungs, and kidney diseases [66, 67]. Also, HGF administration improves the management of hepatic insulin resistance because it reduces the fasting blood glucose, triglycerides, and cholesterol levels in mice [94–96]. The IGF-1 level in blood seems to have an association with the development of diabetes, and the recombinant IGF-1 has proved to drop blood glucose concentration and increase blood insulin [58]. Both effects could be used as a biomarker or as a treatment in diabetes. The IGF-1 has shown a protective effect against the development of Parkinson's disease due to their supplementation restored the damage of brain inflammation, the disminished oxidative damage, apoptosis as well as preserved the integrity of blood-brain barrier, and the cognitive capacities [97]. In another study, the NGF presented therapeutic properties for Alzheimer's disease because it had acted as a neuroprotective agent for PC12 cells when they were exposed to a cytotoxic agent, which is present in this disease [98]. Another relatively new emerging therapy based on a cocktail of GFs has been implemented to treat ocular diseases through plasma application (a blood-derived product), which contained a mix of PDGF, TGF-β, VEGF, IGF-1, and HGF [99].

Recently, GFs have been considered as target molecules for new treatment in cancer development. Despite the beneficial actions that GFs promote under physiological conditions for cell growth and survival, many studies have shown their active involvement in several cancers. Among the leading families of GFs and receptors that are found overexpressed in cancer is IGF, VEGF, TGF-β, HGF, EGF, and FGF [100, 101]. They have been identified in the breast, colon, liver, pancreas, prostate, leukemia, brain, lung, kidney, ovary, stomach, and colon cancers. In these types of cancers, the cells are enormously stimulated by GF inducing an accelerated growth, clonal expansion, angiogenesis, metastasis, and cross-talk with neighboring components [101, 102]. However, GFs are genuinely committed to their natural functions binding their receptors indistinguishably in both standard and tumor cells. The excessive action of GFs in cancer cells only helps for disease development because the real cancer origin is the loss of the cellular cycle regulation due to oncogenic mutations. Cancer cells attract macrophages and create the right conditions for unproportioned release of GFs [103]. Additionally, cancer cells present more transmembrane GF's receptors being hypersensitive to the autocrine action of GFs [101, 103]. After elucidation of the critical role that GFs play in cancer development, they have been identified as target molecules to generate molecular antibodies that prevent the action of both ligands and receptors. Indeed, the genes of these molecules have been detected and selectively silenced by short interfering RNAs [104]. Some

GFs-receptors systems highly present in cancers have been considered as an ideal biomarker for the prognosis or diagnosis of these diseases [105].

2 Challenges and Approaches to Develop Devices for GFs Delivery

The promising therapeutic results of GFs showing in several research works and clinical trials have led to the search for adequate administration ways to generate new and improved commercial products. Nowadays, many recombinant humans (rh) GFs proteins, such as rhFGF, rhIGF, rhKGF, and rhEGF, are commercially available, allowing their use in medical products. However, only one product has been approved by the United States Food and Drug Administration (FDA), this is the Regranex® gel with becaplermin (rhPDGF) at 0.01%, as a topical treatment for diabetic wounds [11]. Other products based on rhGFs are the INFUSE® gel with rhBMP-2 and rhBMP-7, used to treat lumbar spine fusion and open tibial fracture [106]; the Heberprot-P®, a freeze-dried product, the Regen-D™ gel, and the Easyef® spray contains rhEGF to treat ulcer diabetic wounds; while Fiblast® spray contains rhbFGF for being administrated on the skin with burn ulcers [83]. The direct application of GFs using injections, solutions, creams, lotions, gels, sprays, and ointments generally resulted in inefficient delivery due to the gradual deactivation of GFs [79]. In this case, repeated doses of medications are demanded, turning them expensive. However, the constant use of these treatments has several warnings due to adverse effects and consequences they can cause [11, 107]. For that reason, it is imperative to consider the suitable concentrations of GFs to obtain the expected results without inefficient or excessive GFs action. The main concerns about the efficacy of GFs in clinical products are the poor stability and the short-half life that these molecules have [108]. Generally, they are unstable to pH and temperature changes, suffer oxidation of amine groups, and they are very susceptible to enzymatic degradation or inactivation [107]. Many efforts trying to solve these drawbacks are focused on GFs modification to make them stable at physiological conditions, whereas others have concentrated in the development of delivery systems able to preserve GFs the native forms and functions, and carry them to the target cells.

2.1 Approaches for Intrinsic Improvement of Stability and Action of GFs

Many efforts have been made to extend the functional activity of exogenous GF, which are mainly based on structural modification through genetic and protein engineering [11]. Several random, site-direct and combinatory mutations on GFs genes have been done attempting the production of more stable GFs and with prolonged half-life [109]. The final GF produced with genetic changes must remain the similarity enough to the natural GFs to be functional. The most effective variations are the creation of relativity small and less complicated GFs and the removal of protease cleavage sites in the structure. Other changes imply an enhanced binding affinity of GFs by their receptors, and the recycling action of the receptors after their internalization to come back to the membrane [106, 109]. Besides, genetic management also envelopes the production of recombinant proteins due to all engineering GFs are settled, detected, and isolated from surfaces of bacteria, yeast, or mammalian cells [106].

Other structural modifications have been done in mature GFs with the same purpose of stabilization and increase their half-life. In small GFs, the amino-terminal end is used to cycle the structure and join it with the carboxyl-terminal being less prone to the temperature or protein degradation [106]. Another common modification is grafting polyethylene glycol (PEG) on the GFs chain to expand its half-life. PEGylation of GFs provides them thermal stability and avoids their enzymatic degradation due to the PEG increase of hydrodynamic radius, which masks the cleavage sites [110]. The FDA has approved some of these PEGylated-GFs for their usage in therapeutic products among them are found PEG-IGF-1 and FGF [111, 112]. A possible disadvantage of these modifications are their interferences with the receptor-binding site. Figure 2 shows some of the benefits of the modifications of GFs.

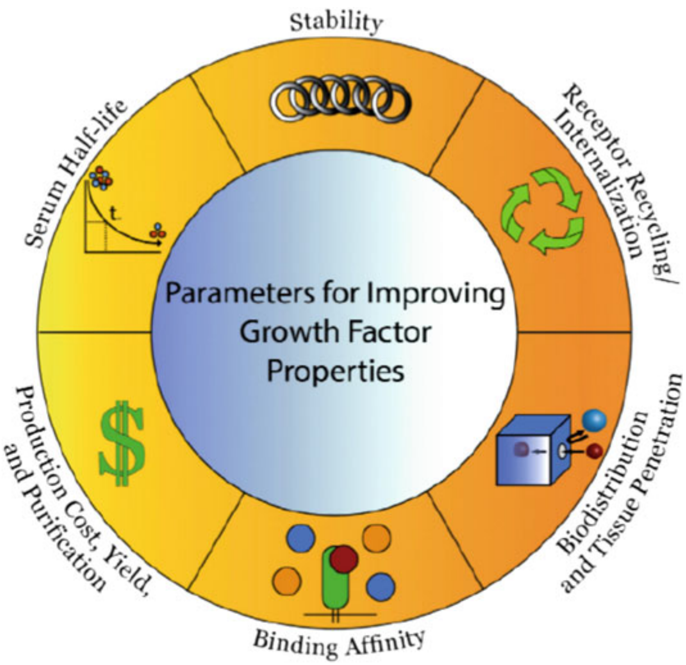

Fig. 2 Main advantages obtained after modification of GFs. *Source* Mitchell et al. [106]

2.2 Approaches for GFs Delivery Systems

Another sophisticated strategy, to preserve the intrinsic properties of GFs to use them in the clinical area, is their encapsulation in delivery systems. The incorporation of GFs in matrices of diverse natures has been considered as a practical approach to separate them within a particular microenvironment that conserves their integrity. There is specific interest in lipid particles (mussels and vessels), nanofibrous assemblies, polymeric materials (natural and synthetic), or well, a combination of them [83]. The main advantages of using delivery systems for GFs encapsulation are protection from physical and enzymatic degradation, direction to the target cells, and release in sustained-manner [113]. Additionally, stimulus-responsive delivery system scan be fabricated to modulate the GFs release after they sense physiological changes or a disease-specific stimulus [10]. Another advantage of these systems is the possibility of dual or multi GFs encapsulation to obtain a synergistic effect and better therapeutic results [82]. Besides, depending on the nature of the delivery system, similar ECM conditions can be created, pretending to extend the bioactivities of GFs. In this way, the spatial-temporal delivery of GFs can be controlled to reduce the continuous applications and the side-effects as well as to improve their therapeutic and tissue-forming capacities, being them more cost-effective [81, 108]. However, the engineering of these delivery systems is challenging, since mimicking the ECM environment and controlling the release rate of GFs, involves the consideration of several biological and physicochemical aspects.

Notably, three aspects have been recognized as necessary for the correct design process of delivery materials. (1) Biological requirements. The selection of adequate GF before starting is imperative. It is essential to keep in mind their functions, size, structure, and biochemical features because they affect their interaction with the materials [12]. The pretended morphological and histological outcomes also need to have visualized. Additionally, the biological, anatomical, and functional characteristics of the target cell, tissue, or organ should know. (2) Material properties. Depending on the place where the material will be used is essential to select it based on its nature, chemical structure, molecular weight, mechanical properties, compatibility, degradability, and binding/interaction ability with GFs [13, 107, 114]. (3) Delivery strategies. Structure shape and size of the material need to be considered not only for its introduction, direction, or colocation in the place of interest, these features are critical for loading efficiency and release kinetic of GFs [12]. The loading step is vital for GFs activity because it involves the manipulation (vulnerability to denaturalization) and estimation (correct quantity for expected results) of GFs, so the process needs to be simple [13]. The amount of GF loading limits the amount of GFs released and its release kinetic. This latter topic is considered the most important to obtain the desired therapeutic results; its control implies avoiding excessive and insufficient dosages or burst and lag phases. Unbalance in loading and releasing stages could involve inactivation of signal pathways, incomplete or incorrect induction of regenerative effect, or well, a cytotoxic and adverse response causing other clinical complications.

2.2.1 Loading and Releasing Strategies for GFs into Biomaterials

Recently, multiple innovative ways have been proposed to seed the GFs into biomaterials, which later facilitate their release. Some loading methods have been described in detail for polymeric materials and they are presented below. The easiest way is the infusive method because it only require the GFs in solution, generally in cell culture media, and then, they are directly added and penetrate the material by diffusion. This incorporation can be performed manually or automatically. Recently, the automatic way is based on the use of bioreactors (spinner flasks, rotation wall vessel, and perfusion bioreactor) [115, 116]. Several considerations should be made when this method is using, for example, the materials require a highly porous structure for good penetration, using the manual method there is the risk that GFs solutions are not well distributed while using the automatic method high shear stress could be generated affecting the cell development [12]. Another method is the immobilization of GFs inside the conveyor material through chemical (covalent) or physical (electrostatic, ionic, hydrogen bonds, among others) entrapments [11, 110, 114]. For this, it is essential to consider the functional groups that are contained in the basic chemical structure to establish the chemical or physical link. Besides, during chemical encapsulation, the GFs could be denaturalized.

As mentioned before, the loading step determines the releasing way of the GFs from the carrier. Physically trapped GFs are commonly released by diffusion, erosion, and swelling. In this case, it is also essential to consider the structural characteristic of the carrier (porosity, pore size, hydrophilicity, etc.). Chemically attached GFs are released by cleaving the interaction site between the GF molecule and the carrier matrix [11, 106, 108]. Enzymatic action is frequently employed for that purpose. Most refined release mechanisms are enclosed in the category of programmed delivery. Here, the materials present the capacity to activate and to control the GF releasing based on biological and physical stimuli, such as temperature, pH, light, magnetic field, and enzymatic [10].

A wide variety of regenerative tissue materials have been exhaustively evaluated to detect the best for GFs delivery. Promising results have been obtained for 3D polymeric materials such as hydrogels, scaffolds, several particulate systems (nano, micro, and macro), and for composite materials (Fig. 3) [83]. Most of them are suitable for GFs encapsulation due to its polymeric composition that resembles the ECM where these molecules are found naturally, allowing them to preserve their bioactivity. Specifically, hydrogels are 3D flexible polymeric networks with high water content that display extraordinary smoothness and moisturizing characteristics which help in the injury treatment. This kind of material also presents porous networks, which are ideal for encapsulation of GFs because they can diffuse through them quickly. Besides, some hydrogels are injectable and can be administered in specific sites, adapting their shape to the surrounding environment. Generally, they are prepared with both natural and synthetic polymers. The performance of hydrogels to release GFs have been evaluated in multiple studies for wound healing or tissue engineering, and very satisfactory results have been obtained [12, 107, 117]. However, hydrogels lack of excellent mechanical properties, so their use may be

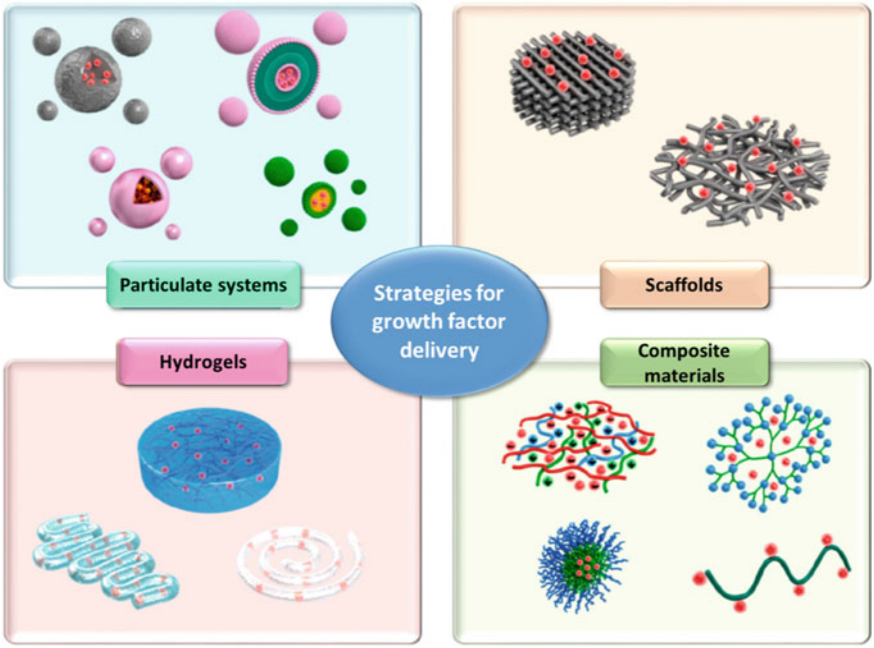

Fig. 3 Classification of the GFs delivery systems according to their matrix structures. *Source* Adapted from Park et al. [81]

limited, avoiding places with a lot of friction or heavy loads. In the case of scaffolds, they are structures designed as 3D culture cells, where differentiation, proliferation, and communication processes are improved [74]. GFs can also be added into scaffolds to enhance the development of cultured cells before implantation as a tissue graft or to be released after grafting to accelerate tissue regeneration. An obstacle to use scaffolds is their complicated implantation in some places inside the human body.

Regarding the GFs release kinetics, both hydrogels and scaffold present burst release effect. The particulate systems may represent a better option to address this undesirable effect. A broad range of particle sizes has been elaborated for the GFs delivery, ranging from 1000 μm (microparticles) to below 1000 nm (nanoparticles) [108]. They have many advantages. For instance, (1) they can be applied in any part of the body; (2) they have shown good releasing patterns of GFs, and (3) they have avoided immunological responses [83]. Their elaboration could be carried out through several efficient methods, although the resulted size distribution sometimes is difficult to control, which makes their commercial production quite problematic. The last class of GFs delivery systems are a combination of the past three; they can even include parts of materials of other nature (non-polymeric). For that reason, they are named composite materials. The desired result of these combinations is a combination of all the benefits, or even improved properties in a single system.

3 Composite Materials for GFs Delivery

Composites have attracted attention in designing drug delivery systems due to the wide variety of material combinations that can be made and result in better capacities. The emergence of these composites materials is based on the drawbacks of isolated materials or systems. For example, liposomal structures serve for the easy incorporation of hydrophobic and hydrophilic drugs and they have low toxicity. However, they are susceptible to be degraded quickly by the action of the reticuloendothelial system, avoiding the sustained drug release. Nevertheless, when they are embedded in polymeric systems, which are stable and do not evoke reticuloendothelial responses, they present prolonged release compared to liposomes alone [118].

Similarly, polymeric systems usually lack of excellent mechanical properties and they are often combined with fibers, metallic, or ceramic particles that reinforce the matrix. Thus, the composite features can be tailored by the types of materials used, their proportion, and distribution in the system [119]. In general, the preparation of composite allows tuning the mechanical properties, to tailor the rate of degradation and adjust the thermal stability problems of the drug delivery systems. As a consequence of these modifications, the release kinetics of drugs, including the GFs kinetics, can be modified. Besides, the adjustable design capacity that composites present allows their use in many places, whether soft and hard tissues [120].

Composites based on the polymeric matrix are widely used in tissue engineering and wound healing care as a strategic delivery system for GFs. Generally, composites are formed by two or more types of materials. One part of them contains a matrix, which is the material that is found in the highest proportion. Based on the nature of this matrix, composites can be classified as polymeric, metallic, or ceramic [119, 121]. The other part is the reinforcements, which are the materials that are found in less quantity and are distributed in the matrix. Depending on the type of reinforcement contained in composites, they can be classified as fiber-reinforced, particle-reinforced, or structural [122–124]. A third part is an intermediate phase (interface) located in the border between the matrix and reinforcement. For tissue engineering and wound healing, the matrix of composite is usually prepared with both natural and synthetic polymers due to their ductility, porous structure, low cost, and secure handling [119]. However, natural polymers have gained attention for the engineering of composites materials due to they are more biocompatible and biodegradables. The main natural polymers employed for composite formation are collagen, alginate, chitosan, hyaluronic acid, heparin, silk fibroin, and PHBV [73].

4 Polysaccharide-Based Composites

4.1 Alginate-Base Composites for GFs Delivery

Sodium alginate is a polysaccharide isolated from seaweed and some bacteria, it is commonly used for tissue engineering or as delivery systems. The high interest to work with this material is due to its excellent biocompatibility and because it is water soluble. It is an anionic polyelectrolyte and its structure is formed by monomeric units of D-mannuronic acid (M) and L-guluronic acid (G) [125]. Alginate-based biomaterials can be assembled quickly and under mild conditions by electrostatic forces using Ca^{++} cations [126]. Frequently, these materials present a high porous structure allowing the incorporation of molecules such as GFs. However, alginate presents several drawbacks, including limited mechanical properties and low degradation rate in mammals (because they do not have the required enzymes) [127]. Many efforts have been focused on the designer of appropriated alginate materials combining it with different ingredients and forming several composites materials.

Alginate-based composites are one of the most used systems for GFs delivery to treat the injuries regeneration or to differentiate stem cells. They have been produced using several methodologies, including a simple drying overnight or more sophisticated electro spinning and 3D bioprinting techniques. By a simple drying overnight method, an EGF-loaded alginate-chitosan composite was created as a potential wound dressing [128]. The membranes were formed by electrostatic interaction between the free amine groups in chitosan chains and the carboxylic groups in alginate. Additionally, they were cross-linked with $CaCl_2$. These membranes were composite because of the incorporation of suture threads of linen and cotton in the layers to improve the mechanical properties. The results showed how the threads increase the elongation at the break about 5–8 times when they were cross-linked. Besides, roughness and opacity were also increased, which could be beneficial for cell attachment. However, the extracts of membranes produced with cotton threads were cytotoxic for human fibroblasts. For that, EGF was only incorporated into alginate-chitosan blend containing linen threads before the dried process, and it proved to be useful for fibroblast proliferation. As mentioned before, combination of several materials allows the modulation of different properties in composites. The cellular adaptability of alginate-based composites has been improved by coupled with sequences of cell attachment peptides as RGD (arginine-glycine-aspartic acid) [129]. That is the case of the 3D alginate composite microspheres used for cell encapsulation and for multiple GFs delivery, which was proposed for muscle regeneration [130]. The result of this modification was an increased cell viability of encapsulated mesenchymal cells after two weeks in an in vitro evaluation. Besides, the combinatory action of several GFs, including FGF, seems to promote the differentiation of the encapsulated cells because they showed muscle cell-like morphology after four weeks of evaluation.

Naturally, sulfated polymeric components in ECM are crucial in several signaling pathways through their interaction with GFs [131]. Based on that, the alginate backbone has strategically been modified with sulfate groups to provide high affinity and specific binding points for GFs trying to modulate their action like in the original medium [132, 133]. Composites of alginate sulfate with polyvinyl alcohol (PVA) have been fabricated by electrospinning technique for TGF-β delivery [134]. PVA, a biocompatible synthetic polymer with excellent mechanical properties, was employed to provide better electro-spinnability to alginate sulfate and high mechanical strength. The incorporation of TGF-β to the electro-spun composite of alginate-sulfate/PVA was by physical absorption and was improved with the addition of sulfate groups to alginate. Moreover, the release of TGF-β was slower in alginate-sulfate/PVA composites than alginate/PVA composites. Regarding the biocompatibility of these composites, they proved the attachment of mesenchymal stem cells to them, and they maintained excellent cell viability (more than 90%).

As mentioned, alginate-based composites can also be fabricated by the 3D bioprinting technique, although for this, it is necessary to adjust some mechanical properties of alginate. Freeman et al. [135] systematically studied combinations between the molecular weight of alginate and types of cross-linking agents to tuning the bio-ink stiffness. This parameter is essential because it improves the printability for alginate, and it also determines the mesenchymal stem cell differentiation as well as the GFs release rate. They showed that only 45–75% of loaded VEGF remains in the printed composites, and the molecular weight of the alginate faster modulated the GF release. They observed that the combination of different molecular weights of alginate retained more easily the VEGF and released it slowly. Besides, the spatially varying mechanical stiffness in the composite influences the differentiation of mesenchymal cells. In the soft portions, one part of the cells undergo to osteogenesis and another part to adipogenesis, while in the stiffer region, most of the cells underwent to osteogenesis.

The use of alginate-based composites with particle-reinforcement is widely studied for dual release of GFs due to they offer a spatial-temporal behavior. An alginate composite contained FGF-loaded poly(N-isopropylacrydamide) nanogels and diclofenac sodium-loaded poly(N-isopropyl acrylamide-co-acrylic acid) nanogels were created to be used as a step-release dressing. The thermos-sensitive pattern of each embed the nanogel showed that about 92% of loaded diclofenac sodium was released at 37 °C in the first three days, corresponding with the inflammation stage. Whereas 80% of the FGF loaded was released at 25 °C after day 3 to day 8, matching within the tissue formation stage. Finally, the in vivo studies indicated that the wound was healing around 96% in 14 days and presented less inflammation and higher angiogenesis than control groups [136]. Another standard version of alginate-based composites used for dual GFs release with particle-reinforcement are the thermosensitive-composites. In these composites, the matrix part, which in this case is alginate, usually presents a sol-gel transition after sensing a temperature change. They are ideal for internal tissue reparation because they can be injected in specific sites to increase the efficacy of GFs, where it is difficult to implant composites in the form of membranes. Several works have been reported the effectiveness of injectable thermosensitive composites for the stepwise delivery of VEGF and BMPs in bone formation. The strategy consisted of loading VEGF in the alginate matrix of the composite, while BMP-9 and BMP-2 were pre-loaded, by absorption on microspheres of chitosan and modified heparin, respectively [37, 137]. Both VEGF and BMP-2 have presented a high affinity for their corresponding polysaccharide due to they showed an encapsulation efficiency greater than 90% [137]. The release profile obtained in both studies was similar since VEGF showed higher concentrations in each evaluation point compared to the BMPs. However, the subsequent release of these GFs can be changed depending on the loading strategy. The BMP-9 release proved to enhance the proliferation and the osteogenic differentiation of mesenchymal stem cells along the surface of microspheres, which were distributed in the composite matrix [37].

4.2 Chitosan-Based Composites for GFs Delivery

Chitosan is another polysaccharide extensively used in the fabrication of composites for GFs release, especially for those engaged with the bone formation. Chitosan is the only natural cationic polysaccharide composed of β-1,4-D-glucosamine and N-Acetyl-D-glucosamine units, containing free amine groups which are available for chemical modifications [138]. DeWitte et al. [139] joined empty poly(methyl methacrylate-co-methacrylic acid) nanoparticles to the chitosan matrix, via carbodiimide-crosslinker chemistry. Specifically, they used the carboxylic acid found on the surface of the nanoparticles to react with the free amine groups of chitosan chains. This method proved to be useful for the retention of nanoparticles for up to four weeks without negative effects on cell viability or proliferation. The nanoparticle retention effect was proposed as a possible system that could serve to delay the release of GFs. Usually, chitosan-based composites with particle-reinforcement are a promising approach for dual administration of GFs because they have proved spatial-temporal control of their releasing. An attempt to the double release of PDGF and BMP-2 was made from alginate microspheres embedded in a chitosan-based composite [140]. BMP-2 was loaded in core-shell alginate microspheres, while PDGF was separately loaded in another type of microsphere. The release kinetic of both GFs was assessed, and the results showed that only 10% of BMP-2 was released in the first week and 50% until five weeks. In contrast, more than 10% of loaded PDGF was released on the first day and around 55% for day 6. Both GFs showed in vitro bioactivity based on cell effects.

Chitosan-based composites have had a particular use to dual delivery of VEGF and BMP-2 because they can act synergistically and orderly to promote osteogenesis and vascularization [141, 142]. The releasing patterns indicate that osteogenesis is supported by BMP-2 released in a slow and maintained manner over time [143]. While for vascularization, VEGF needs to be released in high concentrations at the beginning and then decreased [144]. Besides, the 2-N-6-O-sulphated chitosan (26SCS) have widely used to mimic the polysaccharides found in the

ECM that regulates the GFs activity and their release. Also, some studies point out a pro-angiogenesis effect [142, 145]. A particle-reinforcement 26SCS composite was formed with poly(lactide-co-glycolide) (PLGA) microspheres for the release of VEGF and BMP-2 [142]. The BMP-2 loaded in PLGA microspheres showed a slow and sustained release without burst effect, while VEGF, charged in the composite matrix, displayed a rapid release profile and burst effect in the two first hours. As mentioned before, this release behavior is desirable in these classes of composite, becoming them in a promising approach for bone reparation treatments.

On the other side, an innovative dual-modular scaffold composite based on 26SCS with VEGF and MBP-2 was used for osteogenesis and vascularization development (Fig. 4). The material was formed with 26SCS and a mesoporous bioactive glass (MBG) with hierarchical porous structures (module 1). Inside of the hollowed channels of module 1, GelMA hydrogels columns were in situ fixed (module 2) [145]. Using this unique building composite was possible to have differentiated spatiotemporal delivery modes of BMP-2, which was in module 1, and VEGF, which was embed in module 2. Also, the arrangement of this chitosan-based composite enhanced the osteogenesis and angiogenesis process at in vitro and in vivo studies. Dual delivery of GFs from chitosan-based composites has also had in other applications. In other work, heparin was bound to the residual amine groups of chitosan-g-polyethylene glycol (PEG) composite to increase the electrostatic binding efficiency and of FGF and VEGF to enhance wound healing [146]. The conjugation of PEG to the chitosan

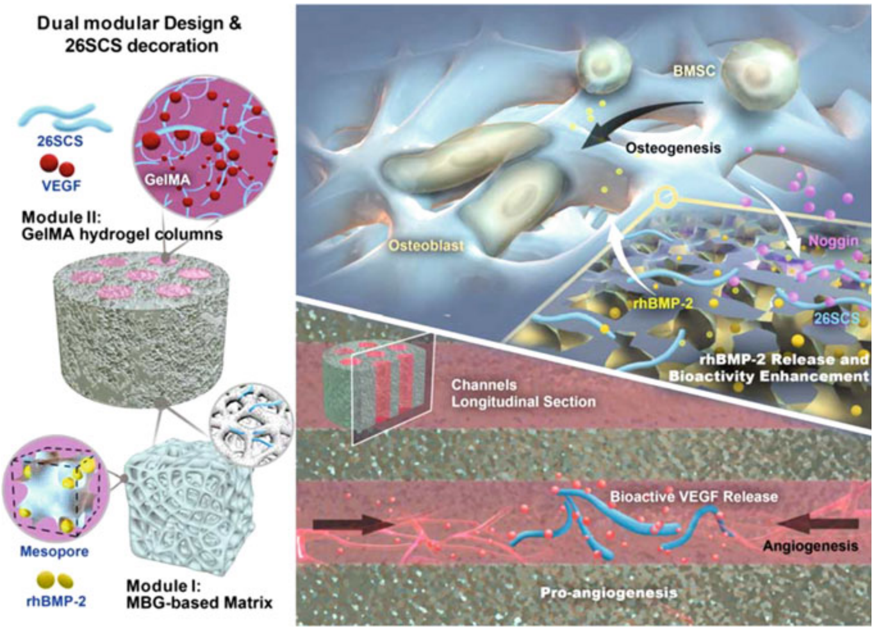

Fig. 4 Schematic representation of dual-modular 26SCS-based composite scaffold for GFs delivery to enhance bone regeneration. *Source* Tang et al. [145]

chains improved the mechanical properties of these composites, which displayed a simultaneous and continuous release profiles of FGF and VEGF to stimulate the proliferation of HaCaT cells. Finally, the dual and simultaneous release of FGF and VEGF increased neovascularization and collagen formation.

Another highly porous structure for BMP-2 delivery was fabricated from chitosan, hydroxyapatite, heparin, and PVA, using glutaraldehyde and cryogelling as cross-linking agents [147]. Incorporation of several components to the mix allowed to have specific functions; for example, the presence of hydroxyapatite in the composite mimics the natural structure for new bone formation. Heparin allowed the electrostatic interaction with BMP-2 to maintain inside the composite. The PVA hindered the formation of polyelectrolyte complexes between chitosan and heparin for more homogeneous matrix assembly, and it improved the mechanical properties. The final composites were biocompatible and released BMP-2 by 30 d to stimulate the differentiation of mesenchymal stem cells into osteocytes.

4.3 Glycosaminoglycan-Based Composites for GFs Delivery

Glycosaminoglycans (GAGs) are a set of structural polysaccharides composed of a random combination of glucuronic acid, glucosamine, iduronic acid, or galactosamine [148]. Some GAGs are found in the ECM of mammals tissues, for example, chondroitin sulfate, heparan sulfate, heparin, dermatan sulfate, keratin sulfate, and hyaluronic acid. In contrast, others, such as carrageenan, fucoidan, and ulvan, are present in seaweeds [149]. Most of them are well-recognized by present highly sulfated chains and to be bonded to several proteins in the ECM (with the exception of the hyaluronic acid). GAGs are considered as regulators of ECM proteins, such as GFs because their unions affect the signalization and functionalization processes that are promoted by these molecules [150]. For that reason, GAGs represent an perfect class of natural polysaccharides to form carrier systems of GFs with particular properties and an enhanced capacity to regulate its delivery in biomedical applications [149].

4.3.1 Heparin/Heparan Sulfate-Based Composites for GFs Delivery

Heparan sulfate or heparin are polysaccharide-origin constituents of ECM, which serve as structural support and have the capacity to join proteins to regulate diverse cellular functions [151]. The primary unbranched chain of heparan sulfate is formed by repeating disaccharide units of D-glucuronic acid (GlcA) and N-acetyl-D-glucosamine (GlcNAc), which are heavily sulfated in a different position [152, 153]. Additionally, the GlcA unit can present 2-O sulfation, changing the disaccharide to L-iduronic acid (IdoA), which is abundant in heparin structure with around 85% [149, 152]. The sulfation patterns that are present in heparan/heparin chains provide negative charge enabling them to bind positively charged amino acids of GFs and moderate their signalization pathways that influence the cell fate [151].

Inspired by the natural function of heparan sulfate or heparin, some composite based on these polysaccharides have been created to protect and deliver GFs in injured tissues. Silva et al. [154] prepared a diblock copolymer with a spontaneous assembly of heparin and polyethylene glycol (Hep-b-PEG) for the protection and delivery of FGF-2. For the engineering of this system, heparin was used without further modifications preserving its biological activity to bind to FGF-2 electrostatically and to form ternary complexes. The composite materials showed in Fig. 5 were microspheres with a particular arrangement of about 400 nm. They were able to encapsulate around 99% of FGF-2 and release more than 80% during 28 d at pH 7.4. The microsphere structure proved to have a protective effect on FGF-2 due to it remains its bioactivity directing the differentiation of MSC onto bone cells. Another polypeptide/heparin-based composite was used to deliver VEGF as a potential treatment in wound healing [155]. In this case, poly(L-lysine) with two shapes (linear and star) were cross-linked with genipin and further ionically complexed with negatively charged heparin, which also bound VEGF. Stronger electrostatic interactions between heparin with VEGF were produced, allowing its stabilization to promote a good healing process.

A novel strategy to create composite materials for sustained delivery of multiple GFs is using the layer-by-layer (LBL) assembly of several polyelectrolytes. Heparin, like other sulfated GAGs, presents a polyanionic character allowing their interaction with polycationic molecules such as GFs or other synthetic polymers. That was the

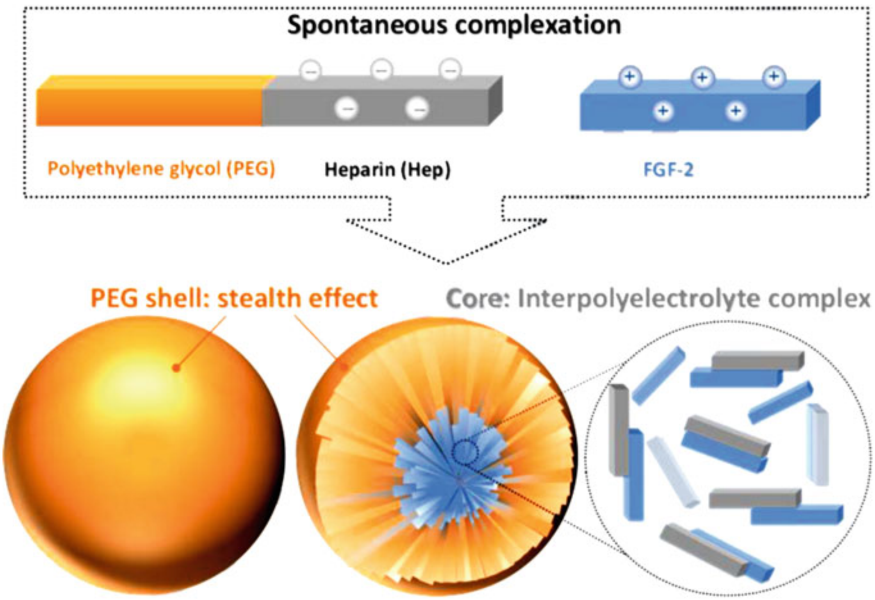

Fig. 5 Representation of self-assembly of polyelectrolyte complexes formed by hep-b-PEG to deliver FGF-2. *Source* Silva et al. [154]

case of a composite based on polymeric implants and subsequent covered with thin polyanionic layers of heparin and dextran sulfate alternated with polycationic layers of several GFs [154]. Specifically, TGF-β, PDGF, and IGF were used. The polymeric implants surfaces were activated by gas plasma (inert argon or reactive oxygen) to provide higher surface area charge for better polyelectrolyte adsorption. Also, the deposition of poly(styrenesulfonate) or poly(ethyleneimine) was performed at the beginning to provide sufficient initial cationic charge. The results showed that the LBL method is an excellent alternative for GFs encapsulation due they can be loaded with around 90% of efficiency, especially in heparin composites. The use of oxygen gas plasma and acidic pH conditions during the engineering process also improves the GFs loading. The LBL composites also exposed an excellent sustain delivery of GFs trough diffusion and erosion processes during 14 days, and they regulated fibroblast proliferation, myofibroblast differentiation, cell morphology, and migration.

4.3.2 Hyaluronic Acid-Based Composites for GFs Delivery

Hyaluronic acid is another GAG that has been used to fabricate composites for GFs delivery. It is a linear polysaccharide composed by repeating disaccharides of D-glucuronic and N-acetyl-D-glucosamine without sulfated units [156]. In this case, the N-acetyl-D-glucosamine units possess carboxyl groups, charging negatively to the hyaluronic acid chains and making them highly hydrophilic. At high molecular weights, hyaluronic acid forms a dense network around the cells, providing mechanical support and interaction with many signal molecules, helping in the regulation of the fate cell [149, 156]. However, hyaluronic acid tends to be degraded fast when it is applied in clinical treatments. In an approach were prepared composites with hyaluronic acid conjugated with sulfate groups, which showed lower degradation by hyaluronidase and improved the retention of TGF. Finally, the new hydrogel composite based on sulfated hyaluronic acid promotes the chondrogenesis being a promising biomaterial for regenerative medicine [157].

5 Protein-Origin Composites

5.1 Collagen-Based Composites for GFs Release

Collagen is a natural polymer considered as the most abundant protein in connective tissue skin, tendon, and cartilage in mammals. In the last years, it has regularly been used as a biomaterial [158]. The collagen presents a distinctive supramolecular structure with many interacting points which allow the design of materials with several applications. However, many times collagen structure requires modifications due to its poor mechanical and thermal stability [159]. To overcome these issues, composite materials based on collagen also represent an alternative, especially for the tissue engineering area. Collagen-based composite contained hydroxyapatite and NGF, showed the capacity to repair craniofacial injuries [160]. In in vitro conditions, the composites presented a burst effect in the first hours of release evaluation, but then they released around 100% during 96 h. The release of NGF from these composites proved to have a stimulation of periosteal and endocortical woven lamellar bone formation, and it improved the remodeling activity in the intracortical region after 30 d of study in Wistar rats. A very similar collagen-based composite with hydroxyapatite was used for the dual release of BMP-2 and VEGF for the effective healing of critical-sized bone defects [161]. In this case, the GFs were soak-loaded onto both sides of the composite. The incorporation of hydroxyapatite in these systems seems to sequester the GFs due to its high affinity with them, causing a longer time of retention inside the composite and a sustained release. These GFs loaded composites demonstrated a complete bridging the gap in the bone defect in a very early time compared with composites without GFs. As in other cases, BMP-2 and VEGF acted synergistically and enhanced the osteogenic and angiogenic processes. One more collagen-hydroxyapatite composite for the release of BMP-2 was presented by Ref. [162]. This composite material was fabricated by the easy and biocompatible methodology of lyophilization and dehydrothermal processes to provoke high porosity and mechanical strength in the material. The obtained results were a successful release of BMP-2, which stimulate osteoblast proliferation and bone repair.

Other types of the collagen-based composite are micro particles combined with porous calcium phosphate and collagen constructed by water in the oil emulsion technique to release BMP-2 [163]. The microspheres of calcium phosphate presented highly interconnected pores where collagen, together with BMP-2, were infiltrated. The in vitro release of BMP-2 was in a sustained manner and caused cell differentiation, while in vivo evaluation displayed bone regeneration with microspheres. The chemical modification of collagen structure whit glutathione and FGF has also been implemented as a strategy to form composites and release, in control manner, the FGF [164]. These composites remain potential therapeutic approaches for myocardial infarction due to their dual function, angiogenesis, and inhibition of cardiac remodeling. The addition of glutathione onto the collagen chain was made by the conjugation of collagen amine groups with the glutathione sulfhydryl groups, with the purpose of increase the binding capacity with the composite. Promising results were obtained with these composites because the modified collagen decreased the degradation of the cardiac matrix and other components as well as the FGF enhanced the vascularization.

5.2 Gelatin-Based Composites for GFs Release

Gelatin is a protein-origin polymer obtained from the denaturalization of collagen, which has shown potential as a biomaterial for tissue regeneration due to its similarity with the ECM [165, 166]. However, gelatin biomaterials present some drawbacks as low mechanical properties and easily enzymatic degradation [166]. An effective way to overcome these issues is using composites, where different materials of several properties can be combined. Also, they represent a manner to improve their therapeutic effectiveness by incorporation of active molecules such as GFs.

Many sophisticated techniques have developed gelatin-based composites with the potential to be used in tissue engineering or wound dressing. A fashionable approach to produce several types of gelatin-based composites that allow the relatively easy incorporation of GFs and preserve their bioactivity is the 3D printing. Especially, FGF has been integrated into 3D-printing gelatin composites used to treat both bone reparation and skin regeneration. Xion et al. [167] produced a gelatin-sulfonated silk composite scaffold with FGF, which proved to trigger the granulation and enhanced the skin regeneration after implantation as well as they encouraged the dermal vascularization. Other 3D printing gelatin-chitosan composite with FGF and magnesium substituted with hydroxyapatite nanocomposites has proved to be biocompatible, presented high cell attachment, and proliferation rate. They also promoted the upregulation of osteogenic-like genes, which evidence their capacities for bone tissue reparation [168]. Another innovative way to prepare gelatin-based composites for GFs delivery was using the modified gelatin methacryloyl (GelMA), which is photocross-linkable with UV light. GelMA-based hydrogel composites reinforced with FGF-loaded chitosan nanoparticles evidenced biocompatibility and fibroblast proliferation during the sustained release of FGF [169].

Other GFs as PDGF, VEGF, and BMPs have also been included in gelatin-based composites with particular characteristics. Specifically, PDGF was absorbed in streptavidin-coated polystyrene microparticles, which were later joined to thermosensitive hydrogels of gelatin using an aptamer [170]. An aptamer is a short sequence of simple-chain nucleic acids able to bind with high affinity to other molecules [171]. The resulted composite showed a gradual dissolution and a slow rate of PDGF release compared with the native gelatin hydrogels. A new injectable nanodiamonds-based composite hydrogel formed by gelatin and chitosan was used to deliver VEGF for wound healing [172]. The nanodiamonds reinforcement improves the mechanical properties of composites, and their complexation with VEGF and subsequent inclusion allowed a sustained release of this GF. On the other hand the electrospinning method has also been employed to produce a gelatin-poly(lactic-co-glycolic acid) composite for dual delivery of VEGF and BMP-2. The composite consisted of randomly-oriented nanofibres that were interconnected in the 3D-reticular structure where cells firmly attached and experienced good morphology and connection [173]. The inclusion of VEGF and BMP-2 and their subsequent and sequential release improved the cell adhesion, proliferation, and differentiation of MSC into osteogenic cells.

Natural-based composites for delivery of growth factors are auspicious materials due to they generally are biocompatible, no toxic, biodegradable, and, don evoke immunogenic responses. In addition, they have great versatility to be combined with materials of serval natures, shapes, and sized to allow their use in any part of the human body. Besides, they can be produced by a wide variety of techniques under mild conditions. Depending on the set conditions used during their manufacturing, many types of composites based in natural polymers could be created. The most significant benefit of this type of system is their control capacity for sequential delivery of two or even more growth factors to produce appropriate clinical treatments for tissue engineering and wound healing. However, there are different points that are need to solve such as the proper dosage of GFs and an uniformly control of the properties of the natural polymers that are used. Regarding the dosages, the synergistic action of several factors on specific tissue must be studied deeply to achieve the right combination of their release patterns. While the natural polymers suffer from intrinsic variations that affect their properties and limit their use, for that, it is necessary standardized some production procedures.

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Biopolymers/Ceramic-Based Nanocomposite Scaffolds for Drug Delivery in Bone Tissue Engineering

K. Lavanya, S. Swetha, and N. Selvamurugan

Abstract Joint repair and reconstructive bone surgeries are growing worldwide. Self-healing of bone is constrained, which entails external stimuli to bolster bone repair and rejuvenation. While conventional approaches (autografts, allografts, or xenografts) have been increasingly utilized to repair bone defects, they all have corresponding drawbacks, thus minimizing their clinical applications. Bone tissue engineering (BTE) is a fascinating approach encompassing bone biology and engineering concepts to combat the flaws associated with grafting, as mentioned above. A variety of biomaterials such as biopolymers (natural and synthetic) and ceramics as scaffolds has been exploited to fabricate the ideal bone constructs using conventional and advanced techniques. Scaffolds loaded with appropriate drugs, including growth factors, bone remodeling molecules, phytochemicals, and other regulatory molecules for sustained and site-targeted delivery, can promote functional bone tissues. Hence, this chapter presents a distinct variety of biopolymer-ceramic-based nanocomposite scaffolds for drug delivery in BTE.

Keywords Bone tissue engineering · Scaffolds · Nanostructure · Drug delivery

Abbreviations

3D	Three dimensional
Alg	Alginate
ALP	Alkaline phosphatase
BG	Bioactive glass/bioglass
BMP	Bone morphogenetic protein
BMSCs	Bone marrow stromal cells
BSA	Bovine serum albumin

K. Lavanya · S. Swetha · N. Selvamurugan (✉)
Department of Biotechnology, School of Bioengineering, College of Engineering and Technology, SRM Institute of Science and Technology, Kattankulathur, Tamil Nadu 603203, India
e-mail: selvamun@srmist.edu.in

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BTE	Bone tissue engineering
CaP	Calcium phosphate
CIP	Ciprofloxacin
Col	Collagen
CS	Chitosan
DEX	Dexamethasone
ECM	Extracellular matrix
FGF	Fibroblast growth factor
GDL	Glucono-d-lactone
Gel	Gelatin
GO	Graphene oxide
HA	Hyaluronic acid
HAp	Hydroxyapatite
HGF	Hepatocyte growth factor
HME	Hereditary multiple exostoses
HPCS	Hydroxypropyl chitosan
IGF	Insulin growth factor
MRSA	Methicillin-resistant staphylococcus aureus
MSC	Mesenchymal stem cell
nHAp	Nano-hydroxyapatite
NP	Nanoparticle
OCN	Osteocalcin
OPN	Osteopontin
PCL	Polycaprolactone
PDGF	Platelet derived growth factor
PEUR	Poly(ester urethane)
PHBV	Poly(3-hydroxybutyrate-co-3-hydroxyvalerate)
PLA	Polylactic acid
PLGA	Poly(lactic-co-glycolic acid)
PLLA	Poly-L-Lactic Acid
PTH	Parathyroid hormone
PTMC	Poly(trimethylene carbonate)
PVP	Polyvinyl pyrrolidone
rBMP2	Recombinant bone morphogenetic protein 2
SBF	Simulated body fluid
SDF	Stromal cell-derived Factor
SF	Silk fibroin
TCP	Tricalcium phosphate
TGF-β	Transforming growth factor-β
TiO_2	Titanium oxide
VEGF	Vascular endothelial growth factor

1 Introduction

The worldwide prevalence of bone diseases and illnesses has been growing. According to comparative statistics, orthopedic grafts' global market value was $3.3 billion in 2017, which is projected to worsen in 2021, representing a vast expense to the national economy [1, 2]. The bone is a complex tissue that aids in movement and is also given its hard and dense structure, safeguarding our body's vital internal organs [3]. It is primarily made up of two regions, cortical and cancellous/spongy bone. The bone extracellular matrix (ECM) predominantly consists of organic (mainly type I collagen fibers) and inorganic [(hydroxyapatite (HAp) like calcium phosphate)] materials [4–6]. Three crucial cells, namely osteoblasts, osteocytes, and osteoclasts, are accountable for bone remodeling [7–9]. Osteoblasts are major players in osteogenesis and are involved in the formation of mineralized bone matrix. Osteoblasts derived from progenitor cells or mesenchymal stem cells (MSCs) form a protein blend called osteoid, which is composed of polymerized collagen and mineralized with calcium and phosphate in the later bone stages growth [10, 11]. In addition to the abovementioned functions, osteoblasts also secrete regulatory molecules responsible for bone formation [12]. Osteocytes are mature forms of osteoblasts that settle in the ECM, interact with the surrounding osteoblasts, and play a crucial role in mineral homeostasis [13, 14]. Osteoclasts are derived from hematopoietic stem cells and are essentially involved in bone resorption [15, 16]. The synergistic interplay of osteoblasts and osteoclasts sustains the native bone mass and regulates terminal remodeling [17, 18].

Bone defects are categorized into various divisions such as old age, hereditary disorders, fractures, cancer, and infection based on their cause. Among the different defects, the most common ones are bone fractures [19, 20]. Bone fractures can occur in any part of the body. Osteoporosis and bone loss caused due to old age can deteriorate the bone's strength and lead to fractures. Osteomyelitis is a major cause of bone infection, and it leads to pain, redness in the affected area. If it is not treated in time, it may lead to amputation, an irreversible trauma [21]. Bone tumors induce rapid bone remodeling, fractures, and anemic patient conditions that might lead to life-threatening situations. Tumor removal is primarily taken care of through surgery, but sometimes defects occur in this site leading to further complications [22]. Genetic bone diseases such as hereditary bone marrow failure syndromes and hereditary multiple exostoses (HME) require stem cell transplantation or surgical resection as treatment modalities [23]. All the aforementioned bone defects can heal on their own to some extent, but external intervention is required for the proper functional bone regeneration once it exceeds the critical size. These different categories of bone defects have to be looked into individually and must be addressed in a customized manner with new innovative technologies for effective bone treatment. Conventional gold standard procedures, i.e., autografts and allografts, hamper their extensive clinical applications due to the risk of higher non-union occurrences with host tissues, possible immune infections, donor site morbidity, and implant-related discomfort [24, 25]. Therefore, designing a system to correctly and effectively restore the damaged bone tissue is of great therapeutic importance for patients with bone-related disorders. Bone tissue engineering (BTE) has gained significant interest from researchers as a promising strategy to alleviate bone defects without the limitations and disadvantages of autografts, allografts, or xenografts [26]. BTE is an interdisciplinary technique that uses various biomaterials as scaffolds to recreate the necessary functions of bone tissue [27]. These scaffolds provide a template for cell adhesion in bone tissue formation. Different vital parameters are used to determine the scaffolds' efficiency, i.e., pore size, pore volume, mechanical strength, and chemical properties. BTE focuses on bone structure, bone mechanics, and tissue development for new accessible bone tissues [28]. A variety of biomaterials such as biopolymers (natural and synthetic) and ceramics have been exploited to fabricate the functional ideal bone constructs using conventional (solvent casting particulate leaching, gas foaming, phase separation, electrospinning, freeze-drying) and advanced techniques (3D and 4D bioprinting) [29, 30]. Native bones have a unique hierarchical structure, ranging from nanoscale to macroscale with specifically designed interfaces [3]. Conventional BTE scaffolds exploited the various pore-forming methods to reproduce the microscale and macroscale characteristics of native osseous tissues [31]. However, nanoscale structures are pivotal to regulating cellular activities such as migration, proliferation, differentiation, and ECM development. In view of conventional therapies' significant weakness, nanomaterials have recently emerged as potential candidates for creating ECM-like scaffolds, which effectively substitute faulty tissues [32]. Nanocomposite-based scaffolds offer a closer architectural approximation to the native bone for the aforementioned cellular functions, which lead to the construction of functional tissues [33]. Most importantly, biopolymer-ceramic-based nanocomposites have the potential to effectively release the drug molecules in a sustained and prolonged manner for orthopedic applications [34, 35]. This chapter summarizes recent advances in the synthesis and implementation of different biopolymer-ceramic-derived nanocomposite scaffolds loaded with therapeutic molecules in the field of BTE. A schematic representation of a biopolymer-ceramic-based nanocomposite scaffold in BTE is depicted (Fig. 1).

2 BTE Scaffolds for Drug Delivery

Targeted delivery of drugs to the deformed bone site can significantly augment the rejuvenation of functional bone tissue. Proper bone tissue repair and regeneration involve a complex interaction of cells and growth regulatory cytokines working in congruence with the delivered drug molecules. Bone remodeling drugs can greatly enhance and induce the bone-forming cells' proliferation and differentiation potential by modulating their cellular activities. These delivered drug molecules selectively bind to the cell surface receptors and translocate to the nucleus, thereby determining

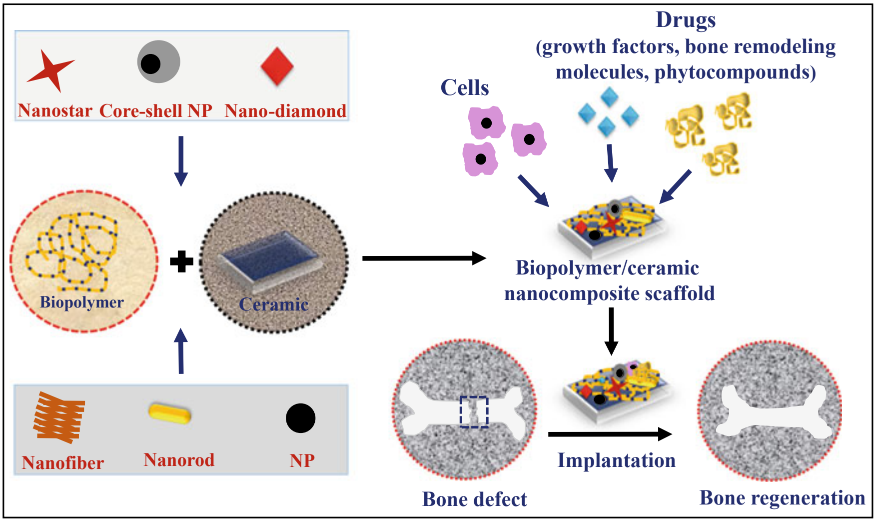

Fig. 1 Schematic representation of biopolymer/ceramic-based nanocomposite scaffolds in BTE applications. Biopolymer/ceramic-based nanocomposites are encapsulated with cells and drug molecules to facilitate bone regeneration effectively

biological responses [36]. In BTE, the multifunctional scaffolds may act as a delivery system for the targeted, sustained delivery of therapeutic drug molecules [37].

2.1 *Types of Drug Molecules Used in Bone Regeneration*

Several therapeutic molecules have been widely investigated for their potential in osteogenic differentiation ability by regulating the cascade of cellular and molecular signaling pathways in bone regeneration [38]. Growth factors, bone remodeling molecules (anti-bone resorptive and bone-forming), phytocompounds, and other regulatory molecules have been used for drug delivery in bone regeneration. The detailed understanding of the bone healing process and proper utilization of these bioactive molecules greatly aid in significant bone regeneration [39].

2.1.1 Growth Factors

Growth factors are soluble signaling peptides secreted by the vast array of cells to stimulate diverse biological responses like cell survival, proliferation, differentiation, migration, and ECM deposition [40]. Growth factors selectively bind to the cell surface receptor of the targeted cells, resulting in the receptors' conformational change and activate the internal signaling cascades, including the transcription factors within the cells. This event is followed by nuclear translocation and results in the regulation of gene expression [41]. Since these growth factors are naturally present in the native bone, the healing process naturally takes place. Several growth factors are being employed in the bone repairing process, which include transforming growth factor-beta1 (TGF-β1), TGF-β3, bone morphogenetic protein (BMP)-2, BMP-4, BMP-7, fibroblast growth factor (FGF)-2, platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), insulin growth factor (IGF)-1, stromal cell-derived factors (SDF)-1, interleukin (IL)-6, and hepatocyte growth factor (HGF) [42–53]. Though these growth factors have much proved their significance in the number of bone regeneration studies, they are often subjected to proteolytic degradation. Additionally, their short half-life with a rapid clearance rate in vivo is also a major concern [54]. The action of growth factors depends upon cells expressing their receptors, indicating their specificity in biological functions [55]. Also, the diffusion rate of the growth factors through the ECM is very low; thus, it requires highly concentrated doses to reach the therapeutic level. But unfortunately, a higher dosage leads to several side effects, including cytotoxicity, heterotopic bone formation, and cancer. Most importantly, the higher cost of these growth factors restricts their usage in more massive amounts. Therefore, the site-targeted and sustained drug delivery kinetics become crucial to address the pitfalls of the growth factors delivery [56].

2.1.2 Bone Remodeling Drugs

BTE involves using a plethora of drug molecules that aid in the significant regeneration of functional osseous tissue. As mentioned in the introduction, bone remodeling is a coupled process involving bone formation by osteoblasts and bone resorption by osteoclasts. Various osteogenic drugs and small molecular weight compounds are used to treat the defect in bone remodeling. These bone remodeling drugs have been predominantly used with bone tissue-engineered constructs to facilitate osteogenesis. Additionally, several antimicrobial and anti-inflammatory drugs are employed to conquer microbial contaminations, and inflammatory responses occur after incorporating the regenerative scaffolds [57, 58].

Osteogenic Drugs

Sustained osteogenic drugs through engineered bone construct at the site of injury can further intensify bone regeneration and control bone resorption. Here we discuss some of the significant osteogenic drugs widely used in BTE. Bisphosphonate, an interesting drug holds a special place in treating osteoporosis and preventing osteoporotic fractures. Bisphosphonates have mainly been utilized in orthopedic applications because of their high affinity toward osseous tissue [59]. They selectively bind to the apatite crystal matrix by establishing strong bidentate bond by chelating phosphonate groups and calcium ions [60]. Also, it is proven that targeted delivery of bisphosphonates at the site of deformation potentially inhibits osteoclastogenesis and promotes osteogenesis [61]. Besides, it prevents the wrapping of fibers on the implants by inducing fibroblasts' apoptosis, thereby enhancing their tenacity [62]. Bisphosphonates, including alendronate, risedronate, ibandronate, zoledronic acid, clodronate, etidronate tiludronate, have been investigated in finding their role on bone regeneration [63–69]. Despite the privileges, bisphosphonates' affinity toward healthy bone may induce certain side effects. Therefore, a sustained and site-targeted delivery of bisphosphonate-related drugs becomes necessary to escape undesirable effects [61]. Sometimes, delayed fracture healing and non-unions occur due to conditions such as osteoporosis, malnutrition, aging, postmenopausal state, or low estrogen. Various bone-building anabolic drugs have been investigated. Parathyroid hormone (PTH) is the primarily approved anabolic drug used to augment the healing process. PTH is an 84 amino acid containing protein secreted by the parathyroid glands. Its predominant role is to regulate the extracellular calcium [70]. Considering PTH's potential biological significances, its derivatives such as teriparatide, abaloparatide, and romosozumab are widely used in bone regeneration therapies [71–73].

The bone regeneration process may also be heightened by administering anti-resorptive drugs to inhibit osteoclast bone resorption. RANKL inhibitors are the selective variant of anti-resorptive drugs, which inhibit osteoclastogenesis by interfering with the binding of RANKL with RANK receptor present on the osteoclast precursors' surface. Denosumab, a monoclonal antibody, is considered as an alternative anti-resorptive drug in the treatment of osteoporosis. It is a RANKL inhibitor, which works by preventing osteoclast cell formation [74].

Low Molecular Weight Drugs

Small molecular drugs and bioactive factors generally have their molecular weight lesser than 900 Daltons. These small compounds are usually stable, display notable osteoinductivity at low doses, and less immunogenic. They are available at an affordable cost [75]. Statins are the most widely used low molecular weight drug in BTE. Statins selectively inhibit 3-hydroxy-3-methyl-glutaryl co A (HMG-CoA) reductase from enhancing BMP-2 secretion of the cells to improve osteogenesis [76]. In bone tissue regeneration, three types of statins, namely simvastatin, lovastatin, and rosuvastatin, are widely utilized. They exhibited fewer adverse effects [77–79]. Other low molecular weight drugs with osteoinductive potential include tilorone, dexamethasone, fingolimod, purmorphamine, and tetracycline [80–84].

Antimicrobial and Anti-inflammatory Drugs

Microbial contaminations and inflammatory responses are of major setbacks that take place during and after the scaffold implantation. These undesirable reactions at the site of scaffold implantation can lead to the serious issue of implant failure. These difficulties can be overcome by the sustained delivery of the antimicrobial and anti-inflammatory agents through the engineered construct. As antimicrobial drugs, tetracycline, vancomycin, doxycycline, rifampicin, gentamycin, clindamycin, and moxifloxacin have been widely investigated in BTE [84–90]. The delivery of divalent cations such as gold, silver, zinc, copper, titanium, magnesium, and strontium can display excellent antimicrobial properties and help in bone regeneration as some of them are integral components of the native bone tissue [91–96]. Furthermore, to mitigate the inflammatory responses raised due to the scaffold implantation, several immunosuppressive and anti-inflammatory drugs are being delivered along with the scaffold. Dexamethasone, aspirin, ibuprofen, naproxen, meloxicam, celecoxib, indomethacin, and diclofenac have been administered in treating bone tissue inflammation [81, 97–103]. Impressively, in addition to the antimicrobial and anti-inflammatory properties exhibited by the drugs mentioned above, they are also proved to enhance the bone modulation by improving cell differentiation, alkaline phosphatase activity, and mineralized matrix formation [81, 104]. But unfortunately, the above-discussed drugs, when delivered in high concentration or long-term systemic administration, can cause invariable side effects. This critical issue can be surmounted by designing a proper drug delivery system with targeted and controlled delivery kinetics.

2.1.3 Phytochemicals

Though numerous studies report the delivery of growth factors and drugs for successful osteogenesis, there are also potential side effects like malignancies and cytotoxicity. Furthermore, the sustained and targeted delivery of these compounds and their escalating cost is of major pitfall to be encountered. These necessitate the search for alternative bioactive molecules to bolster the bone regeneration process. Nowadays, researchers are intrigued by phytochemical-incorporated bone regenerative scaffolds [105]. Phytochemicals are non-nutritive secondary metabolites of plants produced during the period stress condition and injury. Interestingly, these plant-derived compounds play a significant role in enhancing and inducing the osteogenesis process [106]. Moreover, its low-cost, high availability, and non-toxic nature makes them a better replacement in the place of growth factors and drugs. Phytocompounds are classified into various groups based on their structural and functional properties. These are alkaloids, phenolics, carotenoids, nitrogen-containing compounds, and organosulfur compounds [107]. To date, a plethora of phytocompounds with osteogenic potential has been explored. It is also reported to enhance osteogenic marker genes such as ALP, OCN, OPN, OSX, OPG, COL1. These phytocompounds bolster bone regeneration by undergoing bone regenerative signaling pathways, viz. BMP signaling pathway, MAPK signaling pathway, and Wnt/β-catenin signaling pathway [108]. Icariin, silibinin, resveratrol, quercetin, sulforaphane, genistein, sinapic acid, zingerone, chrysin, diosmin, veratric acid, valproic acid, mucic acid are some of the recently investigated phytocompounds with notable osteogenic potential [109–121]. Despite the significances mentioned above of the phytocompounds in bone regeneration, they also express some shortcomings. The hydrophobic nature of some phytocompounds results in low aqueous solubility and bioavailability. On the other hand, hydrophilic phytomolecules may exhibit low absorption and rapid metabolism. These challenges can be catered by using various drug delivery techniques, and understanding of the biological interaction of phytocompounds with stem cells becomes crucial to improve their therapeutic efficacy [122].

2.2 Impacts of Scaffolds for Drug Delivery

Bone regeneration at the deformed site can be promoted by delivering various growth regulatory signaling peptides and drugs [123]. Several techniques have been adopted to deliver the signaling peptides to the site of the fracture. One such traditional approach involves the direct systemic delivery of drugs through oral and intravenous administration [124]. In this strategy, delivered drug molecules are absorbed into the bloodstream and distributed throughout the body with the circulatory system's aid. In this case, a significant quantity of the drug will accumulate in other organs of the body; thus, only a small proportion of the supplied therapeutic dosage range can reach the actual site of the injury [125]. Bone, being a peripheral organ with limited blood supply than other tissues, seriously reduces drug delivery [126]. Additionally, the lack of tissue specificity results in poor penetration of the drug into the targeted tissue, low therapeutic efficacy, systemic cytotoxicity, and several side effects, including liver and renal complications. Moreover, decreased bioavailability, instability, rapid breakdown, and renal clearance rate are major concerns to enhance or maintain the supplied therapeutics [127]. For instance, direct injection of growth peptides at the deformation site is severely prone to degradation as they have a relatively short half-life. When injected intravenously, the half-life of the growth factors such as bFGF, PDGF, VEGF was found to be 3–50 min, 2 min, and 30–45 min, respectively. Additionally, the administered signaling peptides may be susceptible to oxidative damage when the amino acids in them interact with oxygen radicals. Particularly peptides containing tyrosine, tryptophan, histidine, and cysteine residues are more liable to oxidation [128, 129]. These deterrents triggered the need to develop a targeted drug delivery system to facilitate sustained and prolonged drug delivery at the diseased site for a prescribed period. In this regard, site-targeted implantation of the drug encapsulated scaffolds holds an upper hand in overcoming the disincentives mentioned above with systemic drug delivery and providing a comprehensive landscape for successful bone-targeted drug delivery [130].

An ideal scaffold for bone regeneration should (1) be able to biodegrade gradually in congruence with the new bone formation without the release of any toxic by-products; (2) be biocompatible to facilitate cellular attachment, proliferation, and differentiation to fasten bone rejuvenation; (3) be mechanically stable to withstand the load during the amelioration period; (4) possess optimum porosity and pore diameter to facilitate enhanced cellular infiltration, nutrient exchange, and angiogenesis; (5) be able to encapsulate and release drugs in a controlled, sustained manner. Thus, a proper bone tissue-engineered construct should act as a dual function matrix to support new bone formation and act as a carrier for sustained drug delivery in situ [125, 131, 132]. Different types of scaffolds have been widely utilized for controlled drug release in bone tissue repair. The scaffold architecture has been categorized into different types, including hydrogels, porous scaffolds, electrospun fibers, metallic implants, and 3D bioconstructs. These scaffolds can be fabricated using a variety of conventional fabrication techniques (e.g., electrospinning, gas foaming, freeze-drying, melt molding, solvent casting, phase separation, porogen leaching, sol–gel technique) and modern fabrication techniques (3D printing, 3D bioprinting, and 4D printing) [133, 134]. Scaffolds for drug delivery in BTE are fabricated using several biodegradable polymers (natural/synthetic), bioactive compounds, bio-resorbable inorganic biomaterials including calcium phosphate and its derivatives, bioactive glasses, and mesoporous silica [135]. The selection of suitable biomaterial and the fabrication process for drug delivery solely depends on the type of drugs, their stability, bioactivity, and the desired release kinetics [8]. Therefore, a meticulous selection of biomaterials and their fabrication process concerning the type of drug molecules can aid in controlled and sustained drug delivery.

Various drug delivery strategies are being employed to attain sustained drug delivery based on the nature of signaling molecules to be delivered. Different categories of drug molecules demand distinct delivery strategies to accomplish their desired therapeutic effects. Commonly employed drug delivery strategies for BTE applications involve a surface presentation, controlled sustained drug release, pre-programmed drug release, and stimuli-responsive drug release [136]. Surface presentation of the drug molecule on the bone construct can be done either by physical interaction or chemical conjugation approach. This technique enables the drug molecules' contact with the cells migrating toward the construct and potentially reduces off-target side effects [137]. Another intriguing and promising drug delivery strategy for effective bone regeneration involves controlled sustained drug release, which maintains ideal drug concentration at the site of bone regeneration for the desired period. The drug molecules can be encapsulated directly within the polymeric matrix during the fabrication, or it can also be encapsulated in distinct delivery vehicles such as liposomes, micro/nanoparticles. In this, direct drug encapsulation within the polymeric matrix is considered the most straightforward technique, but it may also affect the bioactivity and the release kinetics of the drug. To mitigate these constraints, drug molecules have been introduced separately using specific delivery vehicles to maintain their bioactivity and controlled release kinetics [138]. Another promising drug delivery strategy is pre-programmed drug delivery, an advanced strategy to achieve sequential release of multiple drug components with diverse release kinetics using multi-compartment scaffolds. Supplying multiple drug components can increase bone regeneration at even low dosage concentrations, thus aids in minimizing the potential risk of cytotoxicity associated with delivering a single drug at a high dose [139]. Following the pre-programmed drug delivery, stimuli-responsive drug release is gaining immense attention to reduce the side effects caused due to increased drug dosing and burst release of drugs in conventional drug delivery. Various stimuli-responsive smart biomaterials release drugs based on demand by different external stimuli such as temperature, pH, magnetic field, electric field, and irradiation. But the safety concern associated with the intensity of the external stimuli is a major issue. Therefore, safer and less invasive stimuli are more likely to be utilized in clinical treatments [140]. To overcome the challenges associated with the drug delivery strategy, nanotechnology-based research is widely used presently. Especially, nanocomposites are gaining increasing attention in site-targeted and controlled drug delivery [141].

Nanocomposites combine multiple nanomaterials or nanoparticles encapsulated within bulk biomaterial with a nano-range size of 1–100 nm. Nanostructured particles within the composite system can provide exceptional progress in material properties compared to bulk matrix, micro-composites, and macro-composites. The nanocomposite scaffolds' significances include enhanced mechanical properties, controlled drug delivery profile, and molecular permeability [142]. From the scaffold fabrication point of view, these nanocomposites possess thermal stability, chemical resistance, flame retardancy, electrical conductivity, and optical clarity. Nanocomposites' property varies from normal composite materials in terms of having a remarkably higher surface-to-volume ratio [143]. This property facilitates the transport of large quantities of drug molecules within the bone constructs. Considering nanocomposites' paramount privileges in drug delivery, diverse nanocomposite combinations have been widely developed and investigated for BTE applications. Let us discuss the different combinations of nanocomposites and their orthopedic drug delivery applications in the forthcoming topics.

3 Biopolymer/Ceramic Nanocomposite Scaffolds in Bone Regeneration

Nanocomposites scaffolds are an amalgamation of two distinct biomaterials consisting of two phases, a reinforcing phase (nano-range particles, fibers, sheets), embedded in a matrix phase [144]. Various novel nanocomposite systems have been widely investigated to enhance drug solubility, cell-specific targeting of bone, inhibition of rapid drug degradation, and improvement of drug stability to safely reach its site of action without being eliminated in the systemic circulation. The nanocomposite scaffolds can be categorized based on the carrier matrix and filler material utilized [145]. In recent years, natural and synthetic polymers are the most widely used as carrier matrix for the encapsulation of the drug-loaded nanofillers (ceramic materials) due to its tailorable biodegradability. Figure 2 describes a different combination of biopolymer and ceramic-based nanocomposites for orthopedic drug delivery applications.

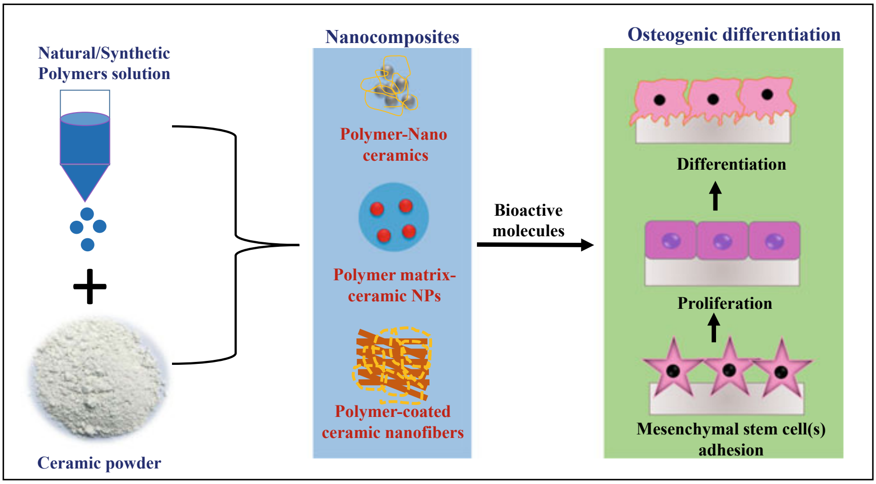

Fig. 2 Different combinations of biopolymers and ceramics are utilized to design biopolymer/ceramic-based nanocomposite bone constructs. Bioactive molecules are included to accelerate the differentiation of mesenchymal stem cells toward osteoblasts

3.1 Biopolymers-Based Nanocomposite Scaffolds

Biomaterials play a predominant role in deciding the scaffolds' characteristics and are categorized into natural and synthetic polymers [146]. The most widely employed natural biopolymer in drug delivery includes chitosan (CS), alginate (Alg), gelatin (Gel), collagen (Col), cellulose, hyaluronic acid (HA), and silk fibroin (SF) [147]. Several biodegradable synthetic polymers like polycaprolactone (PCL), poly(lactic acid) (PLA), polyethylene glycol (PEG), poly(lactic-co-glycolic) acid (PLLA) are increasingly investigated for their drug delivery potential as nanocomposites [148]. These biodegradable polymers can be exploited independently or in combination with one another to achieve controlled drug delivery in various ways, including polymer degradation, polymer disintegration, and drug dispersion within the polymeric network or over a polymer film [149].

3.1.1 Natural Polymers

Natural polymers are increasingly utilized in nanocomposites-based drug delivery for orthopedic applications as they are found to be more biocompatible in nature, biomineralization, and osteogenic potential [150]. Most importantly, natural polymers may contain ligands that facilitate binding with the cell surface receptors to enhance osteogenesis [151]. Here we elucidate the various properties of natural polymers and their current applicability in the fields of orthopedic drug delivery. CS is a biodegradable natural polymer obtained from chitin (shell crusts of crustaceans and fungal cell walls). It is mainly comprised of β-1, 4-linked N-acetyl-D-glucosamine, and D-glucosamine units [152]. Its intrinsic properties, such as biodegradability, biocompatibility, antibacterial activity, cationic nature, and mucoadhesive properties, make them more attractive in BTE applications. Specifically, the CS's structural resemblance with glycosaminoglycans (GAGs) aids in enhancing the bone regeneration rate [153]. But the mechanical instability of CS restricts its usage in native form; thus, this critical issue can be addressed by formulating CS-based nanocomposites scaffolds with ceramics such as HAp, tricalcium phosphate (TCP), and synthetic polymers [154]. In recent years, CS-based nanocomposites are mostly investigated in orthopedic drug delivery applications due to their increased drug loading efficiency, controlled and prolonged drug release profile [155].

Alg is an anionic polysaccharide made of 1, 4-linked β-D-mannuronic acid (M) with 4C1 ring configuration and α-L-guluronic acid (G) with 4C1 conformation [156]. Alg polymer as a carrier matrix in nanocomposites holds a significant place in orthopedic drug delivery. It exhibited improved biocompatibility, drug encapsulation and release potential, biomineralization capacity, porosity, cell adhesion, proliferation, and osteogenic differentiation [157]. Gel is a proteinaceous biodegradable polymer derived from Col. Gel can mimic the components of the native ECM, and this intriguing property helps to improve cell adhesion and proliferation [158]. The excellent biocompatibility, biodegradability, and porous structure of Gel-based nanocomposites gain immense attention in bone-targeted drug delivery [159]. Unfortunately, the Gel's low stability in various physiological conditions triggered the need for the inclusion of other polymeric or ceramic biomaterials to form a stable nanocomposite for drug delivery [160]. Col is one of the abundantly available natural polymers. Type I Col is the chief composition of ECM, especially in tissues such as bone and tendon, which nourishes the osteogenic cells by facilitating proper nutrients infiltration [161]. As a biomaterial, Col holds a significant place in BTE because of its highly porous nature, simple processing, hydrophilicity, biocompatibility, compatibility with other polymers, absorbability in situ, and low antigenicity [162]. Considering the Col's paramount privileges, it has been extensively utilized as a carrier matrix for nanocomposites in controlled drug delivery applications.

Cellulose is the abundantly available natural polymer obtained from bacterial and plant sources. It is made of long-chain polysaccharides with over 10,000 β-(1-4)-connected D-glucose units [163]. Nanocellulose biomaterial possesses unique properties such as enhanced chemical reactivity, mechanical tenacity, the high specific surface-to-volume ratio, and non-toxicity, making it an exquisite candidate for controlled drug release [164]. Cellulose and its derivatives, such as cellulose ether, cellulose esters, and oxycellulose, have been used widely in drug delivery applications [165]. Hyaluronic acid (HA) is a linear glycosaminoglycan that comprises a repetitive unit of N-acetyl-D-glucosamine and D-glucuronic acid with the monosaccharides coupled together by way of alternating β-1,3 and β-1,4 glycosidic bonds [166]. HA and its derivatives have been extensively utilized as carrier molecules for controlled and sustained drug release [167]. HA's specific binding affinity toward receptor for HA-mediated motility (RHAMM) and CD44 cell surface receptors serves as the foundation for the targeted drug delivery of therapeutic proteins and drugs [168]. SF is a protein-based natural polymer derived from the domesticated silkworm *Bombyx mori* and spider *Nephila clavipes*. There is a growing desire to develop protein-based nanocomposites in orthopedic drug delivery due to their exceptional functionalities [169]. SF-based drug carriers possess excellent biocompatibility, biodegradability, and non-antigenic nature [170]. SF has been widely investigated as an ideal drug delivery matrix because of active intrinsic amino groups and tyrosine residues, which facilitates the conjugation of various drug molecules [171].

3.1.2 Synthetic Polymers

Synthetic polymers are another class of biodegradable polymers widely used in BTE applications. According to the application site, its major advantages include the ability to customize degradation kinetics and mechanical properties by modifying the functional groups present in them. They can also be synthesized into different forms with desired pore size geometry that is conducive to the growth of blood vessels and neo-bone [172, 173]. On the contrary, they display reduced bioactivity, osteoconductivity, and cell recognition RGD sequences compared to the natural polymers. In this context, several polymers' surface functionalizations can be done to improve their performance in BTE [174–176]. In the following sections, we discuss the characteristics features of some frequently utilized synthetic polymers such as PCL, PLA, and PLGA in nanocomposites-based drug delivery for BTE applications.

Among the various biodegradable polymers investigated, a special focus is made on PCL mainly because of its broad spectrum functionality and compatibility with other polymers. PCL is an aliphatic polymer with semicrystalline nature that displays desirable biocompatibility and mechanical durability [177]. On the other hand, its slow degradation profile may negatively impact new bone formation. Additionally, its hydrophobic surface affects cell adhesion and proliferation and does not allow facile delivery of hydrophobic drug molecules [178]. This hitch can be conquered using a modified form of PCL with several osteogenic, osteoinductive inorganic compounds such as HAp, bioactive glass (BG), and titanium dioxide. Considering the leverage of the modified form of PCL, several PCL-based nanocomposites have been widely exploited in drug delivery application for bone regeneration [179].

PLA is a non-toxic, aliphatic biodegradable polymer obtained from renewable sources like corn, cassava, and sugar cane, which has been extensively researched as a bone substitute material and drug-releasing carrier in BTE [180]. Based on the microstructure, PLA can be classified into three types such as PLLA, poly(D-lactic acid) (PDLA), and their racemic counterpart D,L-PLA (PDLLA). From the above-mentioned PLA categories, PDLLA is frequently utilized in drug delivery systems because of its monophasic assembly [181]. PLGA is a biodegradable linear copolymer of PLLA and glycolic acid (PGA). PLGA holds the upper hand as a promising carrier matrix for drug delivery applications [182]. PLGA has gained extreme importance in drug delivery applications due to its salient features, including biocompatibility, tunable degradation rate, sustained drug release profile, protection of encapsulated drugs from degradation, and target specificity [183]. Nevertheless, the amorphous nature of the PLGA makes it mechanically unstable and, thus, restricts its usage in load-bearing applications. In this context, PLGA requires the inclusion of nano-sized bioactive ceramics such as HAp, BG, and calcium phosphate (CaP) to create PLGA nanocomposite biomaterials [184].

3.2 Ceramics-Based Nanocomposite Scaffolds

A wide variety of biomaterials have been utilized for orthopedic drug delivery applications. In particular, ceramic-based nanocomposites have gained recognition over the years due to its structural similarity with the native bone tissue's inorganic composition. Ceramics, including HAp, CaP, and BG, have been intensively researched for their drug delivery application [185].

3.2.1 Calcium Phosphate

Among the various biomaterials being utilized to date, CaP has been predominantly scrutinized for its use in the bone regeneration process due to its resemblance to the inorganic mineral segment of native bone, biocompatibility, osteoconductivity, and bio-resorbable nature [186]. CaP is a reservoir of calcium and phosphate ions that persuasively augments stem cells' osteogenic differentiation into the osteoblastic lineage. Depending on the Ca/P ratio and solubility nature, CaP can be classified into different types: HAp, alpha-tricalcium phosphate, and beta-tricalcium phosphate [187]. Considering the preeminent influence of CaP and its derivatives in bone regeneration, CaP has been widely employed as a carrier for various drug molecules and therapeutic cargoes.

3.2.2 Hydroxyapatite

HAp [(Ca)10(PO4)6(OH)2] is the major inorganic composition of the native bone, which holds the potential of regulating the biomineralization process. HAp is increasingly used in bone regeneration applications because of its excellent biocompatibility, osteoconductivity, and tunable physical and chemical properties [188]. Slower degradation kinetics of the HAp much helps control and localizes drug release, thereby minimizing the cytotoxicity [189]. Also, the drug loading capacity, drug release profile, and therapeutic efficacy are depending on the characteristic nature of HAp, i.e., its size, structure, chemical composition, porosity, crystallinity, surface chemistry [190].

3.2.3 Bioactive Glass

BG is an amorphous, silicate-based biomaterial used in BTE applications. The traditional 45S5 bioactive glass comprises 45 SiO_2, 24.5 Na_2O, 24.5 CaO, and 6 P_2O_5 by wt% and has received FDA approval for clinical use as a bone filler in the treatment of deformed bone tissue [191]. The interaction of BG with the physiological fluid results in the release and exchange of soluble ions such as Ca, P, Si, and Na, which binds to the bone to stimulate pertinent intracellular and extracellular signaling cues for enhanced osteogenesis [192]. In recent years, nanocomposites of biopolymers and BGs have been progressively developed for orthopedic drug delivery due to their highly porous structure, large surface area, and bioactive nature biodegradability, and biocompatibility [193].

Each class of the above-discussed biomaterials in drug delivery applications has its advantages and disadvantages. For instance, biopolymers usually serve as an excellent carrier matrix for sustained and prolonged drug delivery due to their native ECM's physicochemical similarities. Nevertheless, some disadvantages, including low mechanical strength, risk of immunogenic reactions, and non-tailorable nature, restrict their native form usage. Similarly, in addition to the ceramics' privileges, it also suffers from certain disadvantages like brittleness, low biodegradability, toxicity, poor drug release profile, and surface morphology. Therefore, to mitigate these catastrophic failures associated with controlled drug release, different biomaterials are being used in combination with one another. In the following sections, we explore the various combinations of the biomaterials mentioned above in orthopedic drug delivery.

4 Biopolymer/Ceramic Nanocomposite Scaffolds for Drug Delivery in Bone Tissue Engineering

Recently, several investigations on biopolymer/ceramic nanocomposite scaffolds have been scrutinized due to their higher mechanical properties, favorable interactions between the material surface and cell membrane, nucleation of the mineralized matrix, and spatially regulated protein binding for cellular adhesion. They offer critical aspects for stem cells' motivation toward specific lineages, compared to either biopolymers or ceramics [194, 195]. As a result, in BTE, this kind of construct can be substituted for innate tissue, as reported in many clinical cases. Single-class materials may not be able to fulfill all the criteria for a given implant application. For this purpose, the composites of multi-scale architectures and the favorable traits for specific applications can be accomplished by combining two or more biomaterials classes. In this section, we address the three-prevailing combination of biopolymer/ceramic nanocomposite scaffolds as follows: natural polymer/ceramic-based nanocomposite scaffolds; synthetic polymer/ceramic-based nanocomposite scaffolds; natural/synthetic polymers/ceramic-based nanocomposite scaffolds.

4.1 Natural Polymer/Ceramic-Based Nanocomposite Scaffolds

The utilization of natural polymers in BTE has received substantial recognition due to its desirable biodegradability, secure, and non-immunogenic [196]. Even though hasty degradation and high solubility of natural polymers combined with the increased risk of loss of biological traits during preparation also hinder their utilization as individual scaffold materials. Several experiments showed that the strengthening of natural polymers with ceramic compounds improves their mechanical properties. Natural/ceramic-based nanocomposites boost the mechanical properties and aid to resemble the nano-topography of the native bone [197].

Among the available natural polymers, Alg is typically used in BTE due to its ability to develop hydrogels when multivalent cations interact with its guluronic residues [198]. The feasibility of mixing both Alg and ceramics enables the synergization of these biomaterials' advantages in the production of BTE composite scaffolds. They possessed high mechanical strength, biodegradability, and biocompatibility [199]. Naik et al. reported the production of chemotherapeutic drug methotrexate-loaded nano-TiO_2-HAp-Alg composite scaffolds by freeze-drying technique. Regulated swelling, enhanced biomineralization, limited degradation, and ideal drug release profile have been observed in the recorded nanocomposite scaffold [200]. Another related study includes discovering Ciprofloxacin's (CIP) persistent release from the HAp/Alg nanocomposite matrix. The reported matrix demonstrated a persistent and extended-release of CIP from the nanocomposite matrix [201]. Several findings have suggested that the antibacterial Alg-HAp-dependent nanocomposite scaffolds have been formulated to treat disease associated infections. In this regard, CIP-integrated Alg-nHAp-based scaffolds were fabricated to assess their bioactivity, osseointegration, and bactericidal activity. Bioactivity and antibacterial properties were explicitly reported in the assay results [202]. On the other hand, in orthopedic drug delivery applications, Alg-based photocrosslinked hydrogels have recently gained a more significant role because cells and bioactive factors can be administered in aqueous macromer solutions in a minimally invasive manner. For example, Maji et al. synthesized photocrosslinked Alg/nHAp paste for BTE to deliver BMP-2. The study of immunocytochemistry showed that applying surface-functionalized nHAp and BMP2 to Alg hydrogel improved the prepared paste's osteogenic ability [203].

Gel is another fascinating natural polymer with many benefits, such as biological origin, relatively low-cost commercial production, and non-immunogenicity [204]. A few research pieces utilized the Gel/ceramic nanocomposite scaffolds to combine individual constituents' synergetic properties. By using the foam replication technique, Reiter et al. developed icariin-integrated Gel-coated, 3D sponge-like scaffolds based on 45S5 BG. The data implied that the multiple crosslinking approaches resulted in different icariin release profile [205]. In another analysis, the zoledronic acid (ZA) primed Gel/β-TCP nanocomposite scaffolds were tested for its osteogenic potential. Approximately 75% of bone regeneration was accomplished after 4 months in rabbits' cranial fossa [206]. Govindan et al. documented that the phosphate glass (PG) reinforced Gel scaffolds mixed with CIP drugs were developed via the freeze-drying technique for BTE. Composite scaffolds revealed an adequate porosity (~73%) and improved mechanical strength with the compression modulus of 4.89 MPa [207]. Besides, to raise bone progress and to duplicate the role of natural ECM for continuous release of multiple growth factors (BMP-2 and FGF-2), the scaffolds containing the surface-functionalized porous Gel nanofibers coated with HAp using an SBF medium were prepared. As verified by the upregulated expression of bone gene markers (RunX2, COL1α1, and OCN), osteogenesis was improved through synergism between multiple growth factor distribution and the nHAp nanofiber coating [208].

CS is one such captivating natural polymer, known to have gained interest over the past two decades as a possible drug and gene delivery carrier because of its unique characteristics. Nonetheless, the loss of uniformity of structure and poor mechanical strength has hampered CS in BTE. To combat these obstacles, CS/ceramic nanocomposites are generally used [209]. Typically, the nanoparticulate composites of HAp and CS were synthesized by ultrasound-assisted sequential precipitation to treat osteomyelitis. There was a controlled release of drug from the matrix, and it also showed antibacterial activity against *Staphylococcus aureus* [210]. Zarghami et al. synthesized similar bactericidal-based nanocomposite scaffolds (CS/BG NPs/vancomycin), and the scaffolds showed a prolonged bactericidal performance [211]. Jolly et al. merged *Phoenix dactylifera* seeds with CS and nHAp and thus prepared the synergistically functionalized nanocomposite scaffolds (PD-CHA) as a bone construct. These nanocomposite scaffolds showed osteoblast cell development and osteogenic differentiation. As early as four weeks in vivo, radiological and histological examination revealed substantial bone regeneration at the deformed site (rat calvarial bone defect) [212]. The use of CS in the production of ceramic-combined organic/inorganic composites for BTE is limited since CS is only soluble in acidic conditions. A semisynthetic CS derivative readily dissolved in water is hydroxypropyl chitosan (HPCS). Due to its antibacterial, antioxidant, biocompatible, and biodegradable substance for cell growth and tissue regeneration, HPCS has recently gained a growing interest in biomedical applications. For instance, Lu et al. developed genipin-crosslinked and fucoidan (FD)-adsorbed nHAp/HPCS composite scaffolds for BTE. These scaffolds accelerated ALP activity and mineralization in osteoblastic cells [213]. We summarize all the reported natural polymer/ceramic-based nanocomposite scaffolds available for BTE in Table 1.

Table 1 Biodegradable natural polymer/ceramic-based nanocomposites for drug delivery in BTE applications

S. No.	Nanocomposites	Fabrication method	Drug	Outcome	Reference
1	nHAp-CS	Nanocrystal-induced biomimetic mineralization method	BMP-2	A sustained delivery of BMP-2 enhanced osteoblast differentiation of BMSCs in vitro	[214]
2	nBG-Gel-Agarose	Freeze gelation	Ciprofloxacin	Controlled drug release reduced the microbial infection and was effective in localized treatment of osteomyelitis	[215]
3	nBG-Alg	Cast molding	Cu^{2+}, Ca^{2+}	Sustained delivery of bioactive ions stimulated rBMSC differentiation toward the osteogenic lineage	[216]
4	GelMA-ND	Photocrosslinking	Dexamethasone	Promoted osteogenic differentiation of hASCs	[217]
5	Heparin-Col-HAp	Fibrillation and mineralization techniques	VEGF	Stimulated vasculogenesis	[218]
6	HA-CMC	One-pot synthesis	Dexamethasone	Enhanced ALP expression and extracellular mineralization	[219]
7	HA-Dextran-Laponite	Sol–gel	BMP-2	BMP-2 release supported spreading, proliferation and osteogenic differentiation of rBMSCs	[220]
8	Gel-β(TCP)	Solvent casting	Zoledronic acid	Effective against metastatic bone cancer	[206]

(continued)

Table 1 (continued)

S. No.	Nanocomposites	Fabrication method	Drug	Outcome	Reference
9	SF/halloysite nanotubes	Ultrasonication	Vancomycin	Sustained release of vancomycin effectively inhibited bacterial infections	[85]

4.2 Synthetic Polymer/Ceramic-Based Nanocomposite Scaffolds

Synthetic polymers have gained more interest in BTE due to their strong biodegradability and biocompatibility. However, the problem with using these synthetic polymers is their inferiority in mechanical properties, bioactivity, and weak tissue adhesion, which does not result in good osseointegration. To prevent synthetic polymers' pitfalls, the use of a mixture of ceramics and biodegradable synthetic polymers has been reported [221, 222].

Among the existing biodegradable synthetic polymers, PCL polymer-based composites have received increasing importance than another synthetic polymer composite for BTE applications due to its low inflammatory response, elastic characteristics, and sustained biodegradability [223]. Various experiments have focused on the mechanical properties of the PCL/ceramic-based nanocomposite scaffolds as a bone graft substitute. PCL nanofibers containing BG NPs and simvastatin were formed by electrospinning. Incorporation of BG NPs in the nanofibers significantly improved tensile strength and induced bone-like apatite on their surfaces [224]. Nithya et al. fabricated the nanocomposite film (HAp-PCL) through the solvent evaporation method to deliver CIP, a widely used antibiotic agent for bone infections. The film had greater water uptake, extended drug release kinetics, and cytocompatibility against the osteoblast cell line (MG-63) and fibroblast cell line (NIH-3T3) [225]. PCL properties can be customized by crosslinking PCL with different amounts of radical initiators to exhibit a two-mode shape memory effect. Such a technique has been attempted by Liu et al., who have formulated a smart shape-memory porous scaffold (c-PCL/nHAp/BMP-2) to restore a mandibular defect rabbit model. In both conditions (in vitro and in vivo), the scaffold shows robust shape-memory retrieval from the compressed shape. The in vivo micro-CT and histomorphometry findings showed that the porous construct could facilitate neo-bone formation in the rabbit mandibular defect [226].

PLA is a well-known biodegradable polymer with multifunctional advantages, including manufacturing surgical devices and as an ideal scaffold in tissue engineering [227]. PLA/ceramic-based nanocomposites have been documented by Zhou et al., where they prepared the BSA containing amorphous CaP nanospheres/PLA composite-coated tantalum scaffolds to treat subchondral bone defects in the rabbit model. They found the biomineralization of the scaffolds in their experimental findings [228]. Poly(hydroxybutyrate-co-hydroxyvalerate) (PHBV) is another useful synthetic polymer, which has been studied for the orthopedic drug delivery system [229]. The surface-functionalized CaP/PHBV nanocomposite scaffolds loaded with rhBMP-2 were synthesized to investigate the sustained release of growth factor. These scaffolds showed controlled growth factor delivery, and there were significantly increased ALP activity and the expression of osteogenic differentiation markers in mouse mesenchymal stem cells (C3H10T1/2) [230]. Almeida Neto et al. successfully developed the scaffolds containing PHBV/nanodiamond (nD)/nHAp-loaded with vancomycin. The formulated scaffolds exhibited sustained release of drug up to 22 days, antibacterial activity, and strong cell attachment capacity [231].

Other synthetic polymers such as PLLA, Poly(ester urethane) (PEUR), and polyglutamic acid also played a role in orthopedic drug delivery combined with ceramic materials. Wang et al. combined rhBMP-2 encapsulated CaP NPs with PLLA to build nanocomposite scaffolds. The dual delivery of Ca^{2+} and rhBMP-2 from the hierarchical porous scaffolds demonstrated superior efficacy in guiding the actions of human bone marrow-derived MSCs. They resulted in enhanced biocompatibility and osteogenic differentiation, suggesting their potential activity in BTE [232]. The bone fractures at weight-bearing sites (Tibial Plateau Slot Defects) in sheep were remedied using biphasic ceramic/nHAp-PEUR nanocomposites. The rate of remodeling of the cement was significantly faster, and there was new bone growth formation [233]. In another study, Shu et al. fabricated nano-doped CaP cement delivery systems (nano-poly(g-glutamic acid)/b-TCP-based CaP) loaded with two growth factors, BMP-2 and IGF-1. This hybrid delivery mechanism offered a controlled release of the two growth factors. The introduction of dual growth factors could enhance bone healing and foster bone ingrowth processes at a low-dose [234]. The details of some of the synthetic polymer/ceramic-based nanocomposites in bone fracture repair are listed in Table 2.

4.3 Natural/Synthetic Polymers/Ceramic-Based Nanocomposite Scaffolds

In the preceding parts, we focused on the individual combination of natural and synthetic polymers with ceramic materials to manufacture nanocomposite scaffolds in BTE applications. This section discusses the complex nanocomposite scaffolds constructed with all three forms of biomaterials to build bone and their related heterogeneous tissue structures to understand their effect on osteogenic development. Nazemi et al. fabricated a nanocomposite drug delivery system by incorporating CS/BG scaffolds with DEX-loaded PLGA NPs. The results revealed that the integration of NPs increased the mechanical strength of the scaffolds and achieved a controlled release drug delivery system [237]. Asadian et al. designed the novel

Table 2 Biodegradable synthetic polymer/ceramic-based nanocomposites for drug delivery in BTE applications

S. No.	Nanocomposites	Fabrication method	Drug	Outcome	Reference
1	PCL-HAp	Solvent evaporation	Ciprofloxacin	Effectively controlled bacterial infections	[225]
2	PCL-HAp	Press molding and sugar leaching	BMP-2	Controlled release of BMP-2 significantly promoted bone defect repair	[226]
3	PLGA-PCL-nHAP	–	BMP-2, bFGF	Combined release of BMP-2 and bFGF significantly improved the proliferation and osteogenic differentiation of BMSCs	[235]
4	PHBV-nHA, ND	Injection molding	Vancomycin	Sustained release of vancomycin inhibited the growth of *Staphylococcus aureus*	[231]
5	PTMC-PLA	Hot pressing method, UV curing	Dexamethasone	Effectively triggered the osteogenic differentiation of MSCs	[236]
6	PHBV-CaP	Selective laser sintering	rhBMP-2	Controlled rhBMP-2 release significantly enhanced the ALP activity and osteogenic differentiation of C3H10T1/2 cells	[230]
7	PLLA-CaP	Cryogenic 3D-printing technique	rhBMP-2	Dual release of Ca^{2+} and rhBMP-2 improved the cell viability, attachment, proliferation, and osteogenic differentiation of hBMSCs	[232]

(continued)

Table 2 (continued)

S. No.	Nanocomposites	Fabrication method	Drug	Outcome	Reference
8	PCL-nBG	Electrospinning	Simvastatin	Controlled simvastatin release resulted in improved apatite layer formation and bone regeneration	[224]

functional CS-graft-poly(acrylic acid)/nHAp scaffolds loaded with naproxen sodium via the freeze-drying method. The reported scaffolds in the presence of nHAp had a significant effect on suppressing the drug's burst release, and their mechanical properties were found close to the density of trabecular bone [238]. Saber-Samandari et al. devised the bioactive chitosan-graft-poly(acrylic acid-coacrylamide)/HAp nanocomposite scaffolds through a novel multi-step route. The results suggested that the prepared scaffolds displayed a biphasic drug release design with a low primary burst and a sustained release up to 14 days [141]. Apart from scaffolds, the layer-by-layer (LBL) assembly film also has a significant impact in BTE, which provides a larger space for high drug loading efficiency and sustains drug release capacity, which helps significant bone recovery.

For example, the nanocomposite films made up of aspirin-loaded graphene oxide (GO)/CS/HAp were fabricated by the LBL assembly technology. The GO/CS/HAp nanocomposite films had good biocompatibility and could be the ideal platform for the growth of mMSCs [239]. In addition to the significance of nanocomposite films in BTE, cryogels also represent the highly interconnected porous microstructure and enhance the mechanical stability required in BTE scaffolds. Saini et al. prepared the antibacterial cryogels composed of nanosilver HAp-loaded Gel/Alg/PVA. The cryogels exhibited antibacterial and non-cytotoxic properties [58]. However, most nanocomposite scaffolds have not been able to accomplish a dual or multiple drug delivery systems for the loading and continuous release of various types of drugs to achieve the foster bone healing process. To overcome this predicament, the microsphere-incorporated scaffold system was proposed. The microsphere-incorporated scaffold system has a fascinating approach as it can provide site-specific drug delivery and regulates drug releases with distinct release pattern. The drugs may be inserted into the microspheres and the scaffold matrix, respectively, in the microsphere-incorporated scaffold system. Hu et al. have attempted such a strategy. They united the advantages of the porous calcium Alg/HAp scaffolds and PLLA-based microspheres to produce the microsphere/scaffold composites for delivering hydrophilic BSA and hydrophobic ibuprofen (IBU). The hybrid composites demonstrated biocompatible nature. The increasing nHAp concentration or D-gluconic acid δ-lactone (GDL) concentration helped improve the compressive strength and Young's modulus of porous scaffolds [240]. Researchers developed a hybrid scaffold obtained

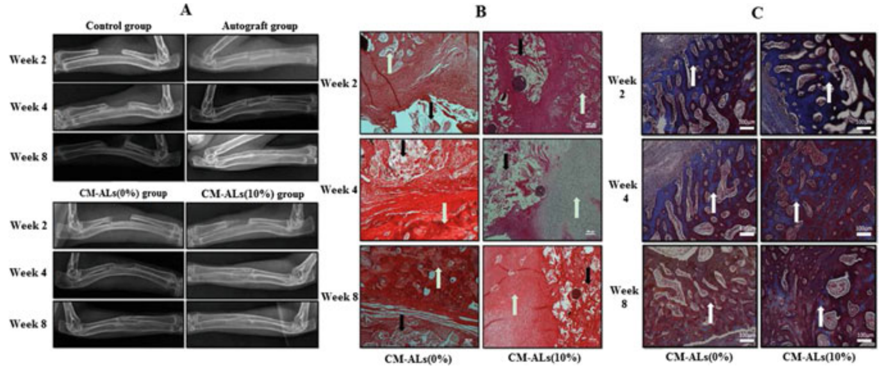

Fig. 3 PLLA/nHAp/CS nanocomposite scaffold-loaded with alendronate was implanted in vivo to treat large-sized bone defects in a rabbit model. **a** X-ray images, **b** HE staining, and **c** Masson staining showed enhanced bone healing and regeneration during 4–8 weeks. Reproduced with permission [241]. Wu et al. 2017, © Copyright 2017, Nature Publishing Groups

from PLLA/nHAp/Alendronate-loaded CS microspheres to repair large-sized bone defects in a similar study. The scaffold showed sustained alendronate release, biocompatible, high mechanical strength, calcium deposition, and ALP activity. Further, in vivo analysis, reported improved hybrid scaffolds' performance with the complete repair of large-sized bone defects within eight weeks in large segmental radius defects of a rabbit model (Fig. 3) [241].

Electrospun-based nanofibrous scaffolds constitute an incredibly attractive subgroup of biomaterials due to special intrinsic features such as high surface-to-volume ratio, which facilitate fundamental cellular roles such as adhesion, proliferation, and differentiation (Fig. 4) [242]. The SF/CaP/PLGA nanocomposite fibrous scaffolds were fabricated by the freeze-drying and electrospinning techniques in one such study. Different kinds of growth factors, namely PDGF and VEGF, were loaded onto scaffolds, and their potential for healing and vascularization were investigated in critical-sized rabbit bone defects in vivo. PDGF and VEGF released from the scaffolds had good bioactivity, facilitating osteoblast attachment, proliferation, and ALP production. Histological analysis revealed the development of a new bone matrix with neovascularization in the angiogenic factors-laden scaffolds after ten weeks of implantation in the rabbit model [243]. In another study, the DEX-loaded mesoporous BG NPs reinforced PCL/Gel osteoinductive fibrous scaffolds were designed to evaluate the long-term therapeutic efficacy in the rat calvarial defect model. Based on in vivo observations, the release of DEX from nanocomposite fibrous scaffolds has been shown to promote bone-forming processes regarding the neo-bone structure's quantity and quality [244].

To facilitate bone regeneration at the deformed site, researchers integrated trace elements into ceramic NPs, which are further strengthened in nanofibers to replicate the natural bone ECM. Kuttappan et al. fabricated nanofibrous scaffolds (silica-coated nHAp/Gel reinforced with electrospun PLLA yarns), encapsulated with FGF2 and

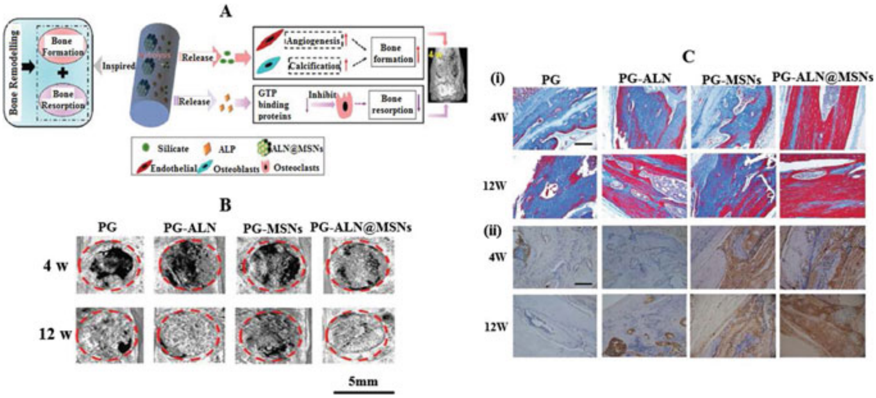

Fig. 4 **a** Schematic representation indicates the dual release of ALN and silicate from the PCL-Gel-Alendronate-Mesoporous silica (PG-ALN@MSN) nanofibers to heighten bone remodeling. **b** From the representative micro-CT images, it was observed that PG-ALN@MSN aids in enhanced bone formation in the 4th week by almost covering 95% of the deformed sites. It became much denser by covering the entire defective sites at the end of the 12th week. **c** Masson staining images of the skull showed a significant increase in new bone regeneration observed at the end of the 12th week in the PG-ALN@MSN complex compared to other reported groups. The images of CD31 immunohistochemistry indicated vascular networks' development during bone remodeling via silicate release from the MSN. Reproduced with permission [242] © Copyright 2018, The Royal Society of Chemistry

BMP2. The scaffold facilitated the delivery of growth factors, improved endothelial and MSCs migration, and proliferation, resulting in angiogenesis and osteogenesis in critical-sized calvarial defects [245]. The therapeutic effectiveness of vancomycin-laden nanocomposite fibrous scaffolds (silica-coated nHAp/Gel reinforced with PLLA yarns) for treating methicillin-resistant *Staphylococcus aureus* (MRSA)-made osteomyelitis in rat femur model was carried out. The scaffolds effectively delivered vancomycin in a sustained mode, which confirmed bactericidal activity against MRSA for 30 days. The implantation of scaffolds into the osteomyelitis rat femur model resulted in substantial bacterial reduction and increased neo-osteogenesis within three months [246]. The descriptions of some nanocomposites-based natural/synthetic polymers/ceramics in BTE are shown in Table 3. Various vital parameters in nanocomposite-based drug delivery are stated in Fig. 5.

5 Conclusions

Effective bone rejuvenation entails a synchronized relationship between therapeutic molecules, cells, and biomaterials. Suitable bone implants should firmly balance the new bone development and resorption across various situations caused by gender,

Table 3 Biodegradable hybrid polymers-based nanocomposites for drug delivery in BTE applications

S. No.	Nanocomposites	Fabrication method	Drug	Outcome	Reference
1	PLLA-nHAp- CS	Particulate leaching method	Alendronate	Sustained release of alendronate resulted in enhanced ALP expression, calcium deposition and osteogenic differentiation of ASCs	[241]
2	SF-CaP-PLGA	Freeze-drying and electrospinning	PDGF, VEGF	Simultaneous release of PDGF, VEGF improved proliferation, ALP production	[243]
3	SF-CaP-PLGA	Freeze-drying and electrospinning	VEGF	Controlled release of VEGF for prolonged time improved angiogenesis, growth and proliferation of osteoblast cells	[247]
4	PCL-Gel-mesoporous nBG	Electrospinning	Dexamethasone	Sustained release of Dexamethasone stimulated the osteogenic differentiation of periodontal stem cells in vitro and in vivo bone regeneration in rat calvarial model	[244]
5	CS-graft- (AA-co-AAm)-nHAp	Freeze-drying	Celecoxib	Model drug anti-inflammatory	[141]
6	Silica-coated nHAp–Gel-PLLA	Aqueous precipitation, electrospinning	Vancomycin	Vancomycin release potentially reduced methicillin-resistant *Staphylococcus aureus* infection	[246]
7	GO/CS-HAp	Layer-by-layer assembly technology	Aspirin	Sustained release of aspirin reduced the inflammation and facilitated bone formation	[239]
8	PLLA-Gel-nHAp-	Electrospinning	Dexamethasone	Enhanced osteogenesis	[248]

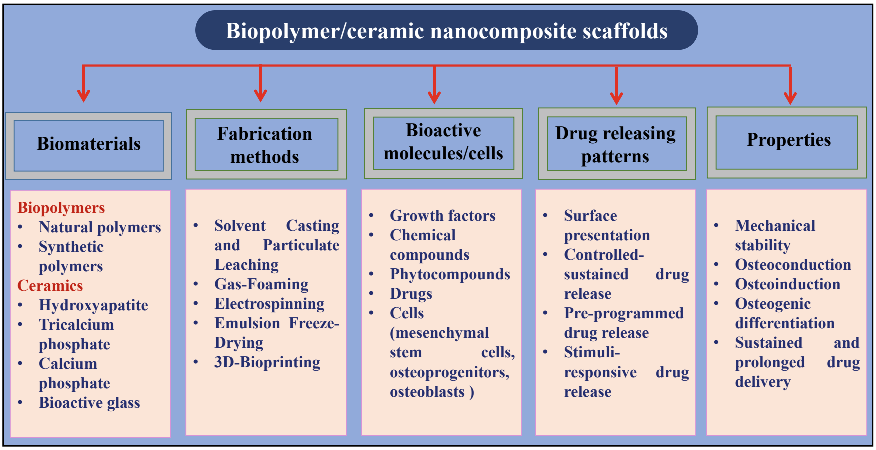

Fig. 5 Biopolymer/ceramic-based nanocomposite scaffolds in BTE applications. Various vital components such as biomaterials, fabrication techniques, bioactive molecules and cells, drug release patterns, and the biopolymer/ceramic-based nanocomposites' properties are illustrated

patient's age, and specific loads at the implantation site. Recent advanced techniques permit great flexibility and more accurate control in the creation of scaffolds. Based on biopolymer/ceramics, nanocomposite scaffolds become an evolving smart approach to balance individual biomaterials' pitfalls and provide effective bone regeneration with sustained/prolonged drug delivery. With a better understanding of biomaterials, bone ECM and cell communication, nanocomposite scaffolds will indisputably become a prevailing tool for the clinical bone defects treatment in the future.

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Biopolymeric Nanocomposites for Orthopedic Applications

Maria Râpă, Raluca Nicoleta Darie-Nita, and Cornelia Vasile

Abstract Among the promising sustainable materials of modern times, biopolymeric nanocomposites have received an increased interest for orthopedic applications due to their excellent mechanical, biocompatibility, bioactivity properties, and special architecture for the cells proliferation, differentiation, and migration in bone tissue regeneration. The aim of this chapter is to summarize the recent research results on the development and applications of various types of biopolymeric nanocomposites utilized in prosthetic devices to bone grafts, for cell delivery, with a special focus on material type, formulations, current design, and performance in bone tissue engineering. Important challenges related to the degradation of biopolymeric nanocomposite scaffolds, wide range of properties, and benefits for bone healing are addressed.

Keywords Bone tissue engineering · Biopolymer · Nanocomposite · Biocompatibility · Orthopedic applications

1 Introduction

Tissue engineering deals with the recuperation, maintenance, or enhancement of tissue functions that are malfunctioning or have been lost due to the different pathological conditions. It is classified into soft tissue (blood vessels, skin, tendons, nerve, and skeletal muscle) and hard tissue (bone) [11]. Bone is the second most transplanted tissue in the world after blood and kidney [46]. The main functions of bones are to assure strength, flexibility and to support the organs in the body. Genetic abnormalities, defects caused by trauma, deficiency in vitamin D, phosphorus, calcium, and hormonal imbalances among other causes are factors that can lead to complications

M. Râpă
University Politehnica of Bucharest, 313 Splaiul Independentei, 060042 Bucharest, Romania

R. N. Darie-Nita · C. Vasile (✉)
Physical Chemistry of Polymers Department, "Petru Poni" Institute of Macromolecular Chemistry, 41A Grigore Ghica Voda Alley, 700487 Iasi, Romania
e-mail: cvasile@icmpp.ro

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in the bone structure and function. It is expected that the bone illness to increase in the future due to the growth and aging population. Therefore, it is a huge need for clear approaches leading to bone healing.

Bone tissue engineering (BTE) has emerged in recent years as a new and applicable approach to repair bone defects. Scaffolds are the three-dimensional (3D) structures characterized by the high surface area to promote the cell adhesion, the mechanical ability to support the newly formed tissue, a framework for healing, inducing new tissue formation, and the biocompatibility with the host environment. Materials utilized for the bone scaffold preparation can be classified into metallic biomaterials, polymers (natural and synthetic), ceramics, and glasses.

Metallic biomaterials such as Ti [56], Mg [35], or Ti alloys (e.g., Ti–Nb, Ti–Mo, Ti–Ta, Ti–Nb-Sn) [54] or other metallic alloys (e.g., Mg alloys, Zn–Mn) [9, 30] possess mechanical properties and corrosion resistance properties necessary for the fabrication of scaffolds. Surgical grade stainless steel has been used in orthopedic applications as fixation elements for the stabilization of fractures or as prostheses [4]. These metal biomaterials may cause some deficiencies like high cost of implant processing, loose of osseo-integration and antibacterial effect, bone distortion, and release of metal ions from the implant to the body fluid.

Among the natural polymers most used for the manufacture of scaffolds are extracellular matrix proteins (collagen), as well as some polysaccharides such as poly (hyaluronic acid), chitosan, and alginate. These polymers are considered promising alternative to metallic biomaterials due to the resemblance to natural elements of the cytoskeleton of regenerated tissues. Collagen is the most abundant protein in the human body [13, 33] and represents 90% of the protein content of the bone [34]. Sulfated polysaccharides derived from the extracellular matrix (ECM) of animal tissues (glycosaminoglycans) or plants, such as marine algae (alginate, carrageenan, fucoidan, and ulvan) could be also exploited as promising biomaterials for orthopedic tissue engineering applications due to their high abundance and sustainability together with limited immunogenicity [16, 52]. Improving in the mechanical strength of the natural polymers is a concern for many researchers.

As synthetic polymers, polycaprolactone (PCL), poly (lactic acid) (PLA), poly (glycolic acid) (PGA), and poly (lactic-co-glycolic acid) (PLGA) polymers are usually used as matrices in bionanocomposites used as biomaterials in BTE. These scaffolds provide an acceptable cell support and proliferation, biodegradability, but cannot be used alone for bone regeneration because to the missing of osteo-promotive capacity [5].

Bioactive ceramics, such as hydroxyapatite (HAp) and tricalcium phosphate (TCP), show unique ability to induce osteogenic gene expression in BTE. HAp $[(Ca)_{10}(PO_4)_6(OH)_2]$ is the essential chemical currently used in function of bone, ensuring the release of calcium and phosphorous ions in a similar way to the occurring of natural bone (Ca/P ratio 1.67) [44]. HAp possesses biocompatibility, cell attachment, and osteo-integration. Its major drawbacks are related to brittle nature, a reduced biocompatibility, and biodegradability. Silica-based bioactive materials have ability to promote osteoblast adhesion, mesenchymal stem cells (MSCs) differentiation, and revascularization leading in a fully functional bone. However, its low degradation rate prevents the formation of new tissue.

Taking into account on the advantages and disadvantages of above-discussed materials for the scaffolds production, one approach to the design of scaffolds for orthopedic applications with improved mechanical strength, biodegradation, bioactivity, and biocompatibility is the combination of ceramics with natural and synthetic polymers.

2 Natural Bone—A Nanostructured Composite

Natural bone is hierarchically structured biomineral with a multiscale arrangement from a macroscopic level (whole bone of various types: long and short bones, flat or tubular), structure of spongy bone tissue, and osteons of compact bone tissue, a microscopic level (cells, matrices, and minerals) to the nanoscale level of single bone apatite crystals and collagen fibers (see Fig. 1) [22, 36].

Bone is a nanomaterial containing 65–70 wt% of inorganic crystals, mainly nanohydroxyapatite (nHAp), trace amounts of carbonate, magnesium and acid phosphate, and 30–35 wt% organic matrix, mainly collagen[11, 57]. Biological nanocrystalline apatites are the main inorganic components of hard tissues like bones.

In the organic–inorganic nanocomposite structure of bone, calcium phosphate nanoparticles are held within a primarily collagen protein matrix (see Fig. 2a). The nanoparticles contain 50–150 nm thick stacks of very closely packed single crystal platelets, with 2.5–4 nm thickness [10], their large faces being parallel to each other and with their c axes strongly ordered (parallel to collagen fibrils). Figure 2b shows the regions containing the disordered phosphate as the interfaces between the apatite layers and the mineral-bound water. The water is prevented to be excluded due to the citrate anions that connect the mineral platelets in bone and therefore maintains the amorphous mineral ion arrangements on the "surfaces" of the mineral platelets intended to arrange themselves in stacks (Fig. 2c) [18].

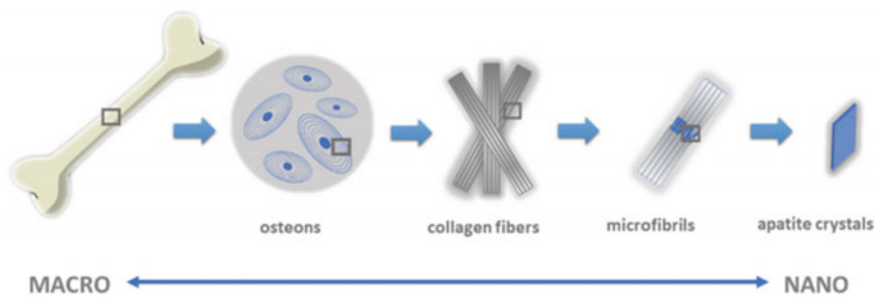

Fig. 1 Multiscale structure of natural bone (**open access** [36])

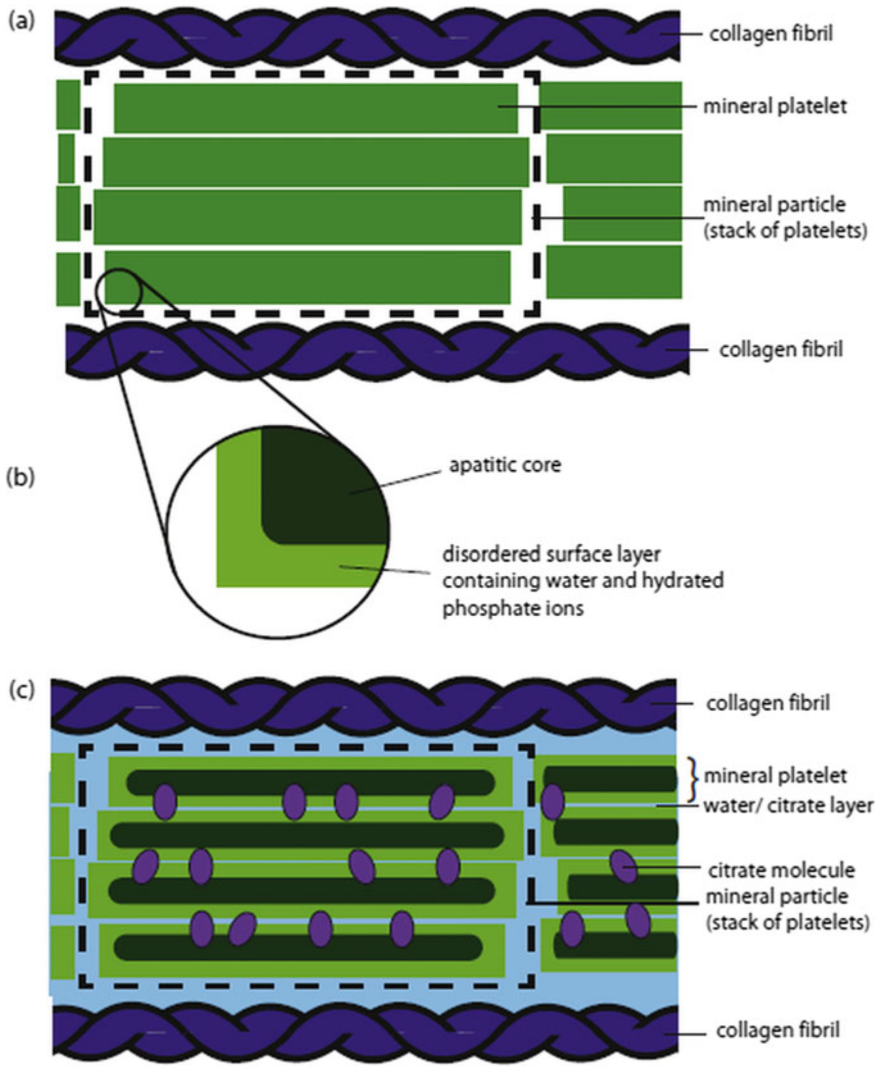

Fig. 2 **a** Scheme of the bone's organic–inorganic composite nanostructure. Polycrystalline mineral particles are sandwiched between collagen fibrils. **b** Schematic view of the structure of a single mineral platelet, with an atomically ordered core resembling the hydroxyapatite surrounded by a surface layer of disordered, hydrated mineral ions. **c** Schematic view of the detailed structural model of bone mineral where citrate anions and water bind the mineral platelets together (Permission from Elsevier [18])

2.1 Bone Defects

Bone defects differ vary greatly by on size, severity, and location of the defects, these factors enabling accurate planning for treatment and rehabilitation [29].

Congenital or acquired conditions can lead to bone defects. The absence of or wrong development of bones are the common reason for the congenital bone anomalies. Acquired bone defects often occur due to trauma, diseases (osteoporosis and osteosarcoma), infection, or surgeries. Osteoarthritis is an osteo-degenerative disease responsible for bone loss over time [32].

Cavity defects and segmental defects are two general groups for bone defects classification. In cavity defects, the limb biomechanics is not affected by the loss, while for segmental defects, normal biomechanics are impeded and the structural stability of the bone as an organ may be endangered [45].

For orthopedics, the most practical and frequently used is Anderson Orthopaedic Research Institute (AORI) classification which predominantly is based on the size of the bone defect originated from the tibia and femur [51]. AORI classifies bone defects into three types as shown in Fig. 3. The major knee defects are occasionally associated with collateral or patellar ligament detachment; therefore, bone grafting or custom implants are usually required.

Bone defects could be remediated by bone grafting because the natural bone tissue is able to completely regenerate; therefore, it can replace the graft material, resulting in fully integrated region of new bone. Bone morphogenetic proteins, bone growth

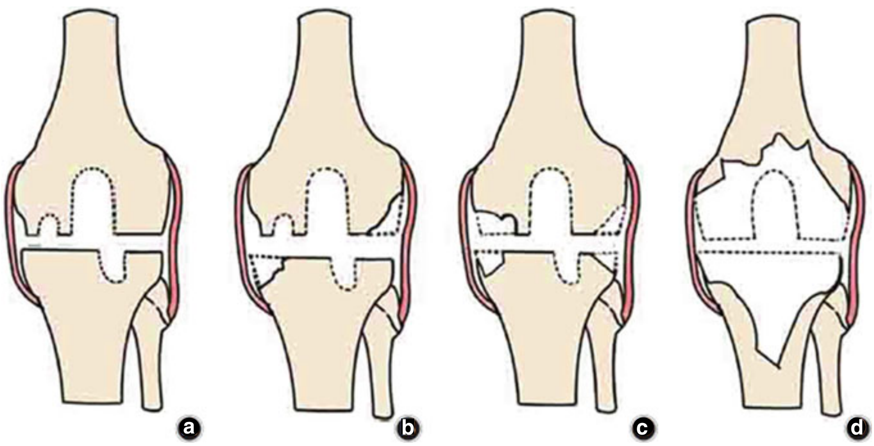

Fig. 3 Bone defects classified according to Anderson Orthopaedic Research Institute: **a** type I (intact metaphyseal bone with minor defects without compromising the stability of a revision component), **b** type IIA (damaged metaphyseal bone with defects in one femoral condyle or tibial plateau), **c** type IIB (damaged metaphyseal bone with more defects in both femoral condyle/tibial plateau), and **d** type III (significant cancellous metaphyseal bone loss with a major portion of the condyle or plateau compromised) (**Open access** [28])

factors, and direct bone anchorage factors strongly influence the biologic mechanisms such as osteo-induction, osteo-conduction, and osseo-integration, powerful interrelated phenomena in bone regeneration[49].

A different classification of bone defects is function of the animal models used to evaluate bone graft substitutes, and the main four types being: the calvarial defect, long bone or segmental defect, partial cortical defect, and cancellous bone defect models [7] (see Fig. 4) (A) Calvarial defects are generally created by introducing a circular burr hole and the subsequent removal of the resulting bone disk. In this case, the underlying dura mater is not damaged by the surgery. (B) The segmental bone defect arises as a large and non-joining wound area (gap) between the bone edges when a segment of the bone is surgically removed. The gap is usually stabilized with a fixation device and/or filled with a tissue-engineered bone substitute to stimulate bone healing and to study bone formation. (C) The burr hole is a partial defect model created when an incomplete hole is drilled into the side of the bone to create a wounded area. The cortical bone is usually penetrated by the burr hole which can extend into the underlying cancellous bone or the bone marrow cavity.

Bone regeneration involves the use of mesenchymal stem cells (MSCs) and bone cells such as osteoblasts, osteoclasts in bone fractures, and defects. Bone grafting techniques are widely used in healing and regenerative therapies of bone defects. Bone grafts are classified based on their source as autograft, allograft (from a donor of the same species), and xenograft (from a donor of a different species) [1]. Autograft for bone repair is the most favorable technique based on the transposition of a patient's bone to the site of the bony defect. Although this graft assures the main characteristics for bone regeneration (osteo-conductive, osteo-inductive signaling molecules, and osteogenic cells properties), it is associated with pain and morbidity at the donor site and the inadequate cellular infiltration and regeneration [53]. Allograft bone refers to the decellularization of bone from human donors. This technique possesses only osteo-conductive properties. Fracture, non-union, infection, and immunogenic response are the possible complications associated to the allograft use. However, the

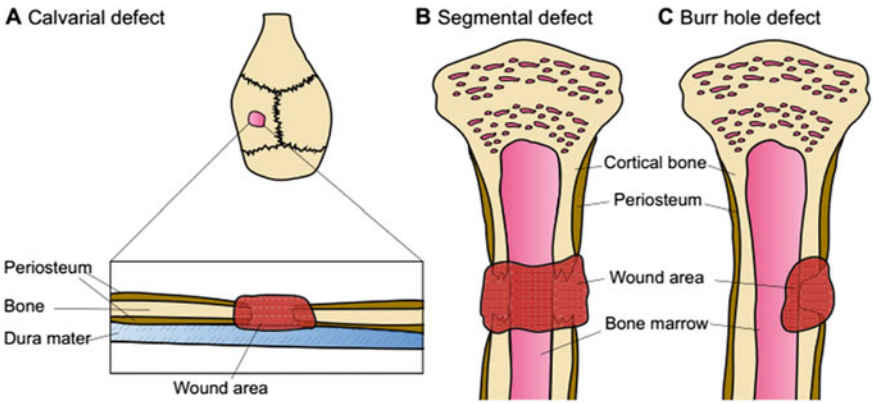

Fig. 4 Bone defect models for evaluation of bone graft substitutes (**open access** [41])

major hurdles of these grafting techniques are donor site pain, limited availability of donor, inflammation, graft incorporation rejection, concomitant vascularization, and pathogen transmission. To address these problems, the bone tissue engineering (BTE) is an optional therapy of the bone graft solutions.

2.2 Requirements for Bone Scaffolds

Three components are necessary for tissue regeneration: an osteo-conductive scaffold for cell adhesion, osteo-inductive signaling molecules to guide bone formation, and osteogenic cells themselves [53]. In addition, the tridimensional scaffolds should provide the mechanical strength so as to transfer the applied load at the implant site, biocompatibility, to be biodegradable, easy to use clinically, as well as low cost processing. The scaffold should present a suitable elastic modulus to avoid bone resorption—stress shielding. The human femoral cortical bone shows an elastic modulus of 17,900 $\pm$ 3900 MPa, fracture toughness of 2–6 MPa, and the average strength of 100–230 MPa [1]. Trabecular bone shows an elastic modulus ranging from 0.02 to 2 GPa, while for compact bone, elastic modulus is situated between 3 and 30 GPa [46].

Pereira et al. [46] performed a study about the properties of three scaffolds from Ti_6Al_4V processed by using a commercial selective laser melting equipment‘, and ZrO_2 and poly-ether-ether-ketone (PEEK) produced by CNC milling. The authors obtained an average pore diameter of 375 μm for Ti_6Al_4V scaffolds and 400 μm in the case of ZrO_2 and PEEK scaffolds. The elastic modulus was of 76.85 $\pm$ 1.43 GPa for Ti_6Al_4V scaffolds, and 141.70 $\pm$ 1.04 GPa and 2.78 $\pm$ 0.06 GPa for ZrO_2 and PEEK scaffolds, respectively. PEEK scaffold exhibited a higher contact angle, leading to a hydrophobic surface and a low cell adhesion and spreading compared to Ti_6Al_4V and ZrO_2 scaffolds. In vitro cytocompatibility and osteogenic ability carried out using SaOS-2 cells showed that the scaffolds were biocompatible and able to host cells for 14 days of the test time [46]. The scaffold’s architecture is critical for healing bone process, and pores have an important role to promote cells penetration, tissue ingrowth, vascularization, proliferation, and differentiation [50]. Hydrophylicity of scaffold surfaces influences the cell attachment and absorption of protein by scaffold. It was established that the contact angles values lower than 90º are favorable for cell adhesion [46], while an optimum value for the contact angle was considered of 55º [6].

An ideal biodegradable implant must have low rates of degradation to ensure appropriate mechanical support until the completion of the healing bone process. It was established that 10% loss of total weight is acceptable for bone tissue engineering [3]. Also, the forming of biological bone-like apatite (carbonate-containing HAp crystallites with defective structure) on the surface of scaffold is critical for the connection between the 3D scaffold and the regenerated bone. The influence of vascularized bone niche, stiffness, roughness, pore size, and porosity of biomaterials

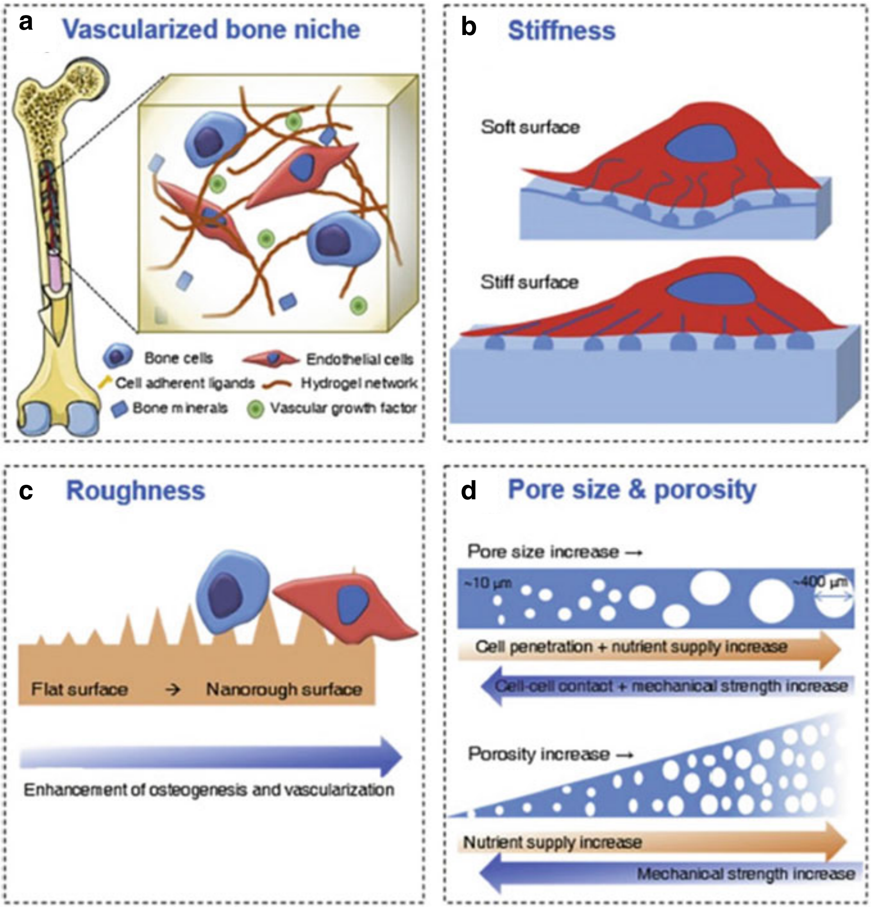

Fig. 5 Vascularized bone niche (**a**); stiffness (**b**); roughness (**c**); and pore size and porosity properties (**d**) facilitating osteogenic differentiation and vascularization (Reprinted with permission from Afewerki et al. [2])

on the facilitating osteogenic differentiation and vascularization was illustrated by [2] (see Fig. 5).

3 Biopolymeric Nanocomposites for Bone Tissue Regeneration

Combinations of biodegradable synthetic polymers, i.e., PLGA [5], poly (l-lactic acid) (PLLA), poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHVB) [17], extracellular matrix-based polymers such as collagen, gelatin, chitosan (CS) [40, 42, 50], or natural biopolymers such as silk fibroin (SF) [31] with nano-sized inorganic materials like HAp, TiO_2, β-tricalcium phosphate (β-TCP), use of polymeric nanosheets [1], or coating of metallic biomaterials with biopolymers [4] and bionanocomposites [20] are widely used for design of bone tissue scaffolds (see Table 1).

The general techniques for scaffold fabrication include solvent casting [57], lyophilization [3, 40], phase separation [31], leaching [14], electrospinning and electrospray [42] extrusion/coextrusion, injection molding [26], 263D printing [48], etc. Different additive manufacturing (AM) [50] approaches such as stereolithography (SLA), selective laser sintering (SLS) [17] (see Fig. 6), and fused deposition modeling (FDM) [5] have been developed in 3D printing. The advances in technology facilitate the use of combinations of methods for obtaining of improved mechanical properties, biocompatibility, and cell migration as polymerization/ copolymerization and cross-linking[43].

The presented formulations in Table 1 show biocompatibility, proved by cell growth and proliferation after direct contact [3, 4, 12, 17, 24, 31, 42].

3.1 Extracellular Matrix Polymeric Scaffolds

As given in Table.1, natural biopolymers such as chitosan (CS), collagen (Col), and gelatin (Gel) are the most used for scaffold preparation due to their similarities with native extra cellular matrix (ECM) [24, 40, 43]. In order to enhance the mechanical strength limiting their applications as the bone to be repaired or substituted, the combination of extracellular matrix with biometallic scaffolds or ceramic nanoparticles has been made. For example, porous composite scaffolds with adequate compression strength and enhanced cell proliferation were fabricated from filling of porous Ti6Al4V part with CS sponge [23]. A content of β-TCP up to 30 wt% into gelatin/CS/nano-β-tricalcium phosphate (β-TCP) (GCT) scaffolds led to obtain pore sizes ranging between 78 and 382 μm (> 80% porosity), and a good compressive strength which correspond to the spongy bone (see Fig. 7) [39].

The inverse relationship between the inorganic amount of nanoparticles and porosity of the scaffolds was also reported by other authors [31]. Increase in the amount of TiO_2–F up to 15 wt% into silk fibroin (SF)/TiO_2–F scaffolds led to decreasing of pore size (88–78%) and increasing in the mechanical properties of scaffolds. Instead, Di Rienzo et al. [14] showed that the mechanical behavior of the poly(para-phenylene) (PPP) porous scaffolds was not significantly influenced by the small or large pore sizes, i.e., 150 μm to 250 μm and 420 μm to 500 μm, respectively.

An innovative approach to enhance the cell viability, proliferation of osteoblast cell on the scaffold, and increase the bearing load after implantation was provided by Atak et al. [3] that modified nHAp with NH_2 group and incorporated them into chitosan scaffold (CS/Hap–NH_2).

Table 1 Some examples of polymeric compositions with relevant properties for orthopedic applications

Composition	Technique	Recommended applications	Significant results	References
Bio-based polymers/inorganic nanoparticles				
Octadecylamine-functionalized nanodiamond (ND-ODA)/PLLA	Solution casting followed by compression molding	Scaffolds for orthopedic regenerative engineering	280% increase in the strain at failure and 310% increase in fracture energy in tensile tests as compared with the mechanical properties of pure PLLA Ca/P ratio of 1.61	Zhang et al. [57]
Magnesium oxide (MgO) nanoparticles/PLLA/HAp	Casting method	Orthopedic tissue regeneration	MgO nanoparticles reduced bacterial growth of *Staphylococcus aureus*, increased the adhesion and proliferation of osteoblasts and fibroblasts	Hickey et al. [27]
PLGA/nHAp	Fused deposition modeling	Bone scaffolds	A reduced inflammatory reaction after subcutaneous implantation of the materials in the rat	Babilotte et al. [5]

(continued)

Table 1 (continued)

Composition	Technique	Recommended applications	Significant results	References
PHBV/Ca–P PLLA/CHAp	Selective laser sintering	Bone tissue engineering	High cell viability and normal morphology and phenotype after 3 and 7 days culture on SaOS-2 cells	Duan et al. [17]
Natural biopolymers/inorganic nanoparticles				
HAp/fucoidan nanocomposites	In situ chemical method	Bone repair/replacement	Slight amount of bone formation by stimulating the osteoblastic activity with rabbit model	Tae Young et al. [52]
Chitosan (CS)/nHAp–NH_2 bionanocomposite scaffolds	Freeze-drying method	Bone tissue engineering applications	10% of total weight loss Cell viability and proliferation of osteoblast cells on the scaffolds	Atak et al. [3]
SF/TiO_2–F	Phase separation method	Bone tissue engineering	Biocompatibility proved by SAOS-2 osteoblast cell line for 1, 3, and 5 days In vitro degradation and bioactivity improved Mechanical properties enhanced by using TiO_2–F amount up to 15 wt%	Johari et al. [31]

(continued)

Table 1 (continued)

Composition	Technique	Recommended applications	Significant results	References
AuNPs/CS bionanocomposite film	Electrodeposition method	Modification of implant surface	98% inhibition efficiency of Ti coated with bionanocomposite film Antibacterial effect	Farghali et al. [20]
$nTiO_2$/gelatin-CS hydrogel		Bone fracture healing	Promote osteoblast Accelerate bone fracture healing	Guo et al. [24]
Gelatin/CS/nano-β-TCP based porous scaffold	Lyophilization method	Nursing bone tissue engineering	> 80% porosity Compressive strength increased from 0.8 to 2.45 MPa Osteogenic potential in vitro without significant inflammatory reaction in vivo	Maji et al. [40]
CS/gelatin/silica-gentamicin	Electrophoretic deposition (EPD) on stainless steel	Coating on removable screws for bone or plate fixation with effective release of antibiotic	Antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* at 24 h, ST-2 bone murine stromal cells proliferation (at 7 days culture) was not inhibited	Aydemir et al. [4]

(continued)

Table 1 (continued)

Composition	Technique	Recommended applications	Significant results	References
Hybrid polymers				
CS-g-PMMA/nano-CaO	Emulsion polymerization technique	Bioadhesive bone cement implants	0.35% nano-CaO led to bioactive bone cement with proper tensile strength and compressive strength	Pradhan and Sahoo [47]
P(HEMA) and P(HEMA-*co*-MMA)/HAp-cartilage powder (CP)/Gel bionanocomposites	Copolymerization	Scaffolds for bone implant	Osteo-conductivity and osteo-inductivity properties; Ca/P ratio of 1.4–1.5 on the surface	Haroun and Migonney [25]
Collagen/PCL-β-TCP scaffold	Additive manufacturing (AM) technology	Bone tissue engineering applications	Improved vascularization	Shanjani et al. [50]
Stimuli-sensitive semi-interpenetrating collagen/poly (*N*-isopropyl acrylamide) polymeric matrix/Dellite® 67G r Cloisite® 93A nanoclays/HAp	Hydrogel preparation: copolymerization and cross-linking		Suitable swelling characteristics for . In vitro cytocompatibility and cell viability revealed that the hybrid nanocomposites were non-cytotoxic for rat osteoblasts. Controllable enzymatic degradation	Nistor et al. [43]
PHB/BC formulations	Melt-mixing/salt-leaching/pressing technique		Support 3T3-L1 pre-adipocytes proliferation New bone growth at 20 weeks	Codreanu et al. [12]

(continued)

Table 1 (continued)

Composition	Technique	Recommended applications	Significant results	References
Ti6Al4V/CS composite scaffold	Electron beam melting and freeze-drying	Scaffold for orthopedic applications	The ultimate compressive strength was 85.35 ± 8.68 MPa Improved cell attachment, higher proliferation, and well-spread morphology as compared to porous Ti6Al4V part	Guo and Li [23]
Synthetic polymer/inorganic nanoparticles				
Poly(para-phenylene) (PPP)	Powder-sintering/salt-leaching technique	Load-bearing orthopedic biomaterial	Both modulus and strength decreased with increasing porosity from 50 to 90 vol%	Di Rienzo et al. [14]
PAN (7%)/AuNPs (1, 2.5, and 5 w/v% concentrations in water)	Blend electrospinning method Electrosprayed method	Bone tissue engineering	MTT and LDH tests were screened using MG-63 cells and revealed non-cytotoxicity and biocompatibility Improved electrical conductivity	Nekounam et al. [42]
UHMWPE/nHAp	Injection molding	Hip liners	Elastic modulus of 1.65 GPa Yield strength 27.6 MPa	Heidari et al. [26]

(continued)

Table 1 (continued)

Composition	Technique	Recommended applications	Significant results	References
HDPE/nGO nanocomposites	Vacuum compression molding technique	Implants for knee and hip replacement	Efficient fatigue, mechanical, and wear properties	Faisal [19]
PPSU/nTiO_2	Ultrasonication and solution casting	Orthopedic and trauma implants	Improved storage and Young's moduli, tensile strength and toughness, glass transition and heat distortion temperature, water absorption and thermal stability Antibacterial activity	Diez-Pascual et al. [15]

(continued)

Table 1 (continued)

Composition	Technique	Recommended applications	Significant results	References
Polypropylene (PP)/carbon nanotubes (CNTs) or carbon nanofibers (CNFs)	Melt compounding	Bone replacements	Addition of low CNT/CNF loadings to nHA/PP composites enhanced their mechanical properties due to the large aspect ratio and remarkable high stiffness of carbonaceous nanofillers	Liao et al. [38]
PP/hexagonal boron nitride (hBN) nanoplatelets/nHAp	Extrusion and injection molding	Polymeric nanocomposites for orthopedic applications/bone replacements	Increased elastic modulus of PP composites and decreased tensile elongation with increasing hBN contents; attachment and proliferation of osteoblastic cells on binary PP/hBN and ternary PP/hBN-20%nHA nanocomposites	Chan et al. [8]

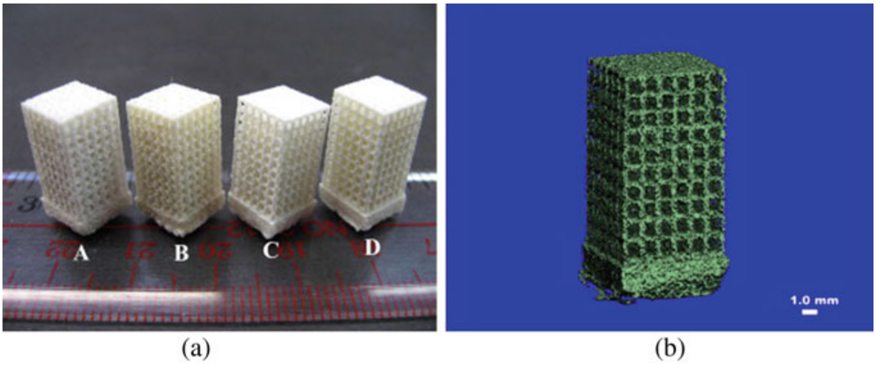

Fig. 6 Scaffolds produced by selective laser sintering **a** (A) PHBV; (B) Ca-P/PHBV; (C) PLLA; (D) CHAp/PLLA; Micro CT image of a Ca-P/PHBV scaffold (**b**) (Reprinted with permission from Duan et al. [17])

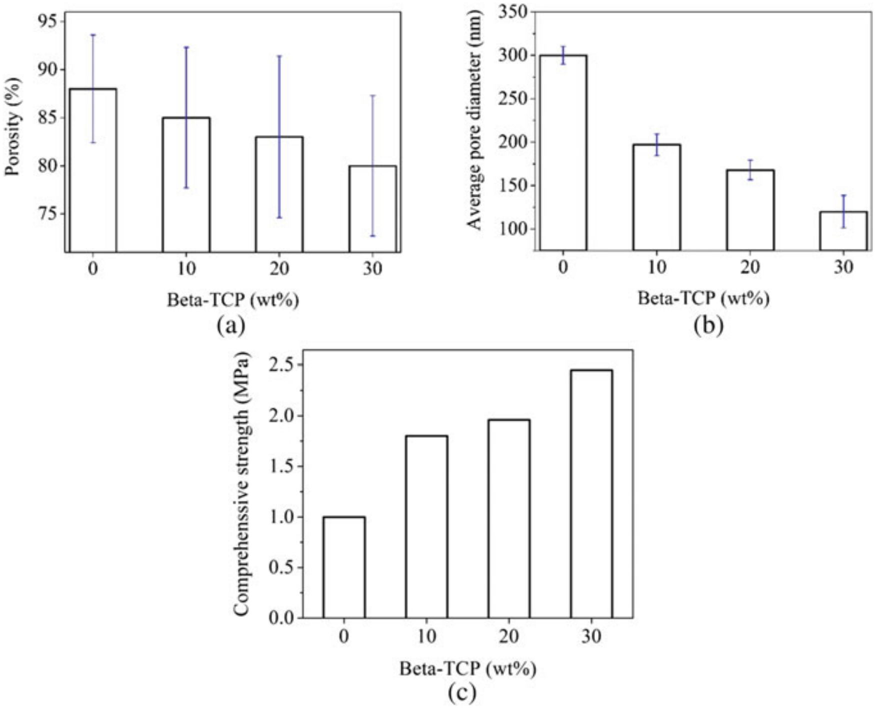

Fig. 7 Influence of β-tricalcium phosphate (β-TCP) bio-filler content on the porosity (**a**) average pore diameter (**b**) and comprehensive strength of GCT scaffolds ([40] adapted from Maji et al.)

3.2 Synthetic Polymeric Scaffolds

Examples of synthetic polymers used for orthopedic applications are unreinforced thermoplastic polymers (poly(para-phenylene)) (PPP) Di Rienzo et al. [14], poly(hydroxylethylmethacrylate) (P(HEMA)) and poly(hydroxylethylmethacrylate-co-methyl methacrylate) (P(HEMA-co-MMA)) loaded with HAp-cartilage powder (CP) and gelatin [25] (see Table 1).

A comparison between the influence of calcium phosphate (Ca–P) and carbonated hydroxyapatite (CHAp) on the biological properties of poly(hydroxybutyrate–co-hydroxyvalerate) (PHBV)/Ca–P and poly(L-lactic acid) (PLLA)/CHAp nanocomposite microspheres revealed that the improved cell proliferation and alkaline phosphatase activity were achieved only in the case of PHBV/Ca–P scaffold [17].

One current trend among researchers to produce scaffolds for orthopedic applications with adequate mechanic properties is to introduce nanofillers into biopolymeric formulations, such as nHAp [26], nanoparticles of titanium dioxide ($nTiO_2$) [15], nGO [19], and gold nanoparticles (AuNPs) [42]. Therefore, the effect of 10 wt% nHAp in an ultrahigh molecular weight polyethylene (UHMWPE) matrix was studied using a finite element analysis (FE analysis) and indicated that this bionanocomposite meets the shrinkage, warpage, wear volume, and yield strength properties and could be used for the manufacturing of hip prosthesis [26]. Specimens for further orthopedic implants fabricated by blending of high-density polyethylene (HDPE) with nano-graphene oxide (nGO) in amount ranging of 0.5–2.5 wt% showed good mechanical properties [19].

In another paper, Diez-Pascual et al. [15] reported that the introduction of titanium dioxide (TiO_2) nanoparticles into polyphenylsulfone (PPSU) led to an antibacterial biopolymeric nanocomposite with reduced the water absorption, increased the mechanical and thermal stability of the polymer.

Nekounam et al. [42] exploited the osteogenic and differentiation to bone lineage advantages of gold nanoparticles (AuNPs) to prepare electrical conductive scaffolds based on carbon nanofibers (CNF) obtained by electrospun of polyacrylonitrile (PAN) solution with gold nanoparticles (AuNPs) by two techniques. First is related to the blend electrospinning method, in which PAN solutions and AuNPs are processed by electrospinning, then the carbonization takes place, and the second, electrosprayed method, accordingly, AuNPs solutions were co-electrospinned with PAN nanofibers. Both methods led to scaffolds with homogenous gold nanoparticle distribution and adequate biocompatibility demonstrated by MTT and LDH assays.

3.3 Hybrid Polymeric Scaffolds

Pradhan and Sahoo [47] explored the chicken eggshell (nano-CaO) as biofiller to improve the mechanical strength and thermal stability of CS grafted with poly (methyl methacrylate) (PMMA) bionanocomposites.

Another approach to induce the differentiation of mesenchymal stem cells (MSCs) into osteoblast cell is to introduce β-tricalcium phosphate (β-TCP), which is more osteo-conductive than HAp, into collagen/poly (ε-caprolactone) (PCL) [50].

Codreanu et al. [12] developed innovative bacterial cellulose-modified polyhydroxyalkanoates (PHB/BC) scaffolds and demonstrated their osteogenic potential in critical-size mouse calvaria defects.

3.4 Multifunctional Biopolymeric Nanocomposites

Current innovations in the materials for orthopedic applications envisage designing of multifunctional biopolymeric nanocomposites with both osteo-inductive and bioactive properties (antimicrobial activity) to protect implants of associated infections, majorly due to bacteria adhesion [2]. In this sense, Ballarre et al. [6] prepared silica-gentamicin (Si–Ge) nanoparticles and incorporated them into a biopolymeric solution containing chitosan and gelatin and coated Ti orthopedic implant with the obtained solution using electrophoretic deposition technique. The new coating of Ti implant showed both occurring of apatite-like deposits after 7 days of immersion in simulated body fluid (SBF) solution and antibacterial activity to *S. aureus* and *E. coli* strains.

Another innovative antimicrobial coating for stainless steel removable screws for bone or plate fixation which could contribute to prevent hospital infections at early implantation times were achieved from chitosan/gelatin/silica-gentamicin [4]. Promising natural bionanocomposites as alternative for regeneration of bone are lignocelulosic materials, which can provide a broad spectrum of antibacterial activity [2, 21].

4 Biomineralization

Controlled biomineralization activity of biomaterials helps avoiding the formation of fibrous capsule, an important issue in bone regeneration and osseo-integration.

An accelerated in bone fracture healing was investigated by Guo et al. [24] that prepared TiO_2/gelatin-chitosan hydrogel sample. The calcium assays in the hydrogel sample was quantified to 4.3 mg highlighting a high mineralization.

Zhang et al. [57] reported for the first time the growth of apatite on the surface specimens with dimension of (4×4) mm^2 prepared from poly(l-lactic acid) (PLLA)/octadecylamine-functionalized nanodiamond (ND-ODA). The biomineralization test was performed by incubation of specimens into SBF at 37 °C, pH 7.4, up to 6 weeks. The incorporation of 10 wt% ND-ODA into polymeric matrix resulted to increase of Ca/P ratio to 1.61 after 6 weeks of incubation. In another paper, the modification of TiO_2 nanoparticles with fluoride ions then its adding into silk fibroin (SF) matrix was evaluated in SBF buffered at 37 °C and pH 7.4 for 28 days [31].

Li et al. [37] realized a bioactive glass (BG)-based hybrid poly (citrate-siloxane) (PCS) elastomer nanocomposite with multifunctional properties for potential bone tissue regeneration, such as elastomeric behavior, bio-imaging tracking, osteogenic cellular response, biomineralization activity, as well as in vivo inflammatory response. Excellent biomineralization activity of PCS-BGN 20% nanocomposites has been proved by characteristic bands of P–O at 563, 603, and 1030 cm^{-1} in FTIR spectra and characteristic crystal pane at 31.8° (XRD) suggesting the formation of biological apatite nanocrystals.

5 Degradation of Biopolymeric Nanocomposites

The degradation of GCT porous scaffolds was studied by Maji et al. [40] using phosphate buffer saline (PBS) at 37 °C for up to 28 days. It was found that the degradation rate of gelatin/chitosan/β-TCP (GCT) hybrid scaffolds took place by the hydrolysis of gelatin and enzymatic process of chitosan and depended on the ratio between organic/inorganic content, being decreased in the case of high content of in β-TCP. This behavior was explained by the authors by role of β-TCP to act as physical cross-linking sites.

Degradation of CS, CS/n–HAp bionanocomposite scaffolds without and with amine functional group by incubation for six weeks in 500 μg/mL lysozyme in PBS was studied by Atak et al. [3]. The study revealed a weight loss up to 15% in the case of CS and CS/nHAp scaffolds, while CS/nHAp–NH_2 scaffolds reached 10% of total weight loss, due to the cross-linking amino group within the scaffold. In the case of SF/TiO_2–F scaffolds, a weight loss ranging from 2 to 5% was observed after 30 days of incubation in PBS medium [31]. This reduced degradation was explained by a compacted structure of SF/TiO_2–F nanocomposite scaffolds. The low degradation rate is a key indicator about the new bone growth.

Low-generation non-immunogenic and non-toxic poly(amidoamine) (PAMAM) dendrimers have been used as initiators to synthesize star-shaped poly(l-lactic acid (SS-PLLA)), which further act as building blocks to assemble nano- and/or meso-scopic structures, as well as to tune the possible surface functionalities and degradation rate [39]. The nanofibrous hollow SS-PLLA microspheres, excellent injectable cell carrier for cartilage regeneration in knee repair, were prepared using a surfactant-free emulsification process, being composed entirely of nanofibers with an average diameter of 160 ± 67 nm, the same scale as collagen fibers. The degradation rate of the nanofibrous hollow microspheres can be tailored by the molecular weight and molecular architecture of the SS-PLLA.

There are some problems with implants during wearing because of various particles formation [55]. Metallic wear particles are associated with adverse local tissue reactions (ALTR) being a significant clinical problem because they affect implant performance increasing of failure rates, so replacements are necessary to metal-on-metal hip and non-metal-on-metal dual modular neck total hip replacement. Bionanocomposites could be a solution.

6 Conclusions and Future Trends

The most used biopolymeric formulations for preparing of 3D scaffolds are bionanocomposites based on natural biopolymers or synthetic biopolymers and inorganic nanoparticles. The cutting-edge innovations in the scaffolds construction in orthopedic bone regeneration based on biopolymeric nanocomposites are notable when the integration of bone scaffolds and vascular networks takes place. High biocompatibility, proper cell attachment, adequate mechanical strength, together with low fabrication costs, in vitro degradation, and biomineralization tests are the most important properties for scaffolds. Tissue engineering should be based on a deep understanding of tissue formation and regeneration, to induce new functional properties of new materials rather than just to implant new spare parts.

Future trends in design of biopolymeric formulations for bone regenerations envisage the use of multifunctional biopolymeric nanocomposites that show both osteo-inductive and bioactive properties (antimicrobial activity) to protect implants of associated infections.

Ongoing research will reveal more details of the inherent qualities of biomaterials and their role in better integration of implants into host tissue or the near-ideal regeneration of host tissue.

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Natural Polymer-Based Composite Wound Dressings

Shreya Sharma, Bhasha Sharma, Shashank Shekhar, and Purnima Jain

Abstract Wound repair is a complicated and firmly synchronized physiological process, entailing the activation of various cell types throughout each succeeding step (homeostasis, inflammation, proliferation, and tissue remodeling). Any impairment within the correct sequence of the healing events could prompt incessant injuries, with probable denouement on the patients' quality of life, and consequential failures on wound care management. Contemporary wound healing treatments like gauzes and bandages primarily are pivoted on passively cushioning the wound and do not proffer properties that escalate the rate of wound healing. Even though these strategies are resilient at safeguarding any infection after application, they are futile at healing a heretofore infected wound or spurring tissue regeneration. The burgeoning of next-generation wound healing treatments aid in enhancing patient care pathways and clinical outcomes. Natural polymers play a significant role in wound care. They deliver a versatile and tunable platform to design the germane extracellular matrix competent to succor tissue regeneration, while contrasting the onset of adverse events. Our goal is to scrutinize the evolution of natural polymers in wound dressing from traditional to modern-day treatment methods. The chief characteristics and properties of a natural polymer, which is widely utilized as biomaterial, are presented. Properties of composite material with peculiar heed on their applications in the skin tissue repair field are discussed. Finally, the unmet needs and developmental perspectives of the new generations of environmentally friendly, naturally derived, smart wound dressings are addressed in light of future research.

Keywords Natural polymer · Biodegradable · Composites · Wound healing · Wound dressing · Sustainability · Biomedical

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S. Sharma · B. Sharma (✉) · S. Shekhar · P. Jain
Department of Chemistry, Netaji Subhas University of Technology, Dwarka Sec-3, 110078 Dwarka, India

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1 Introduction

The skin, the human body's largest organ, serves as a protective shield against the strident environment. Perpetuating the virtue of this deterrent to interdict the intrusion of pathogens and toxins is inevitably pre-eminent for survival. To subsist with the wonted traumas of our world, the skin has adapted an astounding proficiency for healing that depends on the joint effort of a number of cellular and tissue types. The wound damages blood vessels underneath the skin, causing them to leak. The leaking blood vessel forms a clot that plugs the wound to bestow an ephemeral shield. Recruitment of immune cells to the wound is essential for clearing debris and limiting infections. Epithelial cells, constituting the outermost or epidermal layer of the skin, proliferate into the wound bed on a scaffold furnished by underlying dermal cells. These coordinated cellular efforts ascertain that the denuded surface is rapidly sealed or re-epithelialized [1]. Figure 1 depicts different phases of wound healing process. Additionally, the dynamic relationship between skin and microbiome is another major contributor to the outcome of the process [2]. The science of wound healing is reported (since 2000 BC) as "three healing gestures"—wound cleaning, applying plaster, and bandaging the wound [3].The majority of the skin lesions heal within a predictable time and are as a result of unexpected accidents or surgical injury. Howbeit, non-healing, chronic wounds and ulcers corollary of burns, or decline in the healing capacity of skin may have a catastrophic influence on individual's life, spawning pain, diminution of skin function, and in some cases even death.

So as to address all facets of wound care management, suitable materials for wound dressing have been utilized as hydrogels, foams, films, hydrocolloids, or scaffolds. An ideal wound dressing should cohere to the wounded tissue, sustain moist balanced state, permit the exchange of oxygen, and protect from pathogenic intrusion, hence establishing a microenvironment to escalate the healing process. To date, wound

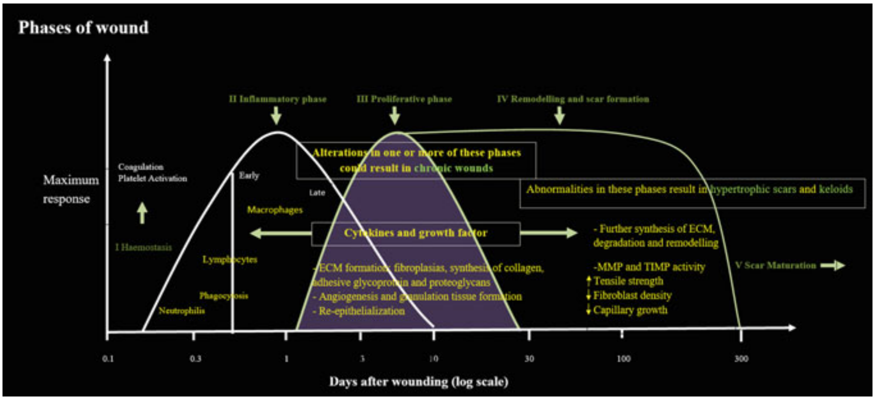

Fig. 1 Phases of wound healing process [ECM: Extracellular matrix, MMP: Metalloproteinases, TIMP: Tissue inhibitors of metalloproteinases]

dressings are categorized as permanent skin substitutes and temporary dressings. The impermanent dressings should extend wound exudate absorption and shielding until wound cessation, while skin substitutes are anticipated to coordinate with the host skin and quicken the recovery cycle [4]. Both synthetic and natural polymers are employed for wound dressing applications. Synthetic polymer, however, are competent only for superficial wounds and abrasions, bestowing paucity of few significant characteristics including; low permeability, absorption, and adherence. Natural polymers including chitosan, cellulose, silk, heparin, keratin, fibrin, alginate, gelatin, fucoidan, etc. similar to macromolecules identified by human body are extensively employed in skin tissue engineering because of their biodegradability, biocompatibility, easy resorption, and capacity for regeneration of the injured tissue [5]. Natural polymers manifest reasonably low mechanical strength in contrast to synthetic polymers and are ordinarily susceptible to microbial contamination. The development and implementation of novel technologies such as cross-linking or blending may enhance the properties of these naturally derived polymers. The composites assist in achieving superior biochemical and mechanical properties over its individual components.

The overarching objective of the chapter is to discuss key aspects of wound healing and to highlight the recent advances in naturally derived polymer composites that contributed to a paradigm shift in wound dressing.

2 Novel Mechanism of Action of Wound Healing

A crucial step during the healing process is the instigation of wound exudate, imitating natural balm that seal wounds from microbes and debris. Exudate production is paramount during the inflammation and proliferation phase of the curing process, but its denouement is not completely perceived. Healing stage wound type, origin, location, and size are the determining factors for variation in the volume of the plasma-derived exudate. Chronic wound *exudate* has a different composition from that of *an acute wound. Acute exudates have higher protein content and essential nutrients for epithelial cells in addition to endogenous proteolytic enzymes and their inhibitors*in an exquisite parity, which aid in degradation of arts of extracellular matrix and wound bed readiness before wound closure and remodeling. Chronic wound exudate in contrast, contain copious proteinases that delay or even obstruct the proliferation of key cells embroiled in the wound healing process. Utilization of wound dressings focuses on the expulsion of unreasonable exudate, debris and potential pathogens from the injury bed, while perpetuating the optimal moisture balance indispensable for cell recruitment and wound healing in due time [6].

A noteworthy facet, ordinarily not exploited, is the mechanical stress at the injury site that incredibly impacts the rate and essence of recuperating. Skin mechanical properties assume a radical job in both intact and harmed skin with involvement in the wound healing process. Several paper report that skin wounds were more susceptible to the development of a scar under mechanical stress during the recuperating cycle [7–9]. These observations have affirmed surgeons to reduce tension post-surgery, at the incision arena [10, 11]. The cells answerable for stress identification in the connective tissue of the injury bed, for example,fibroblasts and myofibroblasts transduce it into a physiological reaction [12]. A few in vitro and in vivo examinations have indicated that escalated tension aggrandized their multiplication [13], repressed fibroblasts apoptosis [14] activated several signaling pathways that instigate an uneven accumulation of extracellular matrix.

3 Types of Dressings

Wound management is a dynamic skill, and dressing selection is both art and a science. When choosing dressings, the choice should be made on rationalization of medicaments, the timeline for care and how progression and denouement will be computed. Present-day dressings intent to proffer strengthened functionality, for instance, the colossal potential for exudate management, within a nadir contour of product with extended wear time [15]. The most commonly used modern dressings (Table 1) in clinical practice include hydrogel dressings, hydrofiber dressings, hydrocolloid dressings, foam dressings, medicated and non-mediated dressings, composite dressings, superabsorbent dressings, and films [16–18].

4 Advances in Natural Polymeric Biomaterials and Composites

Extracellular matrix plays a pivotal role in wound healing and tissue homeostasis, and therefore designing supportive membranes for cells similar to their native niche is imperative for optimizing cell behaviors. Natural polymers imitate the structural, biomechanical, and biochemical functionalities of extracellular matrix and have an immanent bioactivity and biocompatibility, forming marvelous candidates for the development of matrices for wound dressing applications [19]. Natural polymers bestow extracellular matrix support (gelatin, hyaluronic acid, and collagen), present cell-recognition domains and biomolecules binding sites (keratin and silk fibroin), and may possess immanent anti-inflammatory and antibacterial properties (alginate and chitosan) [20]. The preponderant natural polymers employed in wound healing are collagen, alginate, glucan, dextran, hyaluronic acid, cellulose, chitosan, gelatin, silk fibroin, and keratin. Every aforementioned polymers demonstrate efficacious properties indispensable for wound healing. Natural polymer composites induced wound healing is illustrated in Fig. 2.

Table 1 Selection of suitable wound dressing for various wounds (Type, properties, advantages, drawbacks, and applications)

S. No.	Type	General description and physical properties	Advantages and disadvantages	Indication and method of use	Contraindications and precautions	Suitable conditions
1.	Hydrogels	– Composed of three dimensional network of complex hydrophilic polymers with high (90%) water content – Expand in water – Commonly accessible in three forms including sheet hydrogel, impregnated gauze, and amorphous gel – Properties: Clear and transparent, moisturizing, rehydrate eschar, and aid autolytic debridement	– Advantages include; (1) Wound monitoring without removal of dressing (2) Removal of necrotic tissue without damage to epithelial cells or granulation (3) Facilitate cell migration and absorption of exudate (4) Allow adjustment of degradation rate which renders them suitable for use as drug carrier and biologically active substance, – Disadvantages include; (1) Poor microbial barrier	– Suitable for wounds with minimal-to-moderate exudate – Endure for no longer than three days – Safe on neonatal skin. – Marked cooling and soothing effect on skin – Amorphous gels may be covered with secondary dressings such as films or foams.	– Health professionals to be aware of allergic reactions in older population caused by propylene glycol present in amorphous gels – Irrigation with saline solution for easy removal	Skin tears, surgical wounds, burns, pressure ulcers, radiation dermatitis, etc.

(continued)

Table 1 (continued)

S. No.	Type	General description and physical properties	Advantages and disadvantages	Indication and method of use	Contraindications and precautions	Suitable conditions
2.	Hydrofibers	– Composed of nonwoven sodium carboxymethyl cellulose spun into fibers – Variant on hydrocolloid with extra absorbent properties – Form a firm gel in contact with fluid and therefore exhibit properties similar to alginates. -Absorb 25 folds its own weight in fluid	– Advantages include; (1) Aids vertical wicking that protects periwound (2) Removal of necrotic tissue without damage to epithelial cells or granulation – Disadvantages include; (1) Non-adherence and requirement of secondary dressings to endure	– Suitable for wounds with moderate to excessive exudate – Can be kept in place until dressing is saturated – Should gently rinsed away at time of dressing change	– Should not be used in dry wounds – When used with mild exudates, irrigate with saline solution or sterile water to minimize the bed trauma and associated pain	Postsurgical wounds, leg ulcer debridement, partial thickness burns in pediatrics

(continued)

Table 1 (continued)

S. No.	Type	General description and physical properties	Advantages and disadvantages	Indication and method of use	Contraindications and precautions	Suitable conditions
3.	Hydrocolloids	– Composed of absorptive ingredients that form gel(gelatin, pectin, carboxymethylcellulose, etc.) – Occlusive, absorbent and semipermeable to vapor	– Advantages include; (1) Aid autolytic debridement (2) Moisture retention and pain free removal. (3) Barrier to entry of oxygen, water, and bacteria and therefore helps in facilitation of angiogenesis and granulation (4) Reduction in wound surface pH that further inhibits bacterial growth – Disadvantages include; (1) Potential for anaerobic bacteria to grow in a hypoxic environment (2) Not appropriate for deeper wounds, specially wounds with infection that require oxygen to enhance the healing rate	– Appropriate for partial- and full thickness- acute and chronic wounds with minimal-to-moderate exudate – Adhere well to high friction areas, including sacrum and heels – May produce a distinctive odor in some cases, indicative of product breakdown and not infection	– Should not be used in dry and high exudate content wounds – Regular wound tissue assessment to ascertain the discontinuation of dressing before hypergranulation occurs – Hydrocolloids with waterproof backing are not advised on clinically infected wounds	Pressure injuries, ulcers (diabetic foot ulcer, chronic venous ulcer), partial- and split- thickness wounds

(continued)

Table 1 (continued)

S. No.	Type	General description and physical properties	Advantages and disadvantages	Indication and method of use	Contraindications and precautions	Suitable conditions
4.	Foam	– Semipermeable, either hydrophobic or hydrophilic with a bacterial barrier – Made from polyurethane or are silicone-based	– Advantages include; (1) Proffer thermal insulation to the wound, create a moist wound environment, are nonadherent, and allow atraumatic dressing removal. Skin maceration avoided due to excess exudate absorption. (2) Do not need frequent changes due to their properties that conform to wound shape, reduce dead space – Disadvantages include; (1) Increased cost due to the need of secondary dressings in cavity foam adhesive (non-adhesive). (2) Ingrowth of newly formed tissue due to infrequent changes that may result in shearing trauma upon removal. (3) Set size of the foam product may be limited by the size of the wound	– Principally employed as primary dressings and secondary dressings with hydrogel or alginate dressing	– Unfit for use in necrotic wounds, dry wounds, hard eschar and wounds requiring frequent review – Tubular retention bandages or light weight cohesive bandages to fix foam dressing in place proffer a safer option in older population	Chronic wounds, wound-shape cavities, deep ulcers, infected wounds

(continued)

Table 1 (continued)

S. No.	Type	General description and physical properties	Advantages and disadvantages	Indication and method of use	Contraindications and precautions	Suitable conditions
5.	Films	– Composed of adhesive, porous, and thin transparent polyurethane. – Properties: transparent, semipermeable, and vary in size and thickness	– Advantages include; (1) Wound monitoring without removal of dressing (2) Easily conforms to the body, enable moisture evaporation, reduction in pain, and proffer barrier to external contamination (3) Enable autolytic debridement of necrotic wounds and create a moist healing environment for granulating wounds – Disadvantages include; (1) Traumatic on removal (2) Excessive pooling of exudate when used on heavily exuding wounds	– Frequency of dressing change is reliant on wound location, type and size, and may be left unchanged for up to 7 days – – Barrier polymer films should be employed for protection of periwound skin	– Clinicians should be cautious in applying and removing films from fragile skin to avoid skin damage	Radiation induced skin wounds, epithelializing wounds, and superficial wounds with limited exudate
6.	Superabsorbents	– Reduce PMN elastase (biochemical marker for pathologic granulocyte stimulation) concentration and inibit microbial growth	– Advantages include; (1) Reduction in periwound skin maceration, irritation, and inflammation			Moderate to highly exudating wounds, surgical incisions, lacerations, abrasions, burns, donor or skin graft sites

(continued)

Table 1 (continued)

S. No.	Type	General description and physical properties	Advantages and disadvantages	Indication and method of use	Contraindications and precautions	Suitable conditions
7.	Medicated	– Therapeutic agents are incorporated for quick and better recovery of wounds – Antimicrobials are employed for prevention of infections, supplements including minerals and vitamins for removal of dead tissue, growth factors for revitalization of damaged tissues	– Advantages include; (1) Microbes at surface of wound are eliminated	– Antimicrobials to be employed only when bioburden is a barrier to healing – Choice must be influenced by the specificity and efficacy of the agent, its cytotoxicity to human cells, its potential to select resistant strains and its allergenicity	–	Infectious wounds

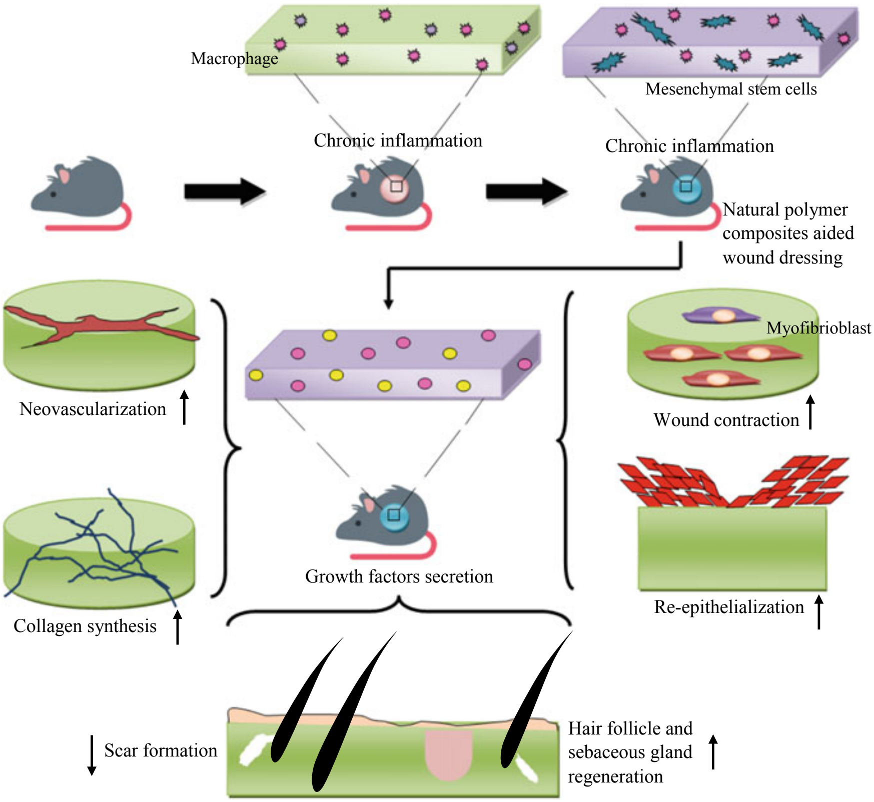

Fig. 2 Natural polymer composites induced wound healing

4.1 *Collagen-Based Wound Dressings*

Collagen is the most copious extracellular matrix macromolecule and is the chief integrant that delivers structural integrity in human skin. Production of collagen molecules by fibroblasts, at the time of wound healing, stimulates the development of new tissue and wound debridement. Collagen can also bind excess proteases, free radicals, inflammatory cytokines that are widespread in wound bed. As animal derivatives may be liable for allergies and pathogen transmission, an alternative is established by collagen from heterologous expression in insect, and yeast cells [5, 20]. Collage-based wound dressings have been recently recognized in modifying macrophage inflammatory response. Enhanced wound macrophage function and epithelialization deliver a precious archetype addressing significance of collagen based dressings [21]. Collagen based hydrogels have procured considerable admiration in wound healing applications. Howbeit, conventional collagen hydrogel are crippled due to the presence of non-reversible bonds. Self-healing collagen-based hydrogel based on dynamic covalent chemistry are able to repair wounds and possess superior tissue regeneration ability [22]. Polymer-nanoparticle composites have been extensively used because of unique features endowed as a result of high surface to volume ratio of the particle and versatility and tenability of the materials' physicochemical properties [2, 23]. Silica-collagen encapsulated with drugs—rifampicin and gentamicin demonstrated medicated dressing to prevent bacterial infections in chronic wounds [24]. *Aloe vera* and ZnO nanoparticles incorporated Zein/PCL/Collagennano fiber exhibited enhanced mechanical an antibacterial activity, revealing promising scaffolds for wound healing issues [25].

4.2 Alginate-Based Wound Dressings

Alginate, a polysaccharide with homopolymeric blocks of 1,4-linked β-d-mannuronic and α-l-guluronic residues is mostly abundant in *brown algae* and some bacteria. The characteristic gelation property enables ionic crosslinking upon exposure to divalent ions, giving rise to a biocompatible 3D polymeric cross-linked scaffold [5, 20]. Alginates deliver a highly absorbent, moist environment to the wound to cease blood flow from wounded vasculature. Ion exchange between sodium ions of blood and calcium ions of the polysaccharide causes the fiber to swell and then partly dissolve to form gel. The gel proffers a moist environment and aids the natural healing process in the removal of necrotic tissue. The role of different counter cations for a series of ammonium salts of alginate have revealed the contribution of different features including branching structure, charge density, molecular weight, linearity, hydrophilicity, etc. in anti-hemolytic, antimicrobial efficacy, and cytotoxity of the dressing [26]. Healing potential of alginate has been recently utilized to deliver moxifloxacin loaded hydrogel for better wound care. The sterile and mucoadhesive nature of the dressings coupled with a controlled and sustained release of antibiotic drug paved path for future research [27].

4.3 Glucan-Based Wound Dressings

β-Glucans isolated from fungi, grain, and yeast manifest the capability of the formation of single and triple-helical resilient gel structures. α-Glucans biosynthesized from starch by the ubiquitous yeast-like fungus *Aureobasidium pullulans.* Both β- and α-Glucans have been employed in wound healing tools because of their non-cytotoxic, quick hemostatic, and antimicrobial characteristics [28–31].

4.4 Dextran-Based Wound Dressings

The non-toxic, hydrophilic polysaccharide composed of linear α-1,6-linked d-glucopyranose residues is resilient to protein adsorption and cell adhesion. Modifications in the polymer backbone tune the discrete properties of biomaterial making them attractive candidates for curing wounds [32–34].

4.5 Cellulose-Based Wound Dressings

Cellulose is utilized as healing matrix for reducing pain and shortening the healing time in chronic wound dressings. The polymers stimulate epithelialization and granulation process for partial and full thickness wounds. Bacterial cellulose, specifically, have been utilized in interesting wound healing tools because of non-toxicity, inability to cause allergies, and prevention of physical rejections. Bacterial cellulose gel films serve as a mechanical barrier, shielding the wound surface from infection and rapid drying. Successful use of bacterial cellulose have been addressed in several publications. However, the polymers require the impregnation of other materials to exhibit antimicrobial activities. Excellent water holding capacity and porosity aids in easy absorption and slow release of the antimicrobial solution. Antimicrobial and wound healing properties of bacterial cellulose gel film and *Bacillus subtilis* has been investigated for delivery of universal wound coating and sanitary hygienic product [35]. Bacterial cellulose impregnated with ε-poly-L-Lysine, a non-toxic biopolymer, has been studied for antibacterial wound dressing tools. The potentiality to render antimicrobial characteristics to the polymer without affecting the mechanical and structural properties pave way for future research and development in the arena [36]. Cellulose materials employed in textile wound dressing can cause maceration of wound accompanied with pain, and microbial deposition and growth. Functionalization with hydrogels have reported to enhance the wearability and drug delivery capacity of the matrix. Additionally, new functionalities such as pH and thermo-sensitivity and antimicrobial properties might be introduced for production of novel interactive wound dressings to improve patient's life quality [37].

4.6 Chitosan-Based Wound Dressings

The semi-crystalline, linear polymer has competent biological property including biodegradability, biocompatibility, non-toxicity, mucoadhesion, antimicrobial, and homeostatic activity. The deacetylated chitin-derivative is found in the exoskeleton of fungi and arthropods and is widely exploited in healing of burn and wound treatment. Chitosan has been processed into the hydrogel, nanofiber microsphere, or porous scaffold in tissue engineering [38]. Chitosan impregnated with nanoparticles such as silver and gold nanoparticles delivers enhanced mechanical properties, well-distributed pores contributing to moisture retention capacity, antimicrobial activity, accelerated re-epithelialization and collagen deposition [39, 40]. Disadvantages of chitosan including poor mechanical properties, fast degradation rate, hardly electrospinnable is improved by cross-linking and blending with other polymers. Polyvinyl alcohol/Chitosan/Starch nanofibrous mats have been investigated for enhanced water retention, porosity, water absorption coupled with water vapor transmission in wound healing tools. Appropriate wound breathing and efficient handling of exudates accelerated the wound healing process effectively [41]. Chitosan-pectin biopolymeric hydrogel presented sound printability, physical integrity, and flexibility to be utilized as appropriate candidates for personalized wound dressing tools [42].

4.7 Hyaluronic Acid-Based Wound Dressings

The non-immonogenic polysaccharide is composed of N-acetyl-D-glucosamine and glucuronic acid. The hygroscopic nature of hyaluronic acid has been exploited in preparation of hydrogel like constructs, to assist the migration of keratinocytes and angiogenesis, and foster a scar-free wound healing. It interacts with CD44 and RHAMM cell receptors to modulate inflammation, stimulate cell migration, formation of new vasculature in injuries. Howbeit, the wound repair mechanism is dependent on the molecular size of the polysaccharides. High molecular weight hyaluronic acids inhibit extracellular proliferation and therefore displayanti-inflammatory and immunosuppressive responses along with causing hindrance to the development of new blood vessels. Whereas, short-chain, low molecular weight hyaluronic acid composed of 3–10 disaccharide units, are potent anti-inflammatory molecules that can stimulate extracellular proliferation, migration, and angiogenesis in wounded tissue [5, 20]. Hyaluronic acid-based wound healing tools, either pure or impregnated with other materials have been propounded for the management of both acute and chronic wounds [43–47].

4.8 Gelatin-Based Wound Dressings

The collagen derived natural polymer is procured by incomplete denaturalization of collagen obtained from skin, boiling bones, and connective tissue. Type A gelatin, procured from acid cured tissue are positively charged at a physiological pH and type B gelatin, procured from lime cured tissue are negatively charged at a physiological pH. Ascribable to its biodegradability, biocompatibility, and lower antigenicity in comparison with collagen, gelatin has been employed as drug delivery systems for growth factors, and crosslinking of gelatin has been addressed to modify the degradation and release rate of encapsulated cargos. With a broad molecular weight, under appropriate conditions of pH, temperature, or solvents,gelatin can espouse different conformations such as microparticles, microspheres, orhydrogels. Gelatin based wound dressings have been engineered with other biomaterials for rendering remarkable next-generation healing properties [48–50].

4.9 Keratin-Based Wound Dressings

Keratin are the most copious group category of filament-forming insoluble proteins produced in epithelial cells of reptiles, birds, mammals including humans. The absence of keratin gene 17 has been reported to delay wound closure whereas down-regulation of keratin gene 16 and keratin gene 6 furnish non-healing phenotypes. Maximum healing was obtained for keratin base dressing on refractory wound (very complicated wounds that are not repairable with conventional dressing alone) population. Integration of antimicrobial products on keratin was also successfully achieved [51]. Keratin extracted from human hair impregnated with carboxymethyl cellulose succeeded in inhibiting growth of bacterial colonies. The effect of keratin addition on fibroblasts revealed better cellular attachment, proliferation, and spreading [52]. Halofuginone-laden keratin dressings proffered novel mechanism for reduction of scarring of severe burn wounds by delivering contracture-inhibiting Halofuginone, a collagen synthesis inhibitor that has been shown to decrease collagen synthesis in fibrosis cases [53].

4.10 Silk Fibroin-Based Wound Dressings

Silk proteins are utilized in wound dressings due to its characteristic properties including flexibility, adherence, absorption of exudates, minimal inflammatory reaction, biodegradability, and biocompatibility. Dressings based on silk fibroin are employed for treatment of a wide variety of chronic and acute wounds. Silk fibroin and its derivatives are prepared as biomaterials are available as sponges, hydrogels, nanofibrous matrices, scaffolds, micro/nanoparticles, and films [54–58].

Table 2 delivers few more instances for better understanding of properties influenced by naturally derived composite matrix.

5 Conclusion

Effective healing of the wound is essential for the personal satisfaction of the patients. This chapter featured several natural polymers and their composites with immuno modulating, bactericidal, and wound healing activities. Both chronic and acute wounds endure disruptions in at least one phase of the recovering cycle, and therefore entail progressed treatment techniques to augment clinical consideration and personal

Table 2 Properties of few naturally derived polymeric composites for wound dressing application

S. No.	Natural polymer	Composite material	Properties	Reference
1.	Silk fibronin	Konjacglucomannan	– Enhanced water retention, water absorption capabilities, and compression strength – Similar compressed modulus with native skin – Excellent biocompatibility for cell proliferation and adhesion	[59]
2.	Chitosan-alginate	Gentamicin	– Enhanced drug delivery, skin regeneration, collagen deposition and formation of new blood vessels	[60]
3.	Vitamin D3 loaded alginate	Calcium carbonate in combination with–glucono- δ-lactone	– Enhanced wound closure, re-epithelialization and granular tissue formation	[61]
4.	Cellulose acetate	Gelatin	– Antimicrobial activity with enhanced biological activities – Proper wound for diabetic foot ulcer healing	[62]
5.	Starch	Polyvinyl alcohol, glycerol, citric acid	– Enhanced combinations of water vapor transmission rate and antibacterial activity	[63]
6.	Hyaluronic acid	Lysozyme	– Remarkable adhesion and appropriate viscocity	[64]
7.	Keratin	Silver nanoparticle	– Accelerated wound closure and epithelialization	[65]
8.	Bovine serum albumin	Polyacrylonitrile	– Promising bioactivity Hs68 fibroblasts and HaCat keratinocytes were found to be more viable in the presence of the biomineralized NFs than when they were co-cultured with the neat polyacrylonitrilenanofibres	[66]

(continued)

Table 2 (continued)

S. No.	Natural polymer	Composite material	Properties	Reference
9.	Silk fibroin-chitosan	Zein nanoparticles with loaded curcumin	– Enhanced stiffness, cell adhesion and proliferation do not have any cytotoxic effect on human gingival fibroblast	[67]
10.	Heparin	Basic fibroblast growth factor	– Diminished inflammation, stabilized basic fibroblast growth factor, upgrade the expressing of vascular endothelial growth factor and transforming growth factor-β in wound site, and better on site cell proliferation	[68]
11.	Bacterial cellulose	Montmorillonite	– Enhanced mechanical and thermal properties of membrane – Decreased the water holding capacity and an improved in the water release rate	[69]
12.	Chitosan	Silicon nanoparticles	– Enhanced the quality of skin regeneration by favorable growth of microvessels, hair follicles and faster formation of epidermis	[70]
13.	Genipin cross-linked gelatin	Cerium oxide	– Cerium oxide act as chemo-attractant to aid in migration of fibroblast cells on the gelatin matrix – Exhibits more infiltration of leukocytes and larger deposition of collagen in contrast to gelatin and control groups	[71]
14.	Sodium alginate	Cellulose nanofibres	– Enhanced structural stability in moist environment and high water/fluid absorption capacity	[72]

(continued)

Table 2 (continued)

S. No.	Natural polymer	Composite material	Properties	Reference
15.	Alginate	Human elastin like polypeptide loaded with the hydrophobic natural antioxidant curcumin	– Controlled release of the model compound curcumin leading to a high antioxidant activity of the material and to maintain, and augment the cytocompatibility	[73]
16.	Collagen and dextran	Zinc oxide	– Enhanced pseudoplastic behavior and antimicrobial activity	[74]
17.	Keratin	Cinnamon	– Enhanced the mechanical compliance of the composite and antibacterial properties – Retained its antioxidant properties and reduced expression of pro-inflammatory factors by 5–7 fold	[75]
18.	Chitosan	Polycaprolactone with nanoclay	– Superior controlled-release of curcumin – Enhanced tensile strength – enhanced biocompatability.	[76]
19.	Soy protein	Cellulose acetate	– Promoted fibroblast proliferation, migration, infiltration, and integrin β1 expression – Demonstrated high water retaining capability – Accelerate re-epithelialization and epidermal thinning as well as reduce scar formation and collagen anisotropy	[77]
20.	Low-methoxy pectin	Zeolite particles	– Zeolite particles served as drug loading entities and enhanced hydrogel stability through swelling – Promoted cell viability	[78]

satisfaction. Contingent on the kind of wound and its location and origin, at least one treatment choices are accessible: effective utilization of antiseptics and antibiotics, innumerable dressing changes every day, and costly medication, for instance, MIST ultrasound therapy and negative weight wound treatment. In spite of the large number of financially accessible items for complex intense and incessant injury therapy, such incurable wounds prevail exigent to manage and require broad clinical intervention. Natural polymers derived wound dressings exhibit intrinsic bioactive properties that can quicken the recuperating process in acute wounds, yet employ a negligible impact in complex acute wounds, for example, severely charred areas or chronic injuries. Combinatorial methodology by fusing engineered or characteristic polymer with remedial bioactive particles like cytokines and development components to advance re-epithelialization, debridement, granulation, and other materials for solid injury healing have proven useful.

The vast majority of these specialists have been utilized forages. Albeit numerous in vivo and in vitro examinations have demonstrated their efficacy in wound healing and recovery, documented clinical trials are as still missing and need to be carried out more often to guarantee the security and adequacy in human application. Contemporary challenge reclines on recognition of dynamic compounds accountable for their injury mending properties and the mechanism followed in question. Management of wound contracture, exudates and bacterial control are still the major challenges in wound healing.Wound contracture is a characteristic method for wound closure; nonetheless, excessive wound contraction prompts functional restrictions, inability, and physical deformity. The majority of wound dressings don't have the ability to retain thick and gooey exudates from wounds. With the emergence of multiresistant microbes, specialists to improve wound mending and forestall intrusion of microorganisms to the body framework are additionally pertinent. The accrued engrossment of the scientific community in the utilization of either protein-based or polysaccharide-derived dressings is striking, and it reflects the growing perspective of giving back what we borrowed from nature.

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A View on Polymer-Based Composite Materials for Smart Wound Dressings

S. Baptista-Silva, P. Alves, I. Guimarães, S. Borges, F. Tavaria, P. Granja, M. Pintado, and A. L. Oliveira

Abstract Wound management challenges everyday thousands of health professionals, mainly due to the constant monitoring and difficulties in deciding the correct treatment options. When considering chronic wounds, selecting the ideal dressing defies clinical knowledge, when facing the large amount of different materials, its distinctive properties and the uniqueness of each patient needs. This chapter presents

S. Baptista-Silva (✉) · I. Guimarães · S. Borges · F. Tavaria · M. Pintado · A. L. Oliveira (✉)
Universidade Católica Portuguesa CBQF—Centro de Biotecnologia E Química Fina—Laboratório Associado, Escola Superior de Biotecnologia, Porto, Portugal
e-mail: sara.baptistadasilva@gmail.com

A. L. Oliveira
e-mail: aloliveira@porto.ucp.pt

I. Guimarães
e-mail: ines.gvguimaraes@gmail.com

S. Borges
e-mail: sandraferreiraborges@gmail.com

F. Tavaria
e-mail: ftavaria@porto.ucp.pt

M. Pintado
e-mail: mmpintado@esb.ucp.pt

P. Alves
Universidade Católica Portuguesa, Instituto de Ciências Da Saúde CIIS—Centro de Investigação Interdisciplinar em Saúde, Porto, Portugal
e-mail: Pjalves@porto.ucp.pt

P. Granja
Instituto de Investigação E Inovação Em Saúde (I3S), Universidade Do Porto, Porto, Portugal

Instituto de Engenharia Biomédica (INEB), Universidade Do Porto, Porto, Portugal

Instituto de Ciências Biomédicas Abel Salazar (ICBAS), Universidade Do Porto, Porto, Portugal

Faculdade de Engenharia Da Universidade Do Porto (FEUP), Porto, Portugal

P. Granja
e-mail: pgranja@i3s.up.pt

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an overview on the challenges and complexity of a chronic wound, exploring the event of awound infection and discussing the large range of polymer-based composite materials and products in use for each specific wound condition, taking into account the key decision aspects defined by the clinicians. Different tissue engineering strategies are also herein addressed with varied reported clinical success, ranging from non-cellularized to considerably sophisticated cellularized products, reproducing the compositional complexity of both dermis and epidermis. Recent advances in smart dressings and sensors are also brought to discussion as sensing the wound can give us new insights about the series of complex biochemical events related to the healing and regeneration process, while contributing for a better wound assessment.

Keywords Polymers · Composite materials · Wound management · Wound dressings

1 Introduction

Wound treatment is based on a complex approach where it is essential to identify the aetiology, make a correct diagnosis, and ensure that the treatment and therapeutic decisions are the most effective for that case. Wounds can be classified as acute or chronic according to their healing process [1, 2]. Acute wounds are associated with a non-complicated process that is generally organized, sequential and able to restore the anatomic and functional integrity of the tissues involved within a reasonable small amount of time. Meanwhile, chronic wounds are associated with difficult or prolonged healing where the reparative process does not follow in a timely or orderly fashion, and thus fails to produce the previous anatomic and functional integrity of the injured site [3]. According to White et al. [4], chronic wounds may be the result of an association between wounds that have an impaired healing process due to the presence of complex underlying pathologies, such as diabetes mellitus, vascular disease or the presence of malignancy. The epidemiological profile of chronic wounds is not fully known, however, it is estimated that there may be more than 20 million chronic wounds worldwide [5], with 1–2% of the population experiencing a chronic wound during their lifetime [6].

Wound care has high associated costs, as studies from Scandinavian countries have reported, they account for 2–4% of the total health care expenses [7], studies from the UK showed that they account for approximately 4.8 billion pounds [8], and studies from the United States showed that they account for an excess of US$25 billion annually in the treatment of chronic wounds [9]. This burden is expected to grow even more due to aging population and chronic diseases, like diabetes. Aspects such as pain, anxiety and social isolation are aspects that may influence the quality of patient's life besides the wound healing process and cost treatment [10, 11]. Nonetheless these aspects are presently difficult to quantify.

This chapter discusses recent advances in the development of synthetic (including biosynthetic) and biologic (tissue origin) ultimate polymer-based dressing materials and composites to promote wound care. In specific, this chapter focuses on the current and new solutions that enhance wound healing and tissue regeneration, keeping in mindimportant aspects such as the improvement of wound care system.

2 Complexity of a Chronic Wound

A chronic wound is related to the complexity and senescence of physiological processes and is associated with stagnation. Chronic wound is described as an interruption in the integrity of skin and underlying tissues progresses through a disorganized and complex shape healing process. These injuries hardly progress through a sequence of normal healing, sequential and timely, and there is a precise balance between production and degradation of molecules [12, 13]; to thereby influence the physiology of healing. Chronic wounds can take several years to heal or even have no cure, causing emotional changes, physical and economic to patients, families and health services.

Healing is an intricate, dynamic and continuous systemic process [14], which requires body activation, production and inhibiting of a large number of molecular and cellular components, the interaction of chemical mediators, and extracellular matrix in an orderly and continuous sequence, conduct the entire process of regeneration/ healing. Its understanding is a critical step towards solving chronic wound problem.

2.1 Different Stages of Healing

The healing process is divided into three continuous phases which overlap temporally: inflammatory, proliferative and maturation [15, 16]. Five steps are known during the healing process, including the induction of inflammatory response by injury, reconstitution, migration and proliferation of parenchymal cells and connective tissue; followed by the synthesis of proteins of the extracellular matrix and restructuring of parenchymal elements to restore functionality and finally the connective tissue remodelling [17].

From the moment in which there is tissue loss, platelets come in contact with exposed collagen and other extracellular matrix components, giving the coagulation cascade and the release of vasoactive substances, adhesive proteins, growth factors and proteases [17, 18]. The formation of this provisional matrix is essential for cell migration, in addition to serving as a reservoir for cytokines and growth factors that will be released during the following phases of the healing process [19, 20]. At this stage the Celsius signals are evident as redness, heat, pain, tumor and possible loss of function [21]. The pro-inflammatory cytokines produce proteases that are present in the exudate and breakthe damaged extracellular matrix proteins [22], a process termed proteolysis. Beyond processes described, there is the phagocytosis of bacteria, cell debris and foreign bodies, as well as the production of growth factors involved in these inflammatory cells to prepare the wound for the proliferative phase when the endothelial fibroblasts and cells also are incorporated [23]. When the inflammatory process does not lapse, a complex response is triggered that can lead to chronic inflammation [24, 25] . The proliferative phase starts with the granulation tissue formation, connective tissue cell proliferation and migration and re-epithelialization of the wound surface. The epithelial cell proliferation starts with a mitogenic and chemotactic stimulation of keratinocytes, with increased microvascular permeability that allows, through the leakage of proteins, cytokines and cell elements, to the formation of a provisional extracellular matrix which is necessary for the migration and proliferation of endothelial cells [26]. The last stage—Maturation is characterized by deposition of extracellular matrix (ECM) remodelling of tissues and wound contraction [27, 28]. In the course of maturation and remodelling process, most fibroblasts and inflammatory cells disappeared from the wound site, giving rise to apoptosis and cell death processes to scar formation.

2.2 Wound Types and Therapeutic Requirements

From the universe of chronic wounds, the most common are pressure ulcers, venousarterial ulcers, and diabetic ulcers (Fig. 1) [29]. Injuries associated with the lower limbs, venous ulcers are the most common type, accounting for about 80–90% of the wounds, and the remaining arterial and neuropathic [30].

The choice of the most adequate dressing is influenced by the aetiology, specific characteristics of the wound bed, type of tissue present, odour, infection signals and amount of exudation [31, 32]. There is a considerable number of different dressings and techniques available for managing wounds according to its characteristics (Table 1).

Thus, the wound bed, and its tissue type are indicative of the required material, the healing phase, progression and treatment effectiveness:

Necrosis: usually black, indicative of tissue death, hard or soft consistency;

Fibrin: yellowish and can be presented adherent to the wound bed (slough);

Granulation: Reddish colored, slightly damp and firm, it is indicative of good evolution of the healing process;

Epithelialization: pinkish tissue, indicative of wound closure and thus usually arises from their edges.

The first principle of wound bed preparation is the removal of this tissue type and should be performed using debridement; which quantitatively reduces bacterial load, toxins and other substances affecting the immune system [33]. It is clear that healing is systemic, but the choice of the most adequate local treatment technique among the available options or the possibility of an infection event are factors that can contribute to accelerate or delay this process.

Fig. 1 Most common pressure ulcers: **a** Calcaneous pressure injury, **b** Venous Leg ulcer and **c** Diabetic foot—Charcot foot

The evaluation of the risk factors of the patient with wound should be an element guide to all decisions for the prevention and treatment of wounds. Several factors that interfere with the healing process are identified in the literature, and they are consensual that this continuous process may be hampered by systemic and local factors [27, 28]. According Nazarko [34], in addition to these local and systemic risk factors, extrinsic factors must be evaluated (affecting the condition of the person) and assessing the intrinsic (referring to the wound characteristics) that interfere with the healing process (Table 2).

3 The Event of a Wound Infection

When the skin integrity is disrupted becomes prone to infection. As a multi-functional organ, skin possesses particular biochemical and physical properties that influence its microbiology. Some of these properties include a slightly acidic pH, low moisture content, high lipid content (which confers hydrophobic characteristics) and the presence of antimicrobial peptides [35].

Table 1 Wound characteristics and established therapeutic options

Color	Tissues	Exudation	Objectives	Considerations	Therapeutic options
	Necrosis Necrotic tissue	Null (dry)	Debride Hydrate	Caution: - Arterial occlusion - Heel - Malignant lesions - Coagulation Levels	Surgical Debridment
		Scarce	Debride Hydrate& Maintain Humidity		Autolytic or enzymatic debridement
	Devitalized \ Fibrin Slough Tissue	Scarce	Removal of necrotic tissue Reduce the bacterial load	Perilesional skin protection	Surgical debridement if necessary Autolytic or enzymatic debridement
		Moderate \ abundant	Removal of necrotic tissue Reduce the bacterial load	Perilesional skin protection	Autolytic or enzymatic debridement Absorbing dressings
	Granulation tissue	Scarce	Promote Granulation & Maintain Humidity	Perilesional skin protection Atraumatic treatments	Hydrogels Absorbing dressings
		Moderate \ abundant	Promote Granulation & Management of exudate	Protect new tissues	Absorbing dressings Tissue regeneration material
	Epithelization	Null	• Promote epithelialization • Protection	• Protect new tissues • Decrease Frequency of Treatments	Silicone films Thin Hydrocolloids

Adapted from WUWHS [31]

Table 2 Risk factors for impaired healing

Intrinsic factors	Extrinsic factors
Age	Aetiologies
Skin condition	Wound site
Associated pathologies	Wound size
Life style	Wound bed tissues—Debris
Nutritional status	Exudation—Maceration
Mobility	Wound edges
Psychological well-being	Surrounding skin
Pain	Pain associated with wound
Immune status	Mechanical stress
	Chemical stress
	Dressing materials
	Medication
	Temperature

The outer surface of adult skin is colonized by a handful of stable inhabitants (resident microorganisms) with rare or transient species contributing to interpersonal variation [36]. Skin physiology determines the pattern of colonization by skin microbes. *Staphylococcus* spp. and *Corynebacterium* spp. are the most abundant in moist sites, while lipophilic microorganisms such as *Propionibacterium* spp. and *Malassezia* spp. dominate in sebaceous areas [37]. The role of transient microorganisms in infection remains largely unknown, although it is likely that they influence the infection life cycle.

Microorganisms are present in all wounds. In acute wounds, the short healing time allows for only a small number of skin contaminates to take residence while in chronic wounds, the continued exposure of devitalized tissue is likely to facilitate the colonization of a wide variety of microorganisms and trigger infection [38]. Bacteria, fungi, viruses or protozoa can cause human infection. However, the presence of microorganisms such as bacteria does not necessarily indicate that an infection exists or that will lead to impair wound healing [39].

The role of bacteria in wound healing has been debated over the years. Some have suggested that bacteria may play a beneficial role in normal wound healing and wounds will heal despite the presence of large numbers of microorganisms [40]. Nonetheless, the detrimental effects of specific pathogens, such as *Clostridium perfringens* and *Streptococcus pyogenes*, have been well recognized. These are typically invasive bacteria that are not normal members of the human skin microbiota. In contrast, some resident microorganisms such as *Staphylococcus aureus*, which are part of the microbiota of many humans, also cause wound infections (Table 3).

Polymicrobial wounds are those containing several potential pathogens. This typically delays wound healing, raising the risk for other complications [42] and is the norm in chronic wounds. The concept of wound bioburden involves the bacterial burden, which is the presence of replication microorganisms within a wound, the bacterial load, the virulence of the microorganism and the host reaction [43]. This leads to increased metabolic load imposed by the multiplying microorganisms in the wound bed, and their ability to spread in tissues and produce toxins [44]. The

Table 3 Bacterial species isolated from chronic wounds

	Chronic wounds (specimens from 19 wounds)*	
Bacterial genus	Swab culture	Tissue PCR
Staphylococcus	28	68
Enterococcus	12	18
Pseudomonas	32	28
Proteus	126	–
Citrobacter	8	28
Corynebacteria	0	68
Anaerobes	0	70

* Frank et al. [41].

interactions of multiple microbial populations in chronic infections are still poorly understood, mainly due to controversies in culturing methods [45]. However, over the past several years, molecular-based methods have been increasingly applied in skin and wound microbiology research. Molecular studies of wound microbiology have revealed very diverse bacterial communities. These studies involved polymerase chain reaction (PCR) amplification of the bacterial gene encoding the small ribosomal subunit RNA (16S). More recent studies have used additional molecular methods including metagenomics for the evaluation of chronic wound microbiota [41]. The results of these studies indicate that chronic wounds contain diverse polymicrobial communities and similar community features, such as the presence of strictly anaerobic bacteria, even though the studies were from diverse geographic regions.

Evidence exists that bacteria colonizing human chronic wounds exist as biofilm communities and not in the planktonic form [35, 46]. Bacteria within a biofilm live in microcolonies encapsulated in a matrix composed of an extracellular polymeric substance. This acts as physical barrier to the permeation and action of antimicrobial agents. Besides this, the biofilm confers a habitat where bacteria can communicate with each other (quorum sensing), which may lead to increased virulence and propensity to cause infection [47]. More than 80% of human infections are caused by biofilms [48]. Chronic wounds have a fertile environment for biofilm formation; necrotic tissues and superficial debris facilitate infection whereas the altered vascularization and subsequent ischemia hinder the immune system to develop an efficient defensive response [49]. It is estimated that 60% of all chronic wounds are colonized by biofilms compared to only 6% of acute wounds [50]. This explains how biofilms can impair the healing process, by being often associated with a persistent inflammatory state, tissue disruption and difficulty in healing. The cascade of events leading to a chronic wound is schematized in Fig. 2. Adding to this, the polymicrobial nature of these infections further complicates diagnosis and treatment. When compared chronic wounds (venous leg ulcers, diabetic foot ulcers and pressure ulcers) with acute, generally are colonized by more anaerobic bacteria and fungi. Diabetic foot ulcers have a high incidence of species of *Bacteroides*, *Peptoniphilus*, *Finegoldia*, *Anaerococcus* and *Peptostreptococcus* [51].

It is tempting for the clinician to start antibiotic treatment, but in case of established mature biofilms, this treatment often has only temporary effect on both inflammation and healing. In addition, the clinician has to rely on the results from a swab or biopsy, which rarely reflects all specimens present in the wound. The bacteria in biofilm are up to 1000 times less susceptible to antibiotics [53], and MIC is not reached in the chronic wound fluid. Even silver as an antimicrobial incorporated in several wound dressings, has limited effect in biofilm *in vitro* [54]. With this in mind, the clinician should exercise restraint in administering antibiotics. This favours biofilm persisting bacteria and promotes resistance. Mechanical removal of wound debris (by ultrasound assisted surgery) and even granulation tissue is an effective way of diminishing the bacterial load and is an important part of treatment protocols. “Biofilm managing strategies” have been implemented, but none have yet proved to be more effective than others [55]. The need for new and more efficient treatment regimens (new biofilm penetrating drugs, new substances to disrupt biofilms) and

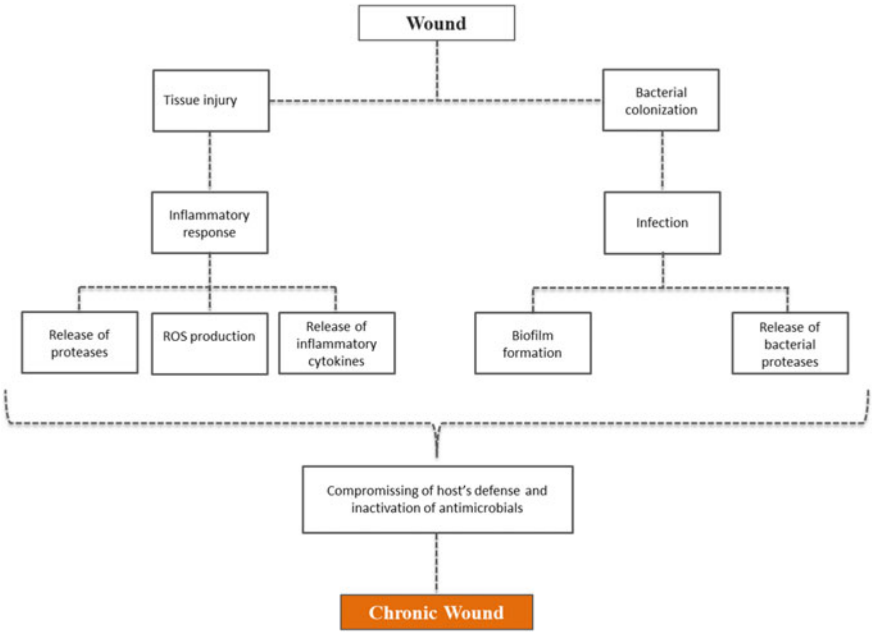

Fig. 2 Cascade of events contribution to a chronic wound. Adapted from McCarty and Percival [52]

research in biofilm (QS manipulation, resistance to antimicrobials) may provide wound care specialists with new, more effective ways to heal wounds.

4 Established Wound Dressing Options

Very few, if any, current wound care products have the capacity to cross the healing process towards tissue restoration [56]. The contexts, realities and needs in wound care around the world are simultaneously equal and different and there are actually a large variety of different dressings for prevention and treatment [57–60] for the most various aetiology's or wound characteristics. These wound care dressings presently can be divided into two broad categories: synthetic (including biosynthetic) and biologic (tissue origin) polymer-based materials and their composites [56].

Biologic-derived polymers for wound dressings are not recent, they are used since the Egyptian's [60], however only after the 60's the first study's started and more specialized biological-derived dressings have been developed since there [56, 61]. Their effectiveness has increased greatly with recent innovative developments, where various skin substitutes were tested over time, such as human skin allograft, xenograft and amnion, are being used at various wound care centres. The skin substitutes provide faster wound coverage solution that may require less vascularized wound bed, increase in the dermal component of healed wound, reduce or removed inhibitory factors of wound healing, reduced inflammatory response and subsequent scarring [62]. Nevertheless, these skin substitutes need specific expertise/experience and have a higher cost [63].

4.1 Synthetic/Biosynthetic Dressings in Wound Care

Synthetic/biosynthetic dressings have been designed primarily to promote moist wound healing and function as a barrier against infection, while simultaneously collaborate in the growth of granulation tissue and epithelisation. Currently, chronic wounds are treated with a broad variety of dressings tailored to the requirements of the wound (dry or exuding, clean or infected, superficial or deep) (Table 2). These materials are generically categorized as textiles, polyurethane films, foams, hydrogels, hydrocolloids, and collagen/alginate combination of wound dressings as example in Table 1 [60]. Nonetheless the most common wound dressing are alginates and hydrofibers, well studied, applied and documented in literature. Alginate has relevant properties as a gel-forming [64] and film-forming [65]. Alginate dressings are highly absorbent, being this dressing a good choice for highly exudative wounds. These dressings are described to absorb 15–20 times their weight of fluid, which can be a substantial lifestyle improvement for patients with draining ulcers [66]. Hydrofibers are dressings highly absorbent, they have a similar function of alginates but can absorb three times more. They have been demonstrated to be useful in partial-thickness donor sites and partial thickness burns. All together, these polymer-based materials and their compositescan present a wide range of properties resulting in interesting possibilities for the final wound dressing product [67]. The selection of various reinforcements and polymer matrices is very critical in designing a desired product for wound care. Figure 3, shows a schematic illustration of a typical decision tree in wound care.

The wound stage and its characteristics will implicate the best dressing choice. While films will be the ideal solution for superficial wounds; foams will be more adequate for exudative and granulating; hydrogels will be best applied in eschar, deep or tunneling wounds and in wounds with slough; and hydrocolloids will be used in superficial, eschar and granulating wounds and also wounds with slough [68]. It is highlighted the key steps prior dressing application, like wound bed preparation (cleanse, debridement and measurement), the wound evaluation (considering the different levels of exudate) and the consequent ideal solution for each type of wound. Likewise, some common commercial products are listed below.

In this chapter novel and promising polymer-based composite dressings in the different categories, are reported for wound healing.

Foams

Foams are one of the most variable and versatile dressings for chronic wounds, including thin and thick, adhesive and non-adhesive, coated or uncoated [69]. Typically, the most used foams are constituted of polyurethane or a silicone core with a

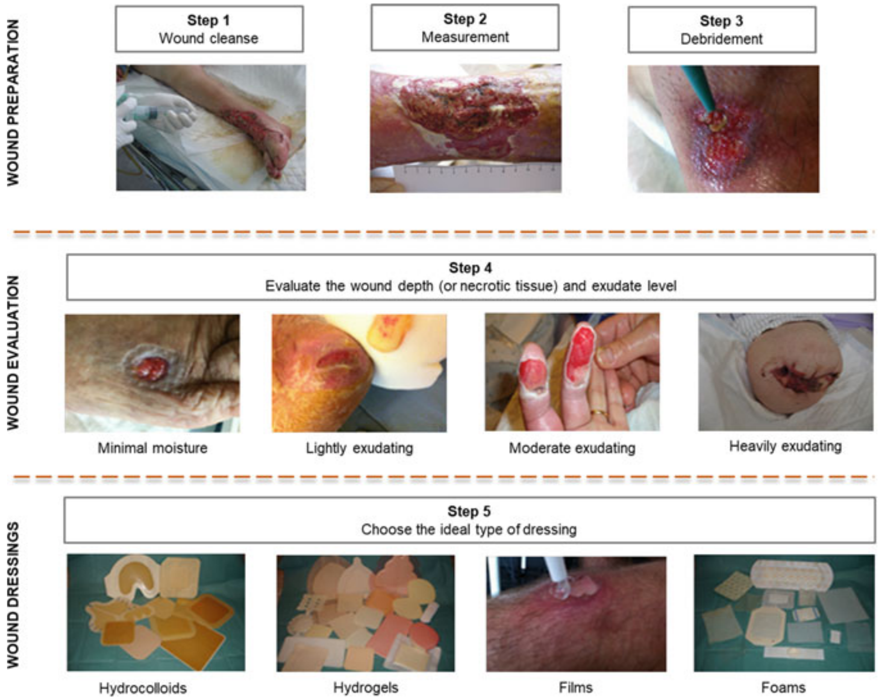

Fig. 3 Schematic illustration of wound care treatment

semi-occlusive out layer. This layer is water vapor permeable and attends to protect against microbial penetration and proliferation, while the polyurethane/silicon serves to give absorptive qualities [66]. Foams are applied in wounds with moderate to high exudate, granulating or necrotic wound, and can be used on infected wounds (Fig. 3). It has been developed foams with antimicrobial activity, as for example silver-containing arabinoxylan foams [70]. There are also available commercial products incorporating silver, such as PolyMem silver® (Ferris Cor.) and Mepilex® Ag (Mölnlycke Health Care).

Foams afford an atmosphere for autolytic debridement and reduce granulation tissue. Thick foams can be used for venous ulcers to offer an improvement of local compression, which allow to control edema and promote healing [69]. Treatment of wounds using negative pressure therapy uses foams that incorporate tubing to a vacuum source. Generally, two types of foams are used: polyurethane foams, for example, VAC GranuFoam® (Kinetic Concepts [KCI], San Antonio, TX); Flexzan® (Dow B. Hickam, Inc.), Hydrasorb® (Tyco Health Care/The Kendall Co.) or polyvinyl alcohol foams, for example, VAC VersaFoam, (Kinetic Concepts [KCI], San Antonio, TX) [71].

Foams can dry wounds with minimal or mild exudate and may require a saline soak during dressing change to avoid pain and trauma [68]. The absorbent capacity of wound fluids is dependent on the polymeric material used and the thickness of the foam. They are extremely absorbent, protective and adaptable to body surfaces. Additionally, foams are easily manipulated and can be adjusted to the wound size [72].

Foams are appropriate for deep wounds with exposed bony areas such as the ankle or sacrum or exudative cavities, however they should be frequently changed, since daily to once or twice weekly, because the dressing becomes soaked with exudate. Foams can be adherent or non-adherent, in the latter case it is necessary a secondary dressing to avoid shifting [66]. Nevertheless, they possess some drawbacks, for example they can dehydrate dry wound and also they are opaque and the wound visualization can be compromised. In addition, adhesive foams may be responsible for some cases of contact dermatitis [73].

Recently, several innovative composite foams have been developed. A new foam combining the attributes of volume filling and rapid coagulation of shape memory polymers (SMP) with the ability to swell and fill hydrogels has been developed by Landsman et al. [74]. This SMP polyurethane foam is coated with n-vinylpyrrolidone hydrogel (NVP) and polyethylene glycol diacrylate (PEGDA). In a new addition, this composite contains iodine in the form of a complex (PVP-I2 or povidone-iodine), widely used as a surgical antiseptic. The iodine-containing hydrogel gives the composite an antibacterial effect (reducing the viability of common bacteria by 80%) while increasing fluid uptake by 19 times over uncoated SMP foams. In another study by Namviriyachote et al. [75] polyurethane combined (PUC) foam dressings with various biomacromolecules (i.e. carboxymethylcellulose, chitosan, alginate, hydroxypropyl methylcellulose) were fabricated with the adsorption of asiaticoside and silver nanoparticles for traumatic wound treatment. The selected PU-alginate combined foam dressing adsorbed with asiaticoside and silver nanoparticles proved advantages for traumatic dermal wound management.A multilayer dressing consisting of polyvinyl alcohol foam (PVA) and electrospun sodium carboxymethyl cellulose (CMC) surface mesh was developed and characterized by He et al. [76] and co-workers. This composite was further loaded into the PVA foam, with the antimicrobial drug stearyl trimethyl ammonium chloride (STAC) for infection control and the CMC surface mesh offered an effective hemostatic function. Another study shows the potential of alginate-pectin composite foams with different blending ratios using calcium ion cross-linking [77]. In this study, the results suggest that controlling the pectin content in alginate-pectin foams is the key to adjust their mechanical properties, water absorption, and drug-release ability. Alginate-pectin composite foams showed to be promising candidates in different wound-dressing applications. A series of foams composed of PVA)/ alginate (PACFs) were prepared through a crosslinking reaction and lyophilization process [78]. The effect of different alginate content on the physicochemical properties and on the hemostatic function of the PACF was analyzed. The results showed that PACF absorbed plasma, but also stimulated blood cells, further promoting blood clotting, with therefore promising results as wound dressings.

Films

Films are thin, elastic and offer a barrier to microbial colonization [68]. Generally, commercial films are transparent, for example Transeal® (DeRoyal), Bioclusive® (Johnson & Johnson Medical), Mefilm® (Molnlycke Health Care), among others. A suitable wound-dressing film must have crucial properties, including capacity to absorb exudate, to regulate the moisture permeation, to maintain the moisture of the wound and to release the retained bioactive compound [79].

Films can be used in superficial wounds, namely burns, catheter sites and epidermal skin graft harvest sites, being suitable for minimally exudative wounds (Fig. 3) [68]. Films permit an environment for softening dry eschar by autolytic debridement, permit a protection from friction and contribute for pain reduction. A quantity of amorphous hydrogel may be added to the film in order to accelerate the autolytic debridement. These dressings may not be applied on wounds with heavy exudate. Films should be changed when exudate escapes onto intact skin or at a minimum of every 7 days [69].

Films are easy to use in wounds with different shapes, generally allowing for an easy wound visualization and flexibility to use as a primary or as a secondary dressing cover in alginates, foams and hydrogels [66]. Due to their adherence, films are easy to apply and do not need a second dressing [73]. On the other hand, they have non-absorbent characteristics that cause an accumulation of exudate and a maceration of wound edges, being necessary to change them frequently. The adhesive properties of films may potentially injury the skin, mostly in patients with delicate skin, such as elderly people and those with cutaneous atrophy. Therefore, in this case they should reduce the dressing changes to minimum or even avoid the use thereof [68].

Antimicrobial protection has been also addressed over the years and there are already films with this functionality in the market, such as 3 M™ Tegaderm™ CHG Chlorhexidine Gluconate I.V. Securement Dressings (3 M Healthcare) and Acticoat 7 (Smith and Nephew) that contains silver. In this sense Kim and coworkers [80], investigated a nitric oxide-releasing chitosan film. That film demonstrated a stronger antimicrobial activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus* and, simultaneously, the film accelerated wound healing and epithelization in a rat model. Nevertheless, innovative solutions are presently under development. Novel chitosan and cellulose acetate polymer composites were prepared by solvent-casting method [81]. The formed films were loaded with nanosized cerium oxide, and the results revealed to be promising as potential wound covering material. Alginate films containing pyrogenic silica supported silver nanoparticles were prepared via solid state sintering route without the use of any solvent and reducing agent [82]. Films exhibited antimicrobial and antibiofilm activities against *S. aureus* and *P. aeruginosa* and showed no cytotoxicity towards human skin keratinocytes and human fibroblasts HuDe, with promising evidences as wound dressing toward infected tissues. Novel adhesive composite films were prepared for mupirocin dermal delivery. Natural polymers as chitosan, sodium alginate and carbopol were used for films' development to evaluate possible interactions and the impaired drug release properties [83]. Solvent evaporation method was used for the films' preparation. The formulation was found more effective compared to the market product (Bactroban® cream) for wound healing at Balb-c mice, which highlights the potential for application as a wound care system.

In a different perspective a series of cross-linked films based on the combination of an elastin-derived biomimetic polypeptide (Human elastin-like polypeptide (HELP)) with alginate (ALG) were studied by Bergonzi et al. to obtain a composite with enhanced antioxidant properties [84]. ALG/HELP composite films loaded with the hydrophobic natural antioxidant curcumin were prepared by solvent casting method followed by the cross-linking with calcium chloride. The antioxidant activity correlated to the increase of HELP content, suggested the applicability of these composites as smart biomaterials for different biomedical applications.

Hydrocolloids

Hydrocolloids are moist wound dressings, that usually comprise a backing material (e.g. semi-permeable films, foams or non-woven polyester fibers) and a layer with hydrophilic/colloidal particles that may contain biocompatible gels made of proteins (e.g. collagen, gelatin) or of polysaccharides (e.g. cellulose and its derivatives) [72, 85]. Hydrocolloids are commonly primary dressings, biodegradable, non-breathable, and adherent to the skin, so that no separate taping is required. They are also waterproof, allowing regular water contact with skin.

Hydrocolloid dressings have been carefully addressed by Broussard et al. [66] in are view on wound dressings. The most commonly used are composed of a polyurethane external layer and an internal layer of a hydrophilic polymer such as gelatin e, pectin or carboxymethyl cellulose [86]. In their native stage they are impermeable to water, but once in contact with the wound exudate they are able to absorb it and form a gel, progressively more permeable to water vapour and air, which allows the excess of fluid to be removed without wound desiccation [87]. The moist conditions produced under the dressing and the control of the exudate are intended to promote fibrinolysis, angiogenesis and wound healing, to encourage the production of granulation tissue and to increase the quantity of synthesized collagen, leading to an increase on tissue regeneration, without causing softening and breaking down of the tissue [86]. On the other hand, these dressings also contribute for a better management of pain, due to the hydration enhancement, which will help the autolytic debridement, and will also provide a physical barrier to external microorganisms [86, 88]. Nevertheless, because these products are non-breathable theyare not recommended prior to infection control [87].

There are a great variety of commercially available hydrocolloid dressings such as Granuflex®, Aquacel™, Comfeel™, Tegasorb™, Exuderm®, Duoderm®, Ultec™ or Tegaderm™ and these are adequate solutions for both acute and/or chronic wounds, moist or dry, to form a semipermeable thin sheet and to produce a flat, occlusive and adhesive dressing [89]. These dressings are made in sheets that can easily be cut to fit the desired size or shape of ulcers, traumatic injuries, surgical wounds, graft donor sites, superficial wounds, and some burns without the need of separate taping [88]. Due to its diversity and availability at a relatively low cost, to introduce innovation on hydrocolloid dressings, becomes a difficult task. Hydrocolloid drug loading has been attempted by several authors [87, 89, 90] Thu et al. [87] developed a novel bilayer hydrocolloid film based on alginate, which was investigated as slow-release wound healing vehicle. The bilayer was composed of an upper layer impregnated with model drug (ibuprofen) and a drug-free lower layer, which acted as a rate-controlling membrane [87]. Successful results suggested that they can be exploited as slow-release wound dressings for low exudate wounds. In another study, novel chitosan (Ch) and hyaluronan (HA) wound dressings were developed loaded tiopronin and captopril as antiinflammatory drugs. Composite biomembranes were examined in skin wounds of ischemic rabbits to accelerate the process of healing. Data proved that the biomembranes composed of Ch/HA/tiopronin or Ch/HA/captopril facilitated healing of skin wounds compared to untreated animals and animals treated with Ch/HA membranes [91]. In a different report, a dressing based on PLGA and Aloe vera was developed containing lipid nanoparticles (NLCs). NLCs were added to prevent dressing from adhering to the wound and improve handling. Consequently, the PLGA-AV-NLC membrane promises to be a promising strategy for the treatment of chronic wounds, since it has improved handling compared to formulations without the lipid character of NLCs [92]. To overlap the adhesion loss of hydrocolloid wound dressings which seriously reduces the therapeutic efficiency and patient experience, hydrocolloid dressings were investigated using sodium carboxymethyl cellulose (CMC)-filled hydrocolloid dressings exposing to physiological environment as model. The results promoted the designing of hydrocolloid dressings with both excellent humidity control and sustained self-adhesiveness [93].

Hydrogels

Hydrogels are commonly defined as polymer three-dimensional networks that may be composed of crosslinked natural polymers (e.g. alginate, chitosan, gelatine, silk) or synthetic macromolecules (e.g. polyethylene glycol, polyvinyl alcohol) [94]. Hydrogels have been reviewed by Moura et al. [72] in a wound dressing report about diabetic wound healing and regeneration.Wound dressing hydrogels can be applied either as an amorphous gel or as an elastic film or solid sheet. These dressings usually require a secondary covering such as gauze, that need to be changed frequently, while hydrogel films or sheets may be used as both primary or secondary dressings [95].

Commercially available hydrogels (ActiformCool®, Stimulen™, Regenecare®, Intrasite Gel, Solosite Gel, Kendall™, 2nd skin®, Tegagel™) are flexible, rubbery and soft, nonreactive or irritant, biocompatible, and permeable to metabolites [96]. Typically, hydrogels are non-adherent and cool to the surface of the wound, which may lead to a better management of pain and therefore a better patient acceptability. Commonly they are suitable for cleansing of dry, sloughy or necrotic wounds by rehydrating dead tissues and enhancing autolytic debridement (Fig. 5). Nonetheless hydrogel dressings due to their high content of water (70–90%) are not suitable to be applied in heavily exuding wounds, once fluid accumulation can lead to skin maceration and bacterial proliferation.

The highly-hydrated network of a hydrogel can be held together via physical or chemical crosslinks, can be made biodegradable, and responsive to specific stimuli such as pH and temperature, and can be engineered to deliver therapeutic cells, drugs and soluble factors in a sustained and controlled way [97]. The success of application of hydrogels as a delivery system in wound healing will largely depend on biomimetic design and engineering, harnessing cell–material interactions in the cell fate and functions [97].

In Table 3 are summarized a few studies on advances in hydrogel formulation for wound healing and regeneration. Shukla et al. [98] studied an apigenin loaded hydrogel using gellan gum—chitosan with polyethylene glycol as a cross linker. Results proven that the prepared hydrogel seems to be highly suitable for wound healing due to adequate properties of biocompatibility, biodegradability, moist nature and antioxidant effectiveness. Agubata et al. [96] developed and evaluated wound healing hydrogels containing hydroxypropyl methylcellulose, ofloxacin and biodegradable microfibres from surgical sutures. These formulations promoted high collagen deposition after twenty-one days of wounding, with minimal scar formation. Evidences support the promising use of these hydrogels containing for effective wound healing.

Zeng et al. [99], developed injectable gelatin microcryogels which could load cells for enhanced cell delivery and cell therapy for wound healing. In this study, human adipose-derived stem cells (hASCs) laden in gelatine microcryogels, were instigated as primed injectable 3D micro-niches for a new cell delivery methodology for skin wound healing. Results showed wound bed recovery and a direct effect on wound basal layer for healing enhancement. Gong et al. [100] studied a biodegradable *in situ* thermosensitive hydrogel as a controlled drug delivery system composed of curcumin loaded polymeric micelles for successful cutaneous wound repair. Despite advances in the design and development of hydrogels it is still a challenge to develop a hydrogel with good stability and strong mechanical attributes for hemostasis and wound healing. In this sense, a recent study has developed a new polysaccharide hydrogel based of fenugreek gum-cellulose composite. A fenugreek gum was combined with cellulose through hydrogen bonding to form a hydrogel to improve the mechanical properties of the compound hydrogel [101]. Notably, hemostasis and wound healing have been confirmed, which highlights the promising medical potential of the compound hydrogel to promote wound healing [101]. The preparation of hydrogel-based materials with high antibacterial activities and good biosafety at the same time can also be challenging. In order to answer this crucial problem, Yang et al. [102] has developed a physical hydrogel composed of multifunctional chitosan/ carboxymethyl chitosan/ silver polyelectrolyte (CTS/ CMCTS/ AgNPs). A physical hydrogel composed and built by in situ photoreduction of silver ions with CMCTS α-hydroxy and acidification sol by semi-dissolving gel transition methods (SD-A-SGT) using natural polymers with no chemical reducer involved. This composite showed desired biosafety and antibacterial activities simultaneously, with great application potentials as wound dressing.

In a different point of view Lin et al,. studied the importance of anti-inflammation and angiogenesis in wound healing [103]. Therefore, the team developed a composite hydrogel dressing with stepwise delivery of diclofenac sodium (DS) and basic fibroblast growth factor (bFGF) to be applied in the inflammation stage and new tissue formation stage respectively for wound repair. The *in vivo* wound healing of rats revealed that this composite hydrogel showed a better healing effect with a wound contraction of 96% at 14 d, less inflammation and higher angiogenesis, than all control groups. This is promising data for hydrogel wound dressings [103]. Hamdi et al developed chitosan and protein isolate composite hydrogels, for carotenoids-controlled delivery and wound healing. The concentration of the protein isolate was increased to turn chitosan hydrogels more elastic, not exceeding 15% (w/w) of protein isolate concentration in thechitosan-protein isolate composite hydrogels revealed low cytotoxicity towards MG-63 osteosarcoma cells. The topical application of adhesives based on this hydrogel compound and enriched with carotenoids, allowed the acceleration of wound healing and complete regeneration being a promising new biomaterial [104].

4.2 Tissue Engineered Skin Substitutes and Advanced Wound Healing

Regenerative medicine is a recent but already widely accepted and expanding field involving the development and/or manipulation of molecules cells, tissues or organs to repair, replace or support injured body parts in order to recover their function [105]. Tissue engineering can be perceived now as among the available regenerative medicine strategies and can be defined as the science of persuading the body to heal itself through the supply of molecular signals, cells and scaffolds, to the adequate anatomic sites [106]. For skin regeneration, it essentially consists in expanding skin or stem cells, cultivating in a biomaterial support structure or scaffold, eventually combining biomolecules of interest such as growth factors, and then implanting the cell-scaffold construct for restoring the barrier function (initial step in burns) or for promoting wound healing (e.g., chronic wounds) [107].

This field has been assuming increasing clinical relevance due to the successful clinical tissue engineering-based products already available, namely for skin regeneration. Its clinically proven potential, associated to the limitation of the previously described technologies, allow tissue engineering, and regenerative medicine in general, to bring hope as solution for several clinical problems presently unsolved.

Tissue engineered skin substitutes, given their potential higher similarity to the natural skin tissue, are capable of overcoming several of the limitations previously described for skin grafts, namely donor shortage, and conventional wound dressings, namely undesirable adhesion to the lesion, impossibility associated to the difficulty in reproducing skin appendages and incapacity to replace the lost tissue, particularly the dermis [85, 108–110].

Tissue engineering has been particularly successful in the field of wound healing, and in particular for the treatment of burns and chronic wounds. This is actually the more mature and only area of application where several different products are already available, recurring to distinct strategies and with varied clinical success, ranging from non-cellularized products, composed of a biodegradable and porous polymeric matrix ready for implantation, to considerably sophisticated cellularized products, reproducing the compositional complexity of both dermis and epidermis

[111–113]. The biomaterial scaffold used can be produced using natural, synthetic or hybrid polymers and serves as a template for cell adhesion, proliferation and differentiation, playing a crucial role in guiding neo tissue morphogenesis [109, 114–116]. Additionally, natural skin healing can be stimulated through the incorporation, into these products, of a myriad of biomolecules such as genes, drugs, cytokines or growth factors [109, 111, 112].

Clinically available skin substitutes can be broadly divided into epidermal, dermal and dermo-epidermal products [109, 111, 117], although other categorization modalities exist. For regenerating superficial wounds, several commercial epidermal substitutes exist, using either autologous or allogeneic keratinocytes, namely MySkin® (CellTran, UK), consisting of a silicone layer seeded with autologous keratinocytes, Epicel® (Genzyme Biosurgery, USA), made of petrolatum gauze covered by autologous keratinocytes sheets, Epidex® (Eurodern, Switzerland), consisting of a silicone membrane cultured with autologous keratinocytes from the outer root sheath, and ReCell® (Avita Medical, Australia), where autologous keratinocytes are directly sprayed into the lesion [118–120]. Although generally providing an efficient epidermal coverage epidermal constructs present several limitations, namely long fabrication time due to the obtention and expansion of keratinocytes, difficult handling due to their fragile nature, variable engraftment rates and high cost [109, 120].

For the regeneration of full thickness wounds dermal tissue is required and mechanical stability is important to prevent wound contraction [109, 121]. For wound coverage dermal substitutes are usually associated to a permanent epidermal substitute using autologous split-thickness skin grafts or cultured epithelial autografts [19]. Available products in the market providing effective dermal regeneration include several cell-free products, such as Integra® (Integra LifeSciences, USA), a nanofibrous composite bilayer mesh composed of crosslinked collagen and glycosaminoglycan layer and a semi-permeable polysiloxane layer, Hyalomatrix® (Anika Therapeutics, USA), a bilayered hyaluronic acid-based scaffold covered with a silicone sheath, Matriderm® (MedskinSolutions, Germany), a composite collagen and elastin scaffold, and AlloDerm® (Lifecell, USA), a donated human dermis processed to remove cells, as well as cellularized scaffolds containing fibroblasts, such as Dermagraft® (Organogenesis, USA), a cryopreserved human fibroblast-derived dermal substitute, generated by the culture of neonatal dermal fibroblasts onto a bioresorbable poly(lactic-co-glycolic acid) (PLGA) mesh scaffold [109, 120, 122, 123].

Dermal-epidermal substitutes are the most advanced tissue engineered currently available in the clinics since they mimic both skin layers (dermis and epidermis) for full skin regeneration. Dermal-epidermal substitutes available in the market include PermaDerm® (Regenicin, USA), constituted by a biodegradable collagen scaffold cultured with autologous fibroblasts and keratinocytes, and Apligraf® (Organogenesis, USA), a composite bi-layered product composed of two distinct nanofibrous layers, being the lower dermal layer constituted by bovine type I collagen cultured with human allogeneic fibroblasts and the upper epidermal layer constituted by cultured human allogeneic keratinocytes [109, 111]. Although providing more effective regeneration of full-thickness skin defects than conventional treatments, these products still present several limitations, namely inefficient wound closure due to rejection of allogeneic cells, high production costs [109].

Overall, tissue engineered skin substitutes present several advantages when compared with conventional treatments, including faster regeneration, increased dermal component in the healed wound, lower vascularization requirements, and reduced presence of inhibitory factors [124]. However, they still also present several limitations as previously pointed out, including lack of skin appendages, such as hair follicles, sebaceous glands and sweat glands, poor cell, inefficient vascularization, wound contraction, fibrosis, scarring at graft margins, use of animal-derived serum in cell culture, and high manufacturing costs [109, 112, 120, 124–126].

To overcome these limitations, several advanced skin regeneration strategies are under development in order to address both the fundamental issues underlying the limited understanding of the phenomena involved, as well as the technological barriers inhibiting their implementation. For instance, in order to promote the formation of skin appendages recent studies explore the culture of specific cells, including stem cells, such as Schwann cells, hair follicle cells, or melanocytes into scaffolds [113, 127]. Other strategies explore the used of advanced fabrication technologies, such as electrospinning or 3D printing, to fabricate scaffolds combined with cells and adequate biomolecules with improved complexity in terms of compositional and architectural biomimicry and providing better control over cell seeding [113, 128–132].

5 Sensing the Wound

The management of chronic wounds can greatly benefit from sensing tools able to predict in real time the need for a specific therapeutic intervention and whether the therapy is working or not. Adding diagnostic and theranostic sensors to wound management is an exciting possibility. The immediate benefits for the clinicians and patients are obvious: an increase of the treatment efficiency, the reduction of treatment time, and in extreme cases, lowering the risk of amputation. On the other hand, sensing the wound can give us new insights about the series of complex biochemical events related to the healing and regeneration process, contributing for a better wound assessment.

5.1 Detectable Biomarkers

Research on biomarkers for the assessment of wound status is of extreme relevance. However, this is a slowly progressing field due to the difficulty on isolating specific biochemical and physiological events that could be used to represent each wound

Table 4 Progress on biomarkers for wound healing Adapted from WUWHS [134]

Main classes of biomarkers (as identified in 2007 consensus meeting)
• Bacterial load/specific microbial species/biofilms • Cytokine release in response to specific microbial antigens • DNA—e.g. gene polymorphisms • Enzymes and their substrates—e.g. matrix metalloproteinases and extracellular matrix • Exposed bone • Growth factors and hormones—e.g. platelet-derived growth factor • (PDGF), sex steroids (androgens/oestrogens), thyroid hormones • Immunohistochemical markers—e.g. integrins, chemokine receptors and transforming growth factor beta II receptors • Inflammatory mediators—e.g. cytokines and interleukins • Nitric oxide • Nutritional factors—e.g. zinc, glutamine, vitamins • pH of wound fluid • Reactive oxygen species • Temperature • Transepidermal water loss from periwound skin
Newly identified biomarkers
• Uric acid • Glucose • H_2O_2

event [133]. Almost a decade ago Harding et al. [134] have gathered in a consensus meeting for discussing the progress of wound monitorization and have generated a list of potential wound markers, which served as basis to the studies in the following years. More recently other markers have been identified and added to this list, as summarized in Table 4.

Some of these markers have been recently gaining importance while others have led to contradictory results and difficulties upon their detection. Recently, Dragaville et al. [135] reviewed the state-of-the-art on some of the most effective markers, either embedded in dressings or as point-of-care (POC) techniques for wound assessment and monitoring. These include temperature, oxygen, bacteria, pH and biochemical signals.

Among the different wound types, chronic non-healing wounds have been particularly studied for biochemical markers, through several clinical investigations [136, 137]. Proteases, protease inhibitors, and pro-inflammatory cytokines are presently under study either locally at the wound site and/or systemically using high-throughput screening (metabolomic, proteomic, genomic and lipidomic analysis) [138]. Of these, proteases (serine, metalloproteinases, cysteine, aspartic) and specifically matrix metalloproteinases have received the most attention in studies of chronic wounds, showing great potential as targets for wound assessment [137, 139]. In a recent review by Lindley et al. [138], some steps are proposed for the validation and implementation of these clinically applicable biomarkers, including those measured in tissue (ex. β-catenin), wound fluid (matrix metalloproteinases and interleukins), swabs, wound microbiota, and serum (ex. procalcitonin and matrix metalloproteinases).

The ongoing research on the above mentioned biomarkers or on others certainly discovered in the future gains more relevance when sustained in the idea of developing appropriated sensor tools. Since a wound is a dynamic environment, there is a strong need to develop systems that can diagnose the wound parameters in a minimally invasive way and report continuously on the type of environment inside the wound. Ideally it would consist of individual or combined sensors for pH, temperature, humidity, oxygen, bacteria sensors, etc [135, 140]. To monitor these parameters is not a difficult task, however the generated information needs to be precisely correlated to the events taking place in the wound bed. For instances a pH variation can occur following an inflammatory process or due to an infection [141, 142]. On the other hand, variations in wound pH will influence proteolytic activity and oxygen content and so measurements of enzyme activity may not be relevant unless correlated with pH [142].

5.2 Multicomponent Biosensor Dressings

The combination of sensors and dressings with active properties is nowadays considered as the gold standard, although numerous challenges still need to be overcome. A biosensor integrated within the dressing should be able to detect low levels of a certain biomarker (ex: resulting from a bacterial contamination) and consequently emit a recognizable output indicative of infection risk. Ideally this smart sensor should then trigger a material response towards a therapeutic effect, e.g., the controlled release of a pre-loaded drug. This could be achieved by integrating switchable surfaces or stimuli-responsive materials into the dressingto generate smart composites [135, 140, 143, 144]. At the same time the sensor should provide information about different parameters indicative of the status of the wound, in particular pH, temperature, moisture, and exudate production, etc. In doing so, these smart dressings will help shifting the paradigm of chronic wound care from routine management and time-based dressing changes toward cost effective personalized care and knowledge-based treatment [140, 144].

There is a significant effort in the research community to develop near-patient or wearable devices to enable wound care professionals to objectively measure the wound status. There have been advances in monitoring moisture [145–147], pH [145, 146], oxygen, protease [140, 144], and bacterial load [142, 146, 148, 149]; however, only a few of these systems are available for commercial use. At the present time, it is striking how few wound care devices have made it into clinical use. Recent advances on sensor research in wound monitoring have been made mostly on generic physiological status indicators such as, moisture [144, 145], pH [142, 146], oxygen [142, 150], and bacterial load [148, 149] and temperature.

In the case of moisture sensors, only slight advances have been made. A sensor to measure moisture content has been commercialized by Ohmedics Ltd (Glasgow, UK) following research on the moisture status of advanced wound dressings [146]. The WoundSense™ device is a disposable moisture sensor, suitable for use in any dressing and allows moisture monetarization without the need to disturb the dressing. Recently, Milne et al. [140] have reported the first large-scale observational study using this system. The results suggest that a large number of unnecessary dressing changes are being made, with disturbance of the wound bed and impact on healing and costs associated.

Presently, a considerable attention is being paid to wound pH monitoring, as it affects fibroblasts/keratinocytes activity, microbial proliferation and oxygen release to the tissues, altering the immune response of the wound [142]. As reported in the literature [151], the pH of healthy human skin is in the range of 4.0–7.0. In chronic venous leg ulcers and in pressure ulcers, an increase in pH (i.e. alkaline or neutral pH) is a sign of infection, if compared with the normal surrounding skin. Although the ideal pH sensor is yet to be discovered, there have been considerable developments in recent years, mostly in response to the limitations of the traditional glass potentiometric system related to its fragility and inability to measure multiple wound regions simultaneously and continuously [143]. These only provide localized measurements and are not feasible for complex measurements across the surface of a heterogeneous wound surface. Other strategies have been explored over the last years for continuous monitoring of the pH, either through electrochemical [148, 149, 151] or colorimetric methods [152, 153]. Electrochemical sensors measure the concentration of H^+ ions based on the rate of electrochemical reactions. Recently, Sharp et al. [150]. has proposed a version of printed electrodes on flexible acetate sheets that incorporate uric acid for monitoring wound pH. This sensor can detect pH in a broad range, from 4.0 to 10. Guinovart et al. [148]have modified a commercial adhesive bandage to create a pH sensor by screen printing Ag/AgCl and carbon electrodes onto it. This wearable pH sensor has shown to detect variations between 5.5 and 8 of pH values. More recently Rahimi et al. [149] have developed an inexpensive flexible array of pH sensors fabricated on a polymer-coated commercial paper, to be integrated in low-cost dressings as a way to map the pH at various wound sites. Another approach that has attracted noticeable attention is the use of pH sensitive materials and dyes for detection of skin pH [138, 140, 152, 153]. These colorimetric sensors are usually easy-to-read and can be utilized without integrated electronics. However, a key challenge for fabrication and use of these systems is to prevent the dye from leaching out of the dressing onto the skin. In addition, the sensitivity of the dye should cover the entire range of pH variation observed in skin disorders and wounds (pH = 4–9). Recently Tamayol et al. [153], developed a composite hydrogel alginate-based microfibers containing mesoporous particles loaded with a pH-responsive dye. T fabricated pH-responsive microfibers were flexible and able to maintain contact with skin, minimizing the leakage of the dye from the fibers. Non-invasive luminescence imaging is also of great interest for studying biological parameters in wound healing. Schreml et al. [154] have developed the first method for 2D luminescence imaging of pH *in vivo* on humans. More recently they have described a sprayable luminescent pH sensor able to be applied to very uneven wound tissues [155].

Oxygen is one of the critical factors regulating the wound healing process [156], Acute hypoxia can cause tissue loss in a chronic wound and negatively impact the wound healing process [157]. Thus measuring oxygen concentration in real-time can be an effective tool for monitoring wound status. However, this parameter has only recently gained attention. Mostafalu et al. [158] have created a localized 3D-printed smart wound dressing platform that enables real-time data acquisition of oxygen concentration. The bandage contained a flexible oxygen sensor in a compact package, incorporating a series of off-the-shelf electronic components including a programmable-gain analog front-end, a microcontroller and wireless radio, an integrated electronic system with data readout and wireless transmission capabilities. This flexible platform can allow for a self-operating remote therapy for chronic wounds.

Uric acid (UA) concentration in wound exudate is another key marker which is recently being explored as specific indicator of wound status and infection since it is highly correlated with wound severity [159] and significantly decreases during bacterial infection [160]. Kassal et al. [161] described a new type of smart bandage for determination of uric acid (UA) status, by screen printing an amperometric biosensor directly on a wound dressing. The smart bandage biosensor interfaces with a wearable potentiostat for on-demand wireless data transfer to a computer, tablet, or Smartphone.

As described in this chapter, chronic wounds are extremely susceptible to infection, as the first defense barrier, the skin, is disrupted allowing for bacteria to invade the underlying tissue. Due to the clinical relevance of this problem to diagnose infection on a dressing at early stages and preferably, without its removal is a critical landmark. As herein referred, many of the above described sensors are used in dressings for detecting bacterial infection. But other strategies have been recently proposed, exploring different biochemical markers. For example, in a study by Krismastuti et al. [162] a porous anodized aluminum oxide (pAAO) based biosensor was developed as a biosensing platform to detect proteinase K, an enzyme which is a readily available model system for the proteinase produced by *P. aeruginosa*. As a proof-of-concept, this platform was successfully tested with human wound fluid, highlighting the potential for detection of bacterial infections in chronic wounds. Hajnsek and coworkers [163] developed an electrochemical sensor for fast detection of wound infection based on the quantification of myeloperoxidase activity as a marker for bacterial infection.

The use of carbon fibre tow as an electrochemical sensing matrix for assessing pyocyanin production, a substance produced by *P. aeruginosa* as a result of quorum sensing during wound colonization, was proposed by Sharp et al. [164]. The proposed small and inexpensive sensor assembly is suggested for use in monitoring *P. aeruginosa* growth. Ciani et al. [165] have reported the design and characterization of an electrochemical biosensor system and impedance detection method capable of the multiparameter detection of TREM-1 (Triggering Receptor-1 Expressed on

Myeloid cells), MMP-9 (Matrix MetalloPeptidase 9) and HSL (N-3-oxo-dodecanoyl-l-HomoSerineLacton), relevant in bacterial quorum sensing. These antigens are used without amplification and with minimal pre-analytical requirements on screen printed electrodes (SPEs), which are cheap, commercially available.

Temperature is an important parameter to assess chronicity, is frequently used for monitoring at-risk patients and anticipate ulceration [166]. However, there is little research using temperature sensors embedded in wound dressings. This can be achieved by incorporating miniaturized wireless sensors within wound dressings, as proposed by Matzeu et al. [167]. They fabricated a sensor using multiwall carbon nanotubes and electrodes produced by electroplating nickel and gold over the copper tracks prepared through a lithography process. Due to the wireless communication ability, this system can be used under a bandage or a wound dressing for minimally invasive, remote monitoring of temperature.

Most of the current methods for incorporating biosensors in dressings to monitoring chronic wounds have been independently developed and therefore are predominantly single-parameter. However due to the complex, multi-stage progression of wound healing the development of wearable integrated systems with sensors and readout telectronics is a major goal in the continuous monitoring of a chronic wound. Some steps are being done in this integrative approach. For instances, Mehmood et al. [167] presented a low-power portable telemetric system for measuring and transmitting real-time information of wound-site temperature, sub-bandage pressure and moisture level from within the wound dressing. Wang et al. [168] reported the use of a sprayable and thermogelating biomaterial (Poloxamer TM; a.k.a. Pluronic) in optical imaging of pH values, local oxygen and temperature. The polymer sensor particles containing molecular probes (such as for sensing O, pH or temperature) are incorporated in the host material, and the resulted sensor cocktail was sprayed onto surface of interest at low temperature. On increasing temperature, the sprayed thin film forms a gel and tightly adheres to form a stable sensor film. Two of the most relevant fluctuating wound parameters during the healing process which are pH and glucose concentration. Jankowska et al. [169] presented a fluorescent sensing system to monitor the wound status and to distinguish between an autonomously healing and a chronic wound at an early stage. The system allows monitoring simultaneously pH and Glucose using a fluorescent pH indicator dye, carboxynaphthofluorescein, and a metabolite-sensing enzymatic system, based on glucose oxidase and horseradish peroxidase, immobilized on a biocompatible polysaccharide matrix.

6 Future Perspectives

Chronic wounds have a deep impact in patient's health, social life, and it means an economical general burden, being therefore a current topical interest worldwide. In this chapter, we have focused on the current state of the art in chronic wound healing medicines involving the active treatment of these wounds. The evolution of the different advanced wound dressings, skin substitutes and available commercially products, have been discussed, highlighting the advantages of combining materials, bioactive compounds and sensors to better face the different stages of the wound. The exact moment of deciding which dressing should be used remains controversial and the existing medical literature is not helpful. Only a few, ifany, prospective randomized control trials conclusively prove the superiority of one type ofwound dressing over another [170]. Therefore, more wound care research providing level A evidence is needed. Nevertheless, in daily clinics, the decisions need to be taken and all strategies attempt to achieve thesame goal: the successful healing.

The chapter covered many advanced wound dressings, including several types of polymeric systems and its composites in the form of foams, films, hydrogels and hydrocolloids for wound healing and tissue-engineered skin substitutes, dressings containing (antibiotics, silver, stem cells, etc). Although there is an enormous diversity of solutions, challenges still remain in tackling the problems associated with chronic wounds, and it is clear that one single advanced dressing does not always address the problems encountered in chronic wounds. Therefore, a combination of the above-mentioned advanced systems will be required, which impliesthat there is no single perfect dressing for allwounds. Nevertheless, the increasingly advanced biomaterials and their composite systems, creates favourable conditions for: better management and retention of the exudate; better adjustment to certain anatomical sites, not limiting the mobility of its users; better ease of removal of material for visual inspection, exchange of material for suspected infection; possibility to monitor parameters such as the pH or temperature.

The future of dressings points to the "interaction" between the material and user, as well as between the material and the clinician. It is not always easy to achieve or control all the variables involved in the process, because the dressing is optimised to control one or two needs, being optimal for the purpose of its design hence the professional's assessment of the best material for the priority variable to be controlled at that time. The future will bring possibilities of monitoring, at the wound bed, important events such as:an infection or exudate increase.

As a concluding remark there is no single ideal dressing code for wound healing. In the future it seems crucial to address advanced multi-component dressings that will tackle the problems of chronic wounds such as: pain, inflammation, odour, infection, delayed healing, and associated costs to health systems and populations worldwide. Therefore, a multi-targeted approach seems to be the best way toward wound care, which also should include a more detailed comprehension by the health professionalson the use of these advanced dressings and their recommendations.

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