Materials Horizons: From Nature to Nanomaterials

S. Snigdha
Sabu Thomas
E. K. Radhakrishnan
Nandakumar Kalarikkal *Editors*

Engineered Antimicrobial Surfaces

Springer

Materials Horizons: From Nature to Nanomaterials

Series Editor

Vijay Kumar Thakur, School of Aerospace, Transport and Manufacturing, Cranfield University, Cranfield, UK

Materials are an indispensable part of human civilization since the inception of life on earth. With the passage of time, innumerable new materials have been explored as well as developed and the search for new innovative materials continues briskly. Keeping in mind the immense perspectives of various classes of materials, this series aims at providing a comprehensive collection of works across the breadth of materials research at cutting-edge interface of materials science with physics, chemistry, biology and engineering.

This series covers a galaxy of materials ranging from natural materials to nanomaterials. Some of the topics include but not limited to: biological materials, biomimetic materials, ceramics, composites, coatings, functional materials, glasses, inorganic materials, inorganic-organic hybrids, metals, membranes, magnetic materials, manufacturing of materials, nanomaterials, organic materials and pigments to name a few. The series provides most timely and comprehensive information on advanced synthesis, processing, characterization, manufacturing and applications in a broad range of interdisciplinary fields in science, engineering and technology.

This series accepts both authored and edited works, including textbooks, monographs, reference works, and professional books. The books in this series will provide a deep insight into the state-of-art of Materials Horizons and serve students, academic, government and industrial scientists involved in all aspects of materials research.

More information about this series at http://www.springer.com/series/16122

S. Snigdha · Sabu Thomas · E. K. Radhakrishnan · Nandakumar Kalarikkal
Editors

Engineered Antimicrobial Surfaces

Springer

Editors
S. Snigdha
International and Inter University
Centre for Nanoscience
and Nanotechnology (IIUCNN)
Mahatma Gandhi University
Kottayam, Kerala, India

E. K. Radhakrishnan
School of Biosciences
Mahatma Gandhi University
Kottayam, Kerala, India

Sabu Thomas
Centre for Nanoscience and Nanotechnology
Mahatma Gandhi University
Kottayam, Kerala, India

Nandakumar Kalarikkal
Mahatma Gandhi University
Kottayam, Kerala, India

ISSN 2524-5384 ISSN 2524-5392 (electronic)
Materials Horizons: From Nature to Nanomaterials
ISBN 978-981-15-4629-7 ISBN 978-981-15-4630-3 (eBook)
https://doi.org/10.1007/978-981-15-4630-3

This Springer imprint is published by the registered company Springer Nature Singapore Pte Ltd.
The registered company address is: 152 Beach Road, #21-01/04 Gateway East, Singapore 189721, Singapore

Preface

The alarming increase in incidences of resistance of microorganisms to antibiotics has created a great demand for alternative modes of combating harmful microbes. This decade has witnessed a great increase in the research and development of antimicrobial substances and surfaces, which can aid in curtailing the resistant microorganisms. This book aims to present the recent and trending developments in this dynamic field to a wide readership. In this book, we present the various techniques used to achieve effective and lasting antimicrobial activity, methods to analyse the efficacy of such surfaces and how these surfaces affect the target microorganisms. The book will also try to evaluate the possible effects on the environment created by such engineered surfaces. In brief, this book will prove to be a cutting-edge multidisciplinary book specifically focused on engineered antimicrobial surfaces and its allied fields. This book will be a very useful reference source for graduates and post graduates, engineers, research scholars (primarily in the fields of material science, microbiology, polymer chemistry, biotechnology and tissue engineering, nanoscience and nanotechnology and the medical field), material scientists, polymer engineers and polymer technologists from industries. Great opportunity lies in the future for developing new and reliable antimicrobial surfaces. These antimicrobial materials find applications in every field imaginable such as biomedical area, environmental remediation and agriculture.

I take this opportunity to introduce the book titled *Engineered Antimicrobial Surfaces*. This is an edited book which provides insights into the world of research where alternates or supplements to conventional antibiotics are being innovated. Antibiotic resistance is a major problem in developed as well as underdeveloped countries. This has enabled the development of various strains of multiple drug-resistant pathogens, which are capable of resisting even the last resort antibiotics. This has elevated microbial infections to the top five causes of global mortality. This alarming trend has led to increased incorporation of antimicrobial materials to various material applications. The current trend is to incorporate materials with multiple modes of action against microorganisms, which will reduce the chances of microorganisms developing resistance.

The book is divided into seven chapters that cover some of the leading problems and research areas associated with antimicrobial technology.

Chapter 1 discusses the need for developing antimicrobial materials and surfaces for combating the increasing microbial resistance that is being spread worldwide. The chapter is titled "The Need for Engineering Antimicrobial Surfaces" and explains why it is crucial for researchers to deal with the antibiotic strains of bacteria. This chapter provides an overall introduction to the book.

Chapter 2 is titled "A Thirst For Polymeric Antimicrobial Surfaces/Coatings For Diverse Applications" and explains in detail the worldwide status in terms of antimicrobial coating development and various means used to achieve the desired results.

Chapter 3 details the potential sites that can be targeted by antimicrobial surfaces and materials. The chapter is titled "Potential Target Sites That are Affected by Antimicrobial Surfaces", and the role and structure of these sites in designing antimicrobial surfaces are discussed in detail.

Chapter 4 is "Carbon Nanotube-based Antimicrobial and Antifouling Surfaces" and discusses the use of CNT in the design of antimicrobial and antifouling surfaces.

Chapter 5 describes the use of phyllosilicate clay minerals for supporting antimicrobial materials and the enhanced activity of these hybrid materials. The chapter is titled "Engineered Phyllosilicate Clay Based Antimicrobial Surfaces" and discusses montmorillonite and laponite based hybrid materials.

Chapter 6 "Antimicrobial Metal-based Nanomaterials and Their Industrial and Biomedical Applications" provides insights into the field of antimicrobial metals for industrial and biomedical applications.

Chapter 7 describes the importance of surface engineering for producing antimicrobial surfaces. It is titled "Modulating Surface Energy and Surface Roughness for Inhibiting Microbial Growth". It is of utmost importance to pay attention to surface properties while designing microbicidal surfaces.

Chapter 8 titled "Potential Environmental Effects of Engineered Antimicrobial Surfaces" looks at the potential dangers to the environment that could be caused as a result of artificially engineered surfaces with microbicidal activity.

The highly specific nature of the book would prove to be very useful for material scientists and biologists who are working to curb the spread of infectious bacteria.

Kottayam, India

S. Snigdha
For Editors

Contents

Editors and Contributors

About the Editors

S. Snigdha completed her Ph.D. at the International and Inter University Centre for Nanoscience and Nanotechnology (IIUCNN), at Mahatma Gandhi University, Kottayam, India, where her research focused on nanostructured materials for microbiological applications.

Sabu Thomas is currently Professor and Pro-Vice Chancellor at Mahatma Gandhi University, Kerala, India. Prof Thomas' research has spanned many areas of nanocomposite and polymer science and engineering, and he has edited more than 70 books, holds 5 patents and has authored over 750 research publications.

E. K. Radhakrishnan is an Assistant Professor in the School of Biosciences at Mahatma Gandhi University, Kerala, India. His research focuses on biological and metabolic processes in bacteria, bioactive compound synthesis, biofilm formation, metal nanoparticles synthesis and thin films for antimicrobial applications.

Nandakumar Kalarikkal is Professor & Head of the Advanced Materials Laboratory in the School of Pure and Applied Physics as well as the Honorary Director of IIUCNN at Mahatma Gandhi University, Kerala, India. His research focuses on the synthesis, characterization and applications of nanomaterials, phase transitions, and the effect of ion irradiation on novel materials.

Contributors

M. I. Abou-Dobara Botany and Microbiology Department, Faculty of Science, Damietta University, Damietta, Egypt

A. B. Arun Yenepoya Research Centre, Yenepoya (Deemed to Be University), Deralakatte, Mangalore, India

M. Gomes LEPABE - Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Porto, Portugal

Manohara Dhulappa Jalageri Department of Chemistry, Manipal Institute of Technology, Manipal Academy of Higher Education, Manipal, India

Nandakumar Kalarikkal International and Inter University Centre for Nanoscience and Nanotechnology, Mahatma Gandhi University, Kottayam, Kerala, India;
School of Pure and Applied Physics, Mahatma Gandhi University, Kottayam, Kerala, India

B. N. Kumara Nanomaterial Research Laboratory (NMRL), Nano Division, Yenepoya Research Centre, Yenepoya (Deemed to Be University), Deralakatte, Mangalore, India

Sasmita Majhi Materials Science and Engineering, Indian Institute of Technology Gandhinagar, Palaj, Gandhinagar, Gujarat, India

Pooyan Makvandi Institute for Polymers, Composites, and Biomaterials (IPCB), National Research Council (CNR), Naples, Italy;
Department of Chemistry, College of Science, Shahid Chamran University of Ahvaz, Ahvaz, Iran

F. J. Mergulhão LEPABE - Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Porto, Portugal

Abhijit Mishra Materials Science and Engineering, Indian Institute of Technology Gandhinagar, Palaj, Gandhinagar, Gujarat, India

Akshatha Nagaraja Department of Chemistry, Manipal Institute of Technology, Manipal Academy of Higher Education, Manipal, India

A. Nikhitha Yenepoya Research Centre, Yenepoya (Deemed to Be University), Deralakatte, Mangalore, India;
Department of Dermatology, Yenepoya (Deemed to Be University), Mangalore, Karnataka, India

N. F. Omar Botany and Microbiology Department, Faculty of Science, Damietta University, Damietta, Egypt

K. Sudhakara Prasad Nanomaterial Research Laboratory (NMRL), Nano Division, Yenepoya Research Centre, Yenepoya (Deemed to Be University), Deralakatte, Mangalore, India;
Yenepoya Research Centre, Yenepoya (Deemed to Be University), Deralakatte, Mangalore, India;
Centre for Nutrition Studies, Yenepoya (Deemed to Be University), Deralakatte, Mangalore, India

Yashoda Malgar Puttaiahgowda Department of Chemistry, Manipal Institute of Technology, Manipal Academy of Higher Education, Manipal, India

E. K. Radhakrishnan School of Biosciences, Mahatma Gandhi University, Kottayam, Kerala, India

K. Sapna Nanomaterial Research Laboratory (NMRL), Nano Division, Yenepoya Research Centre, Yenepoya (Deemed to Be University), Deralakatte, Mangalore, India;
Yenepoya Research Centre, Yenepoya (Deemed to Be University), Deralakatte, Mangalore, India

Manjunath M. Shenoy Department of Dermatology, Yenepoya (Deemed to Be University), Mangalore, Karnataka, India

S. Snigdha International and Inter University Centre for Nanoscience and Nanotechnology, Mahatma Gandhi University, Kottayam, Kerala, India

J. Sonia Nanomaterial Research Laboratory (NMRL), Nano Division, Yenepoya Research Centre, Yenepoya (Deemed to Be University), Deralakatte, Mangalore, India

R. Teixeira-Santos LEPABE - Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Porto, Portugal

Sabu Thomas International and Inter University Centre for Nanoscience and Nanotechnology, Mahatma Gandhi University, Kottayam, Kerala, India;
School of Chemical Sciences, Mahatma Gandhi University, Kottayam, Kerala, India

Ehsan Nazarzadeh Zare School of Chemistry, Damghan University, Damghan, Iran

Chapter 1
The Need for Engineering Antimicrobial Surfaces

S. Snigdha, Nandakumar Kalarikkal, Sabu Thomas, and E. K. Radhakrishnan

1 Introduction

According to the World Health Organisation (WHO) global health estimate, "half of all deaths in low-income countries in 2016 were caused by the so-called Group I conditions, which include communicable diseases, maternal causes, and conditions arising during pregnancy and childbirth, and nutritional deficiencies" (Fig. 1) [1]. The WHO and Centre for Disease Control (CDC) have expressed deep concerns over the widespread increase in the number of multidrug-resistant pathogens. Increased microbial resistance is a prevailing global crisis. Drug abuse, lack of development of new antibiotics, and the time-consuming process to market a new drug are major stumbling blocks in treating infectious diseases [2]. The alarming number of infection-associated deaths demands attention for improving and developing highly effective multi-targeted antimicrobial systems. Highly contagious and resilient bacteria such as *Staphylococcus aureus* tend to survive on inanimate objects indefinitely. Such materials cannot be protected by the use of antibiotics, which can further accentuate the antibiotic crisis. These materials can be packaged/coated aseptically by using nanomaterials with multiple modes of actions.

S. Snigdha · N. Kalarikkal · S. Thomas
International and Inter University Centre for Nanoscience and Nanotechnology, Mahatma Gandhi University, Kottayam, Kerala 686560, India

E. K. Radhakrishnan (✉)
School of Biosciences, Mahatma Gandhi University, PD Hills (PO), Kottayam, Kerala 686560, India
e-mail: radhakrishnanek@mgu.ac.in

S. Thomas
School of Chemical Sciences, Mahatma Gandhi University, Kottayam, Kerala 686560, India

N. Kalarikkal
School of Pure and Applied Physics, Mahatma Gandhi University, Kottayam, Kerala 686560, India

S. Snigdha et al. (eds.), *Engineered Antimicrobial Surfaces*, Materials Horizons: From Nature to Nanomaterials, https://doi.org/10.1007/978-981-15-4630-3_1

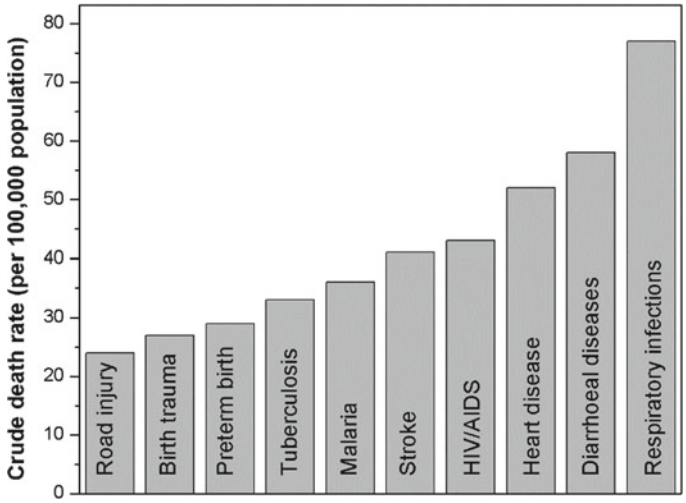


Fig. 1 Top ten causes of death in low-income countries Adapted from: Global Health Estimates, 2016, Deaths by cause, age, sex, by country, and by region, 2000–2016, Geneva, World Health Organisation 2018

The significance of research and development for antibiotic substitutes or supplements can be highlighted upon inspecting the global priority list of antibiotic-resistant bacteria published by the World Health Organization (WHO) in February 2017 [3]. The press released lists of 12 families of bacteria that present the greatest risk to human health. These bacteria are listed below:

Priority 1: (critical)

- *Acinetobacter baumannii*, carbapenem-resistant
- *Pseudomonas aeruginosa*, carbapenem-resistant
- *Enterobacteriaceae*, carbapenem-resistant, ESBL-producing

Priority 2: (high)

- *Enterococcus faecium*, vancomycin-resistant
- *S. aureus*, methicillin-resistant, vancomycin-intermediate and resistant
- *Helicobacter pylori*, clarithromycin-resistant
- *Campylobacter* spp., fluoroquinolone-resistant
- *Salmonellae*, fluoroquinolone-resistant
- *Neisseria gonorrhoeae*, cephalosporin-resistant, fluoroquinolone-resistant

Priority 3: (medium)

- *Streptococcus pneumoniae*, penicillin-non-susceptible
- *Haemophilus influenzae* , ampicillin-resistant
- *Shigella* spp., fluoroquinolone-resistant

The severe threat from these bacteria is due their resistance to known antibacterial drugs and the more dangerous strains tend to be resistant to multiple drugs, thereby being potentially untreatable. The WHO has called for various organisations to support research that aims at developing new antibacterial agents against the listed deadly bacterial strains.

2 Design of Antimicrobial Surfaces

Microorganisms tend to preferentially migrate towards and colonise a solid surface. This adherence and proliferation on a solid surface results in microbial biofilm formations which can be observed in a number of natural environments (soils, aquatic ecosystems, food sources), biological tissues, industrial settings, water piping systems, and on medical implants and instrumentation [4]. There is a great interest in finding strategies to inhibit biofilm formation as microbial biofilm causes trauma and economic loses [5, 6]. The biofilm forming organisms have been extensively studied, and it has been established that they pose great threat. However, the tremendous resistance of biofilms to conventional antibiotic therapy has led to research on synthetic surfaces and coatings that resist bacterial colonisation [7]. Controlling the topography and hydrophobic properties of materials surfaces can influence bacterial interaction with the surface and must be taken into account when developing novel, anti-infective biomaterials. Such surfaces can by synthesised using chemical approaches or physical methods. Chemical modification, derivatistion, functionalisation, or coating with microbicidal material such as nanoparticles, polymers, antibiotics, among others form the basis of chemical approaches to antimicrobial design. The chemical and coating methods are limited by the possibility of development of antimicrobial resistance against the coated antibiotic and possibility of toxicity [8, 9]. The physical methods bring about changes in the materials surface topography to prevent microbial colonisation. High aspect ratio antimicrobial surfaces utilise naturally occurring, surface chemistry-independent, physico-mechanical mechanisms to control microbial growth [10, 11]. These engineered surfaces are sustainable and safe alternatives for preventing biofilm formation. The surface structure and microbial attachment are found to depend on surface roughness, smoothness and grain size, nano-patterning, or nano-architecture, other surface characteristic variables such as geometry, size, height, periodicity, aspect-ratio, surface irregularity, and substrate chemistry [12–15]. Various studies indicate that the micro-/nano-topography of the surface plays a critical role in determining microbial attachment on the substrate. An in-depth understanding of such variables could aid in the manufacture of need-based antimicrobial surfaces that can minimise or prevent the formation of biofilms or for contact killing of microorganisms [16, 17]. Various factors that contribute to successful engineering of anti-infective surfaces are shown in Table 1.

Table 1 Factors affecting design of antimicrobial surfaces

Properties	Factors	References
Surface morphology	Macroporosity, microporosity, microscale roughness, nanoscale roughness	[18–24]
Physicochemical properties	Surface energy, hydrophylicity/hydrophobicity, superhydrophylicity/superhydrophobicity, functional groups: hydrophobic, polar groups, charged and possessing specific activities, degree of hydration	[25–30]
Environmental factors	Temperature, pH, electrolyte concentration, host proteins, viscosity	[31–34]
Type of pathogen	Gram ± strains, genus, shape, surface energy, strain type, cell wall components	[35–38]

Scientific and industrial interest in antimicrobial surfaces have greatly escalated in the current scenario of persistant microbial infestations [39, 40]. The microbial contamination involving the health care and biomedical industries, water purification systems, and food packaging and storage is a cause for great concern [41, 42]. The prevalence of antimicrobial resistance has become extremely challenging. The continued antibiotics abuse in conjugation with rapid evolution of multi-resistant microbial pathogens has increased the incidences of therapy-resistant diseases. These developments are affecting conventional therapies in a drastic manner [43]. Therefore, it is imperative that antimicrobial therapies and agents utilise multiple modes of attack on the pathogenic bacteria. Management of drug-resistant bacteria through multi-modal action can be achieved by careful study and engineering of nanostructured materials [44, 45].

3 Drug-Resistant Bacteria

Persistent use of antibiotics, self-medication, and exposure to hospital infections has led to the emergence of multidrug-resistant (MDR) bacteria which are responsible for 15.5% hospital acquired infection in the world. The term "ESKAPE" is used to describe six pathogens with growing multidrug resistance and virulence: *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp. [46]. ESKAPE pathogens are responsible for majority of nosocomial infections and are capable of evading the action of antimicrobial agents. Each passing year witnesses the decrease in overall number of antibiotics effective against ESKAPE, predisposing the world towards a future where antibiotics will be rendered ineffective [47, 48].

The lack of treatment options to deal with the MDR pathogens leads to the use of last-line therapies such as carbapenems and antimicrobials previously discarded due to toxicity, such as polymyxins [49]. Nanoparticles have shown effective antimicrobial activity against MDR bacteria, such as *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Mycobacterium tuberculosis*, vancomycin-resistant enterococci, methicillin-resistant Staphylococcus aureus (MRSA) and others [50–53].

3.1 Microbial Biofilms

Device-associated infection are a serious global problem with one out of every four patients experiencing a device-associated infection. These implant-associated infections are usually due to the ability of infecting bacteria to form biofilms on the surface of implanted biomedical devices [54]. Such bacteria living in a biofilm colony have diverse genotypes and phenotypes resulting in physiologic heterogeneity leading to improved ability to resist conventional antimicrobials and their unique biofilm properties also compromise the immunity of the host organism. In addition, the extracellular matrix components and the roughness act as barriers to most available antibiotics. *S. aureus* and *S. epidermidis* are the most common and widely prevalent biomaterial colonizers responsible for device-related infections [55].

4 Antimicrobial Nanomaterials

The widespread use of broad-spectrum antibiotics has led to the appearance of MDR isolates that worsen the situation in hospitals [56]. With majority of available antibiotics becoming ineffective due to mounting drug resistance and the biofilm recalcitrance, a pressing need for alternate drugs is ever increasing [57]. Nanomaterials have shown great promise in killing microbes due to their unique physical and chemical attributes. Their large surface area relative to volume enables increased interactions with microbial membrane; additionally, the surface functionalisation also helps in developing superior antimicrobials [58, 59].

4.1 Antimicrobial Metal Nanoparticle

Several nanoparticulate metals, metal oxides, and metal halides have been exhibited antimicrobial activity. It is believed that the bacteria are less likely to develop resistance to nanomaterials. These include NPs of Ag, Au, Zn, Cu, Ti, Mg, Ni, Ce, Se, Al, Cd, Y, Pd, and super-paramagnetic Fe [60–62]. Among the metal-containing NPs, Au NPs have moderate antibacterial activity, when their surface is functionalised

[63], but Ag NPs are the most effective against bacterial infections [64]. Multi-metal composite NPs were found to be highly effective in inactivating bacteria [65–67].

4.2 Antimicrobial Carbon Nanoparticles

Carbon nanostructures such as graphene oxide (GO) sheets, carbon nanotubes, and fullerenes have demonstrated antimicrobial properties when used in conjugation with other methods [68]. Doping nanotubes or fullerenes with silver or copper nanoparticles may prove to be extremely effective in preventing the ability of microbial cells to grow and replicate DNA [69]. The exact mechanism which promotes their synergistic activity is not clearly understood, but it is believed to be linked to the unique surface chemistry of carbon nanostructures [70].

4.3 Antimicrobial Nanoparticle-Drug Hybrids

Nanoparticles have been found to enhance the action of traditional antibiotics towards which a microorganism could have gained resistance [71]. In addition, they can also help decrease the overall minimum inhibitory concentration (MIC) required for the drug [72]. Silver nanoparticles have been found to increase the potency of amoxicillin, penicillin, and gentamicin in bacteria by altering membrane permeability. Many traditional antimicrobial herbs and extracts have also been used in conjugation with various NPs [73]. Similarly, the potency of the NPs against microorganisms was also found to be enhanced when used in conjugation with drugs or herbal extracts [74, 75].

4.4 Antimicrobial Clay Minerals

Clay are potentially harmless against microorganisms, but they can be engineered to produce antimicrobial hybrid structures [76, 77]. In past few years, the synthesis of MMT-based antibacterial materials and their application in industrial, environmental, and biomedical fields has increased [78]. Numerous reports of MMT modified with antibacterial materials have been published. Some of the materials such as antibiotics, silver, copper, and zinc ions have been immobilized on MMT [79, 80]. Cetylpyridinium, alkyl ammonium, cetyltrimethylammonium, 2-mercaptobenzimidazole, tetradecyltrimethyl ammonium, chitosan, and chlorhexidine acetate been intercalated into the MMT layers [81–83]. In addition, studies indicate that MMT layers are able to adsorb bacteria such as *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*), and immobilized cell toxins [84, 85].

5 Antimicrobial Polymer Nanocomposites

Polymer nanocomposites (PNC) have become key materials in modern nanotechnological applications. Their popularity in various applications can be attributed to their superior performance, improved properties, design flexibility, lower costs, and wide applicability in various industrial fields [86]. These polymeric nanocomposites are essentially composed of organic/inorganic nanoparticles incorporated into polymer matrix to yield superior materials [87]. Antibacterial polymeric nanocomposite essentially consists of antimicrobial nanoparticles incorporated into the polymer matrix [88, 89].

The surface properties of PNC determine the nature of interaction between the microbe and the materials. The bacterial adherence to a biomaterial surface is influenced by chemical compositions, surface charge, hydrophobicity, and surface roughness or physical configuration. Hydrophobicity of the bacteria and the PNC surface determines the eventual adherence of the bacteria to the surfaces [90–92].

Better understanding of the interaction between microorganisms and the biomaterial topography may improve our current knowledge and aid in development of highly effective antimicrobial surfaces [93]. Combined use of multiple antimicrobial mechanisms and modes of action may improve the performance of these antimicrobial agents and circumvent bacterial adaptation. Since bacterial adhesion is a very complex process, surface engineering based on nanostructured materials can act as potent alternatives to conventional antibiotic therapies or to antimicrobial-coated or antimicrobial-loaded biomaterials [94]. Nanostructured materials effectively alter the nanotopology, reducing the surface available for bacterial attachment or through generation of superhydrophobic surfaces.

6 Conclusions

There is a dire need for developing effective antimicrobial materials in view of the alarming spread of MDR strains of bacteria. The microbial infections are becoming one of the leading causes of mortality in developing countries and this calls for the development of antibiotic materials with multiple modes of actions, which will help in reducing the chances of resistance development. In order to prevent the spread of pathogenic microorganisms, working surfaces should also be coated with effective antimicrobial coatings. Nanostructured materials could be potential candidates for such surface coating applications as evidenced from numerous promising reports published every year. The effective utilisation of nanomaterials could aid in the fight against infectious and multiple drug-resistant strains of life-threatening bacteria.

Acknowledgements The authors are grateful to the facilities provided by the International and Inter University Centre for Nanoscience and Nanotechnology, School of Chemical Sciences, School of Pure and Applied Physics, and School of Biosciences, Mahatma Gandhi University.

References

1. WHO (2018) Deaths by cause, age, sex, by country and by region. Available via World Health Organisation. https://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death.2000-2016
2. French G (2010) The continuing crisis in antibiotic resistance. Int J Antimicrob Agents 36:S3–S7
3. WHO (2017) World Health Organization (WHO) publishes list of bacteria for which new antibiotics are urgently needed (2017)
4. Garrett TR, Bhakoo M, Zhang Z (2008) Bacterial adhesion and biofilms on surfaces. Prog Nat Sci 18(9):1049–1056
5. Thomas JG, Litton I, Rinde H (2005) Economic impact of biofilms on treatment costs. In: Biofilms, infection, and antimicrobial therapy. CRC Press, pp 39–56
6. Abdullahi UF, Igwenagu E, Mu'azu A, Aliyu S, Umar MI (2016) Intrigues of biofilm: a perspective in veterinary medicine. Vet World 9(1):12
7. Bowler PG (2018) Antibiotic resistance and biofilm tolerance: a combined threat in the treatment of chronic infections. J Wound Care 27(5):273–277
8. Zeng Q, Zhu Y, Yu B, Sun Y, Ding X, Xu C, Wu Y-W, Tang Z, Xu F-J (2018) Antimicrobial and antifouling polymeric agents for surface functionalization of medical implants. Biomacromol 19(7):2805–2811
9. Rauner N, Mueller C, Ring S, Boehle S, Strassburg A, Schoeneweiss C, Wasner M, Tiller JC (2018) A coating that combines lotus-effect and contact-active antimicrobial properties on silicone. Adv Func Mater 28(29):1801248
10. Van Loosdrecht M, Lyklema J, Norde W, Schraa G, Zehnder A (1987) The role of bacterial cell wall hydrophobicity in adhesion. Appl Environ Microbiol 53(8):1893–1897
11. Van der Westen R, Sjollema J, Molenaar R, Sharma PK, Van der Mei HC, Busscher HJ (2018) Floating and tether-coupled adhesion of bacteria to hydrophobic and hydrophilic surfaces. Langmuir 34(17):4937–4944
12. Schubert A, Wassmann T, Holtappels M, Kurbad O, Krohn S, Bürgers R (2019) Predictability of microbial adhesion to dental materials by roughness parameters. Coatings 9(7):456
13. Andreotti AM, De Sousa CA, Goiato MC, da Silva EVF, Duque C, Moreno A, Dos Santos DM (2018) In vitro evaluation of microbial adhesion on the different surface roughness of acrylic resin specific for ocular prosthesis. Eur J Dent 12(2):176
14. Idumah CI, Hassan A, Ihuoma DE (2019) Recently emerging trends in polymer nanocomposites packaging materials. Polymer-Plast Technol Mater 58(10):1054–1109
15. Absolom DR, Lamberti FV, Policova Z, Zingg W, van Oss CJ, Neumann AW (1983) Surface thermodynamics of bacterial adhesion. Appl Environ Microbiol 46(1):90–97
16. Yuan H, Zhang X, Jiang Z, Chen X, Zhang X (2018) Quantitative criterion to predict cell adhesion by identifying dominant interaction between microorganisms and abiotic surfaces. Langmuir 35(9):3524–3533
17. Mello TP, Oliveira SS, Frasés S, Branquinha MH, Santos AL (2018) Surface properties, adhesion and biofilm formation on different surfaces by *Scedosporium* spp. and *Lomentospora prolificans*. Biofouling 34(7):800–814
18. Zou S, Wei Z, Hu Y, Deng Y, Tong Z, Wang C (2014) Macroporous antibacterial hydrogels with tunable pore structures fabricated by using Pickering high internal phase emulsions as templates. Polym Chem 5(14):4227–4234
19. Shirbin SJ, Lam SJ, Chan NJ-A, Ozmen MM, Fu Q, O'Brien-Simpson N, Reynolds EC, Qiao GG (2016) Polypeptide-based macroporous cryogels with inherent antimicrobial properties: the importance of a macroporous structure. ACS Macro Lett 5(5):552–557
20. Tan K, Obendorf SK (2007) Development of an antimicrobial microporous polyurethane membrane. J Membr Sci 289(1–2):199–209
21. Hill BR, Watson Sr TF, Triplett BL (1991) Antimicrobial microporous coating. Google Patents
22. Choi BG, Park HS (2012) Superhydrophobic graphene/nafion nanohybrid films with hierarchical roughness. J Phys Chem C 116(5):3207–3211

23. Díaz C, Schilardi P, Salvarezza R, Lorenzo Fernández, de Mele M (2007) Nano/microscale order affects the early stages of biofilm formation on metal surfaces. Langmuir 23(22):11206–11210
24. Preedy E, Perni S, Nipiĉ D, Bohinc K, Prokopovich P (2014) Surface roughness mediated adhesion forces between borosilicate glass and gram-positive bacteria. Langmuir 30(31):9466–9476
25. Hallab NJ, Bundy KJ, O'Connor K, Moses RL, Jacobs JJ (2001) Evaluation of metallic and polymeric biomaterial surface energy and surface roughness characteristics for directed cell adhesion. Tissue Eng 7(1):55–71
26. Atefyekta S, Ercan B, Karlsson J, Taylor E, Chung S, Webster TJ, Andersson M (2016) Antimicrobial performance of *Mesoporous titania* thin films: role of pore size, hydrophobicity, and antibiotic release. Int J Nanomed 11:977
27. Bazaka K, Jacob MV, Crawford RJ, Ivanova EP (2011) Plasma-assisted surface modification of organic biopolymers to prevent bacterial attachment. Acta Biomater 7(5):2015–2028
28. Chouirfa H, Bouloussa H, Migonney V, Falentin-Daudré C (2019) Review of titanium surface modification techniques and coatings for antibacterial applications. Acta Biomater 83:37–54
29. Hasan J, Crawford RJ, Ivanova EP (2013) Antibacterial surfaces: the quest for a new generation of biomaterials. Trends Biotechnol 31(5):295–304
30. Ping X, Wang M, Xuewu G (2011) Surface modification of poly (ethylene terephthalate) (PET) film by gamma-ray induced grafting of poly (acrylic acid) and its application in antibacterial hybrid film. Radiat Phys Chem 80(4):567–572
31. Chung Y-C, Wang H-L, Chen Y-M, Li S-L (2003) Effect of abiotic factors on the antibacterial activity of chitosan against waterborne pathogens. Biores Technol 88(3):179–184
32. Vasilev K, Cook J, Griesser HJ (2009) Antibacterial surfaces for biomedical devices. Expert Rev Med Devices 6(5):553–567
33. Tavaria FK, Costa EM, Gens EJ, Malcata FX, Pintado ME (2013) Influence of abiotic factors on the antimicrobial activity of chitosan. J Dermatol 40(12):1014–1019
34. Jung EJ, Youn DK, Lee SH, No HK, Ha JG, Prinyawiwatkul W (2010) Antibacterial activity of chitosans with different degrees of deacetylation and viscosities. Int J Food Sci Technol 45(4):676–682
35. Shan B, Cai Y-Z, Brooks JD, Corke H (2007) Antibacterial properties and major bioactive components of cinnamon stick (*Cinnamomum burmannii*): activity against foodborne pathogenic bacteria. J Agric Food Chem 55(14):5484–5490
36. Krishnamoorthy K, Veerapandian M, Zhang L-H, Yun K, Kim SJ (2012) Antibacterial efficiency of graphene nanosheets against pathogenic bacteria via lipid peroxidation. J Phys Chem C 116(32):17280–17287
37. Brown DG, May-Dracka TL, Gagnon MM, Tommasi R (2014) Trends and exceptions of physical properties on antibacterial activity for Gram-positive and Gram-negative pathogens. J Med Chem 57(23):10144–10161
38. Krishnamoorthy G, Leus IV, Weeks JW, Wolloscheck D, Rybenkov VV, Zgurskaya HI (2017) Synergy between active efflux and outer membrane diffusion defines rules of antibiotic permeation into Gram-negative bacteria. MBio 8(5):e01172–e01117
39. de Abreu PM, Farias PG, Paiva GS, Almeida AM, Morais PV (2014) Persistence of microbial communities including *Pseudomonas aeruginosa* in a hospital environment: a potential health hazard. BMC Microbiol 14(1):118
40. Bogdanos DP, Sakkas LI (2019) Infections: viruses and bacteria. In: Mosaic of autoimmunity. Elsevier, pp 203–213
41. Bolduc J, Nagel C, Li J, Hanson C, Fernholz P (2019) Performic acid biofilm prevention for industrial CO_2 scrubbers. Google Patents
42. Gustavsson R, Mandenius C-F, Löfgren S, Scheper T, Lindner P (2019) In situ microscopy as online tool for detecting microbial contaminations in cell culture. J Biotechnol 296:53–60
43. White BP, Patel S, Tsui J, Chastain DB (2019) Adding double carbapenem therapy to the armamentarium against carbapenem-resistant Enterobacteriaceae bloodstream infections. Infect Dis 51(3):161–167

44. Baker S, Perianova OV (2019) Bio-nanobactericides: an emanating class of nanoparticles towards combating multi-drug resistant pathogens. SN Appl Sci 1(7):699
45. Hasan N, Cao J, Lee J, Hlaing SP, Oshi MA, Naeem M, Ki M-H, Lee BL, Jung Y, Yoo J-W (2019) Bacteria-targeted clindamycin loaded polymeric nanoparticles: effect of surface charge on nanoparticle adhesion to MRSA, antibacterial activity, and wound healing. Pharmaceutics 11(5):236
46. Mulani MS, Kamble EE, Kumkar SN, Tawre MS, Pardesi KR (2019) Emerging strategies to combat ESKAPE pathogens in the era of antimicrobial resistance: a review. Front Microbiol 10
47. Post S, Shapiro J, Wuest W (2019) Connecting iron acquisition and biofilm formation in the ESKAPE pathogens as a strategy for combatting antibiotic resistance. MedChemComm
48. Zhen X, Lundborg CS, Sun X, Hu X, Dong H (2019) Economic burden of antibiotic resistance in ESKAPE organisms: a systematic review. Antimicrob Resist Infect Control 8(1):1–23
49. Santajit S, Indrawattana N (2016) Mechanisms of antimicrobial resistance in ESKAPE pathogens. BioMed Res Int
50. Huang X, Chen G, Pan J, Chen X, Huang N, Wang X, Liu J (2016) Effective PDT/PTT dual-modal phototherapeutic killing of pathogenic bacteria by using ruthenium nanoparticles. J Mater Chem B 4(37):6258–6270
51. Kumari M, Pandey S, Giri VP, Bhattacharya A, Shukla R, Mishra A, Nautiyal C (2017) Tailoring shape and size of biogenic silver nanoparticles to enhance antimicrobial efficacy against MDR bacteria. Microb Pathog 105:346–355
52. Bellio P, Luzi C, Mancini A, Cracchiolo S, Passacantando M, Di Pietro L, Perilli M, Amicosante G, Santucci S, Celenza G (2018) Cerium oxide nanoparticles as potential antibiotic adjuvant. Effects of CeO_2 nanoparticles on bacterial outer membrane permeability. Biochimica et Biophysica Acta (BBA)-Biomembranes 1860(11):2428–2435
53. Siemer S, Westmeier D, Barz M, Eckrich J, Wünsch D, Seckert C, Thyssen C, Schilling O, Hasenberg M, Pang C (2019) Biomolecule-corona formation confers resistance of bacteria to nanoparticle-induced killing: Implications for the design of improved nanoantibiotics. Biomaterials 192:551–559
54. Tattevin P, Flécher E, Auffret V, Leclercq C, Boulé S, Vincentelli A, Dambrin C, Delmas C, Barandon L, Veniard V (2019) Risk factors and prognostic impact of left ventricular assist device-associated infections. Am Heart J 214:69–76
55. Chen J, Howell C, Haller CA, Patel MS, Ayala P, Moravec KA, Dai E, Liu L, Sotiri I, Aizenberg M (2017) An immobilized liquid interface prevents device associated bacterial infection in vivo. Biomaterials 113:80–92
56. Roca I, Akova M, Baquero F, Carlet J, Cavaleri M, Coenen S, Cohen J, Findlay D, Gyssens I, Heure O (2015) The global threat of antimicrobial resistance: science for intervention. New Microbes New Infect 6:22–29
57. Ventola CL (2015) The antibiotic resistance crisis: part 1: causes and threats. Pharm Ther 40(4):277
58. Costa-Gouveia J, Ainsa JA, Brodin P, Lucia A (2017) How can nanoparticles contribute to antituberculosis therapy? Drug Discov Today 22(3):600–607
59. Rai M, Ingle AP, Pandit R, Paralikar P, Gupta I, Chaud MV, dos Santos CA (2017) Broadening the spectrum of small-molecule antibacterials by metallic nanoparticles to overcome microbial resistance. Int J Pharm 532(1):139–148
60. Khan ST, Musarrat J, Al-Khedhairy AA (2016) Countering drug resistance, infectious diseases, and sepsis using metal and metal oxides nanoparticles: current status. Colloids Surf B 146:70–83
61. Parham S, Wicaksono DH, Bagherbaigi S, Lee SL, Nur H (2016) Antimicrobial treatment of different metal oxide nanoparticles: a critical review. J Chin Chem Soc 63(4):385–393
62. Hoseinzadeh E, Makhdoumi P, Taha P, Hossini H, Stelling J, Amjad Kamal M (2017) A review on nano-antimicrobials: metal nanoparticles, methods and mechanisms. Curr Drug Metab 18(2):120–128

63. Zheng K, Setyawati MI, Leong DT, Xie J (2017) Antimicrobial gold nanoclusters. ACS Nano 11(7):6904–6910
64. Franci G, Falanga A, Galdiero S, Palomba L, Rai M, Morelli G, Galdiero M (2015) Silver nanoparticles as potential antibacterial agents. Molecules 20(5):8856–8874
65. Li S, Wang E, Tian C, Mao B, Kang Z, Li Q, Sun G (2008) Jingle-bell-shaped ferrite hollow sphere with a noble metal core: Simple synthesis and their magnetic and antibacterial properties. J Solid State Chem 181(7):1650–1658
66. Tung LM, Cong NX, Huy LT, Lan NT, Phan VN, Hoa NQ, Vinh LK, Thinh NV, Tai LT, Mølhave K (2016) Synthesis, characterizations of superparamagnetic Fe3O4–Ag hybrid nanoparticles and their application for highly effective bacteria inactivation. J Nanosci Nanotechnol 16(6):5902–5912
67. Zaharia A, Muşat V, Ghisman VP, Baroiu N (2016) Antimicrobial hybrid biocompatible materials based on acrylic copolymers modified with (Ag) ZnO/chitosan composite nanoparticles. Eur Polymer J 84:550–564
68. Rezić I, Haramina T, Rezić T (2017) Metal nanoparticles and carbon nanotubes—perfect antimicrobial nano-fillers in polymer-based food packaging materials. In: Food packaging. Elsevier, pp 497–532
69. Maas M (2016) Carbon nanomaterials as antibacterial colloids. Materials 9(8):617
70. Dizaj SM, Mennati A, Jafari S, Khezri K, Adibkia K (2015) Antimicrobial activity of carbon-based nanoparticles. Adv Pharm Bull 5(1):19
71. Moorcroft SC, Jayne DG, Evans SD, Ong ZY (2018) Stimuli-responsive release of antimicrobials using hybrid inorganic nanoparticle-associated drug-delivery systems. Macromol Biosci 18(12):1800207
72. Zheng K, Setyawati MI, Lim T-P, Leong DT, Xie J (2016) Antimicrobial cluster bombs: silver nanoclusters packed with daptomycin. ACS Nano 10(8):7934–7942
73. Snigdha S, Rahul M, Kalarikkal N, Thomas S, Radhakrishnan E (2019) Poly (ε-caprolactone) microsphere decorated with Nano-ZnO based phytoformulation: a promising antimicrobial agent. J Inorg Organomet Polymers Mater 1–11
74. Ildiz N, Baldemir A, Altinkaynak C, Özdemir N, Yilmaz V, Ocsoy I (2017) Self assembled snowball-like hybrid nanostructures comprising *Viburnum opulus* L. extract and metal ions for antimicrobial and catalytic applications. Enzyme Microb Technol 102:60–66
75. Senthilkumar R, Bhuvaneshwari V, Ranjithkumar R, Sathiyavimal S, Malayaman V, Chandarshekar B (2017) Synthesis, characterization and antibacterial activity of hybrid chitosan-cerium oxide nanoparticles: As a bionanomaterials. Int J Biol Macromol 104:1746–1752
76. Xue J, Niu Y, Gong M, Shi R, Chen D, Zhang L, Lvov Y (2015) Electrospun microfiber membranes embedded with drug-loaded clay nanotubes for sustained antimicrobial protection. ACS Nano 9(2):1600–1612
77. Stavitskaya A, Batasheva S, Vinokurov V, Fakhrullina G, Sangarov V, Lvov Y, Fakhrullin R (2019) Antimicrobial applications of clay nanotube-based composites. Nanomaterials 9(5):708
78. Reddy AB, Manjula B, Jayaramudu T, Sadiku E, Babu PA, Selvam SP (2016) 5-Fluorouracil loaded chitosan–PVA/Na^+ MMT nanocomposite films for drug release and antimicrobial activity. Nano-micro Lett 8(3):260–269
79. Rapacz-Kmita A, Bućko M, Stodolak-Zych E, Mikołajczyk M, Dudek P, Trybus M (2017) Characterisation, in vitro release study, and antibacterial activity of montmorillonite-gentamicin complex material. Mater Sci Eng C 70:471–478
80. Zhang L, Chen J, Yu W, Zhao Q, Liu J (2018) Antimicrobial nanocomposites prepared from montmorillonite/Ag. J Nanomat
81. Pielichowski K (2016) Modern polymeric materials for environmental applications
82. Al-Samhan M, Samuel J, Al-Attar F, Abraham G (2017) Comparative effects of MMT clay modified with two different cationic surfactants on the thermal and rheological properties of polypropylene nanocomposites. Int J Polymer Sci
83. Edraki M, Zaarei D (2018) Modification of montmorillonite clay with 2-mercaptobenzimidazole and investigation of their antimicrobial properties. Asian J Green Chem 2(3):171–280, 189–200

84. Hu C-H, Xia M-S (2006) Adsorption and antibacterial effect of copper-exchanged montmorillonite on *Escherichia coli* K88. Appl Clay Sci 31(3–4):180–184
85. Yan Y, Li C, Wu H, Du J, Feng J, Zhang J, Huang L, Tan S, Shi Q-S (2019) Montmorillonite-modified reduced graphene oxide stabilizes copper nanoparticles and enhances bacterial adsorption and antibacterial activity. ACS Appl Bio Mater
86. Harito C, Bavykin DV, Yuliarto B, Dipojono HK, Walsh FC (2019) Polymer nanocomposites having a high filler content: synthesis, structures, properties, and applications. Nanoscale 11(11):4653–4682
87. Liu H, Brinson LC (2008) Reinforcing efficiency of nanoparticles: A simple comparison for polymer nanocomposites. Compos Sci Technol 68(6):1502–1512
88. Nigmatullin R, Gao F, Konovalova V (2008) Polymer-layered silicate nanocomposites in the design of antimicrobial materials. J Mater Sci 43(17):5728–5733
89. Palza H (2015) Antimicrobial polymers with metal nanoparticles. Int J Mol Sci 16(1):2099–2116
90. Cloete TE (2003) Resistance mechanisms of bacteria to antimicrobial compounds. Int Biodeterior Biodegrad 51(4):277–282
91. Ji J, Zhang W (2009) Bacterial behaviors on polymer surfaces with organic and inorganic antimicrobial compounds. J Biomed Mater Res Part A: Off J Soc Biomaterials, Jpn Soc Biomater, Aust Soc Biomater Korean Soc Biomater 88(2):448–453
92. Abdollahi M, Damirchi S, Shafafi M, Rezaei M, Ariaii P (2019) Carboxymethyl cellulose-agar biocomposite film activated with summer savory essential oil as an antimicrobial agent. Int J Biol Macromol 126:561–568
93. Joo SH, Aggarwal S (2018) Factors impacting the interactions of engineered nanoparticles with bacterial cells and biofilms: Mechanistic insights and state of knowledge. J Environ Manage 225:62–74
94. Liu Y, Shi L, Su L, van der Mei HC, Jutte PC, Ren Y, Busscher HJ (2019) Nanotechnology-based antimicrobials and delivery systems for biofilm-infection control. Chem Soc Rev 48(2):428–446

Chapter 2
A Thirst for Polymeric Antimicrobial Surfaces/Coatings for Diverse Applications

Akshatha Nagaraja, Manohara Dhulappa Jalageri, and Yashoda Malgar Puttaiahgowda

1 Introduction

It is significantly accepted that microorganisms such as bacteria, yeast, fungi, and algae inhabit our world, which dominate us in number and size. We only happen to wander for a certain period of time in their world. In spite of our own cell count, even our own body is outnumbered by 10:1 microbial cells and we are living only because we can accept this fact and seek to coexist [1]. Sometimes, the presence of microorganisms is essential like in the growth factors of insects and animals. In fact, microbes are used in fermenting food products (like yeast used in the preparation of beer, wine, bread, etc.), in addition to this, microbes are also used in the treatment of preventing microbial infections in which they are used in the form of antibiotics and vaccines [2]. However, modern human culture still needs some control over the microbial community and it applies especially to pathogenic microbial strains, which still cause millions of deaths each year. Nevertheless, it is becoming more difficult to treat microbial infections as the number of antibiotic-resistant microbial strains and patients is growing much faster than the number of antibiotics that we use. The number of deaths caused by the resistant microbial strain methicillin-resistant Staphylococcus aureus (MRSA) has been reported to surpass the number of deaths caused by HIV in the USA [1]. A statistical analysis from US Centers for Disease Control and Prevention (CDC) reported not less than two million people are infected by antibiotic-resistant bacteria, and at least 23 thousand people die yearly due to infections in the USA. One of the global strategy recommendations dictated by the World Health Organization (WHO) is to make the control of antimicrobial resistance a prime concern for National Governments and Health Systems. Therefore, new prevention and control strategies are urgently required [3–6]. In addition, the microbial infections in developed countries are also escalating because of antibiotic-resistant

A. Nagaraja · M. D. Jalageri · Y. M. Puttaiahgowda (✉)
Department of Chemistry, Manipal Institute of Technology, Manipal Academy of Higher Education, Manipal 576104, India
e-mail: yashoda.mp@manipal.edu

S. Snigdha et al. (eds.), *Engineered Antimicrobial Surfaces*, Materials Horizons: From Nature to Nanomaterials, https://doi.org/10.1007/978-981-15-4630-3_2

microbes. Antimicrobial resistance (AMR) scares the effective prevention and treatment of ever growing infections caused by bacteria, parasites, viruses, and fungi. For example, Gram-positive *Staphylococcus aureus* which has become a global epidemic that is responsible for the main surgical site infections [7–10].

Antimicrobial coatings are rapidly growing as a foremost element to the global mitigation strategy of bacterial pathogens. Thanks to present-day developments in materials science and biotechnology methodologies and a growing understanding of environmental microbiology, a wide range of options are now available for the design of antibacterial surfaces. Progress toward broader use in clinical settings, however, depends crucially on resolving the primary remaining issues [11]. The global market of antimicrobial coatings (AMCs) will reach $4.5 billion and nearly 590 kt of production volume by 2020, forecasted by International Antimicrobial Council, 2015 [12]. Biofouling and biocontamination of surfaces are of important threat due to undesirable growth of microorganism on solid surfaces in diverse surface settings like in solid–water interfaces like taps, shower caps, drains, and so on, and also in solid–gas interfaces like door handles, curtains, computer keyboards, clothes, and so on, especially in hospital environment [5, 13, 14]. There are numerous other areas of interest for antimicrobial surfaces in addition to medical devices and implants, including food protection, household hygiene, water towers, air conditioners, and sportswear [1].

The look for the term antimicrobial and coatings in the period 2007–16 resulted in 2882 documents. In the last 10 years, there has been a linear inclination in the increase in the amount of publications with a value of four times in 2016 to that in 2007. The majority of publications originated in the USA (30%), followed by China (12.7%), India (11.2%), Germany (9.7%), and the United Kingdom (7.0%), with a limited number of articles published in close collaboration between these nations (Fig. 1).

In materials science, nearly 47% of papers were published, followed by about 27% in chemistry. Published articles accounted for about 47.7% of chemical engineering and other engineering disciplines. It is appealing to note that the science and engineering disciplines are equally comparable in this field [15] (Fig. 2). More than 76%

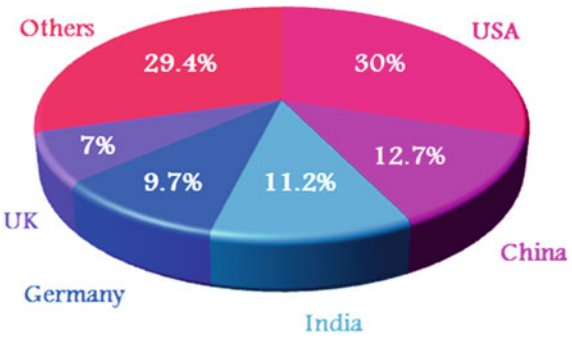


Fig. 1 Statistical analysis on antimicrobial surface development by academic researchers in last decade

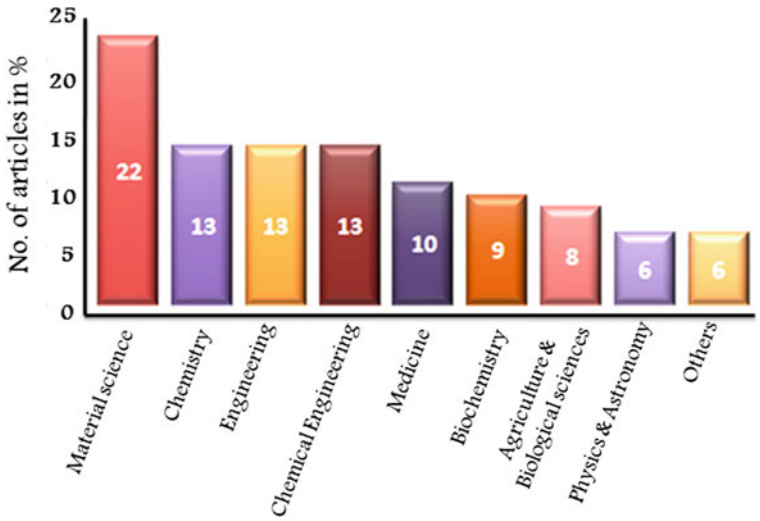


Fig. 2 Bar graph representing statistical data of research articles related to antimicrobial coatings published in different fields of science and technology

of these publications appeared in journals or trade magazines as research papers, followed by 9.4% as review papers and 8.6% as conference papers. The USA research publications indicated a tendency toward the work of silver metals. On the other hand, China reported an extraordinary increase in the number of publications per year, with the majority of articles from the field of materials science. Approximately, 26% of these papers are published from China in cooperation with foreign countries and mostly with the USA. Likewise, about 17% of India's publications have been written in partnership with various countries. Indian research articles addressed the use of silver, chitosan, and herbal products as a sustained release of antimicrobials in coatings. This clearly shows that there has been fair collaboration between global academic institutions in the field of research and development [15]. Over the period 2007–16, the search for patents for the words antimicrobial and coatings stated that over 15,000 patents have been issued over the past 10 years. This means that over the last decade, more than 1000 patents have been granted each year. The statistics reveals that over 65% of these patents were filed in the USA patent office, while about 18% were filed in the European patent office. The third highest number of applications has been submitted by the Australian patent office. In the number of patents granted over the past 10 years, there has been nearly fourfold increase. In 2016, more than 2500 patents were issued on this topic, showing the enormous demand for such materials [15].

The staggering demands of customers have inspired the industry to constantly scan for new effective antimicrobial materials. Many companies market several antimicrobial coatings with various promising attributes. The below tabular column briefly explains the active agents in the current marketing products. Most of the companies

use metal ions/metal ion nanoparticles (silver, zinc, etc.), halides (Iodine, bromine), and also active agents like *o*-phenylphenate, polyvinylidenedifluoride, 2,2-dibromo nitropropionamide, Diiodomethyl-*p*-tolylsulfone, etc., for their application in various sectors like antifouling coatings for boat, ship hulls, paper coatings, inks, adhesives, sealants and cordage, metal working fluids, paints and coatings, slurries, adhesives, latex, and resin emulsions and in industrial products including inks, polishes, waxes, detergents, cleansers, and soforth [15] (Table 1).

2 Basic Concepts of Antimicrobial Coatings

Antibacterial coatings have become a very vigorous research area, strongly encouraged by the increasing urgency of finding substitutes for conventional antibiotic administration. The key techniques for antibacterial coatings design: antibacterial agent release, contact-killing, and anti adhesion/bacteria repelling.

2.1 Anti Adhesion/Bacteria Repelling

Polymer layers and surface brushes are the most common strategies for protein- or microbial-repellent coatings. These polymers can be attached to the surface in many different ways. The polymers used may often differ from linear to branched, such as star-shaped polymers, and from homo-polymers to block copolymers. All these polymers have in common the fact that they are hydrophilic in most situations. Often, non-covalent strong interactions trap the polymers onto the surface [16]. Anti-adhesion coatings use non-cytotoxic mechanisms to prevent the earliest step of biofilm formation (Fig. 3a). Bacterial adhesion on biomaterial surfaces is often explained using a two-stage model: an initial, quick, and reversible stage (stage I), mediated by non-specific physicochemical interactions, followed by a second stage of 'locking' (phase II) involving species-specific bacterial adhesion proteins [11]. Since the polymers are attached to the surface in a high density, they are generally called polymer brushes. The better anti-adhesive surfaces are achieved at the higher the surface density of the polymer chains and also the longer chains are more effective in preventing the bacterial adhesion [17, 18]. Since most of the polymers used for brushes are hydrophilic, the water will be drawn into the brush layer and form a repulsive layer on the surface. The water is held on the substrate via hydrogen bonding to the polymers. As a result, there is steric hindrance for proteins or microorganisms to adsorb on the surface. As a result, protein adsorption is reduced by several orders of magnitude [16]. Surface immobilization of molecules capable of resisting protein adsorption, such as PEG and zwitterion, has shown great in vitro anti-adhesion properties and is generally considered the standard approach for anti-adhesion coatings, despite stability issues.

Table 1 Use of antimicrobial polymers in developing antimicrobial coated surfaces by various companies and their current applications

Company	Products	Active agents	Applications
AkzoNobel	Interlux Micron	Copper, copper oxide, zinc oxide, and carbon black	Antifouling coatings for boat and ship hulls
PPG	SilverSan	Silver	Antimicrobial coatings
BASF	Irgaguard B6000	Silver and zinc zeolite	Adhesives and biocidal coatings
Sciessent	Agion Active XL	Silver ions and zeolite	Coating technologies
Dow chemical	Silvadur	Silver ions	Fabric coatings
	BiobanIPBC 100	3-Iodo-2-propynylbutylcarbamate	Paper coatings, inks, adhesives, sealants and cordage and metalworking fluids
	Amical	Diiodomethyl-*p*-tolylsulfone	Adhesives, paper coatings, plastics, tanned leather, caulks, metalworking fluids, textiles, coatings and wood preservation
	Dowicide	*o*-phenylphenate	Hide-parting operations and in sizing, finishing, and dressing materials
	Vinyzene IT	10,10′-oxybisphenoxarsine (OBPA)	Plastic processing and coatings
	DOWICIL QK-20	2,2-dibromo-3-nitrilopropionamide (DBNPA)	Paints and coatings, slurries, adhesives, latex and resin emulsions and in industrial products including inks, polishes, waxes, detergents, and cleansers
DuPont	Alesta	Silver	Powder coating
Sherwin-Williams	Microban	polyvinylidenedifluoride	Antimicrobial coatings

(continued)

Table 1 (continued)

Company	Products	Active agents	Applications
Dunmore	DUN-SHIELD	Silver ion	Antimicrobial coatings
Troy Corporation	Mergal 530	2,2-dibromo nitropropionamide (DBNPA)	Paints and coatings
Ashland	Nuosept 14	5-chloro-2-methyl-2H-isothiazolin-3-one (CMIT) and 2-methyl-2H-isothiazol-3-one (MIT)	Water treatment/cooling system, fuel, and metal working fluids
	NuoseptBMc 412	1,2-benzisothiazol-3(2H)-one (BIT), 5-chloro-2-methyl-2H-isothiazolin-3-one (CMIT) and 2-methyl-2H-isothiazol-3-one (MIT)	
	Bodoxin TG	Aliphatic hemiacetal and 1,2-benzisothiazol-3(2H)-one (BIT)	
Lonza	Proxel range	1,2-benzisothiazol-3(2H)-one (BIT),sodium pyrithione (NaPT),2-bromo-2-nitropropane-1,3-diol (BNP), zinc pyrithione (ZPT) and 2-methyl-2H-isothiazol-3-one (MIT)	Paper coatings, water-based adhesives, printing inks, emulsion paints
	Dantogard range	DMDM hydantoin and MDM hydantoin	Paper coatings
	Vantocil range	Poly(hexamethylenebiguanide) hydrochloride (PHMB)	Adhesives, coatings and selants
	Omacide range	3-iodo-2-proponyl-*n*-butylcarbamate (IPBC)	Paints and coatings
	Densil range	Diuron, zinc pyrithione(ZPT), n-butyl-1,2-benzisothiazolin-3-one (BBIT), and chlorothalonil (CTL)	Surface coatings
	Reputain	2-bromo-2-nitropropane-1,3-diol (BNP) and 2,2-dibromo-3- nitrilopropionamide (DBNPA)	Paints and coatings

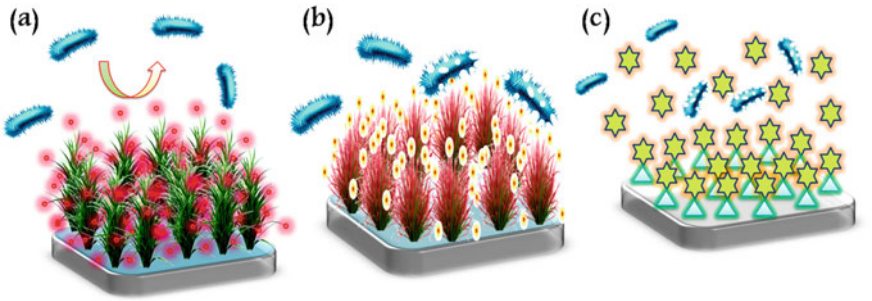


Fig. 3 Schematic illustration of **a** anti-adhesive/bacteria-repelling surfaces, **b** contact killing surfaces, and **c** antibacterial agent releasing surfaces

The use of physical surface modifications (especially surface topography) as non-specific strategy for modulating bacterial adhesion, however, is most likely more complex than previously thought [11, 19–21]. In the latter days, the use of thermo-sensitive polymers such as poly(*N*-isopropylamide) was addressed as a controlled repelling mechanism allowing temperature-sensitive switching between adhesive and repelling state for biofilms [1, 22]. In addition to the various synthetic and natural polymers ideal for repelling microbes from surfaces, the negative protein albumin can also reduce bacterial adhesion [23, 24].

In addition, the nature of the polymer repellant attached to the surface and its mechanical properties both seem to play a role in attracting microbes. This was shown by Lichter et al., who studied multilayer poly(allammonium hydrochloride) (PAH) and poly(acrylic acid) (PAA) and found that the coating's rigidity positively correlates with *E. coli* adhesion [25].

2.2 Contact Killing

Contact-killing coatings were developed to eschew the exhaustion of the reservoir from release-based materials [26]. In this process, antimicrobial agents are covalently bound to the material surface by flexible, hydrophobic polymeric chains. Adhered bacteria are believed to be destroyed by the adhered antimicrobial agents due to destruction of their cell membrane, breaking through the microbial envelope, caused by long binding chains [27] (Fig. 3b). Since the main mechanisms of action are based on membrane interactions, such as physical lysing or charge disruption either cationic compounds (QACs, chitosan, AMPs, etc.) or enzymes were the most active compounds for contact-killing coatings [11, 28]. Isquith et al., who modified glass substrates with silane 3-(trimethoxysilyl)-propyldimethyl octadecyl ammonium chloride, also referred to as DOW5700, identified the first contact-killing surface [22]. The model was constructed on the idea that a surface-grafted membrane-active biocide on a polymer spacer could penetrate a Gram-positive bacterial cell wall, reaching its cell membrane and killing the microorganism. This was studied by surface grafting of poly (4-vinyl-*N*-hexylpyridinium bromide) an antimicrobial polymer to glass and later to several plastics [26, 29].

Yet another highly potent polymer for this application was found to be poly(ethyleneimine), which efficiently kills microbes and even deactivates certain influenza viruses when grafted to surfaces quarternized with dodecyl and methyl groups [30, 31].

The elaborate surface modification of all coatings so far has been overcome by the use of block copolymers containing hydrophobic and hydrophilic antimicrobial blocks as emulsifiers for the emulsification of styrene and acrylates in water. The developed paint was helpful in acquiring contact-active antimicrobial coatings from aqueous suspensions. In parallel, polymeric additives have been developed for polyurethane and acrylate coatings that migrate during the preparation process to the surface of the coating [32, 33]. This way, antimicrobial contact-active surfaces can be

achieved without a finishing procedure. Recently, coatings based on single-walled carbon nanotubes have also been claimed to be antimicrobially active as the nanotubes could poke through the cell walls of approaching microbial cells, according to the authors [1, 34].

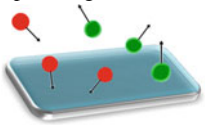
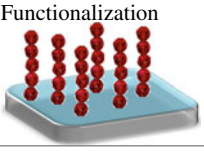
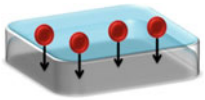
2.3 Antibacterial Agent Release

Release-based coatings exert their antibacterial activity by releasing loaded antibacterial compounds overtime, allowing both adhered and adjacent planktonic bacteria to be killed (Fig. 3c). The release of induced antibacterial agents is attained by diffusion into the aqueous medium, erosion/degradation, or hydrolysis of covalent bonds [35]. In contrast with conventional antibiotic delivery methods, direct elution of antibacterial agent from the material surface offers the potentiality to deliver a high concentration locally, without crossing systemic toxicity or ecotoxicity limits. It only offers antibacterial action where appropriate, thus reducing resistance production and preventing potentially harmful systemic repercussions. Nevertheless, because coatings have essentially small supplies of antibacterial agents, their activity is only temporary [11]. A wide range of antibacterial compounds for release-based systems have been developed over the past decades. The oldest and still commonly used method of providing such substances consists of simply impregnating surfaces, soaking a porous substrate or covering with the desired antibacterial product. The lack of a particular bonding mechanism to the coating results in a quick release [1]. Delivery systems have since developed to include a wide range of carrier materials (i.e., any substance that can be loaded into an antibacterial compound) and methods of deposition. The most commonly used carriers include poly(methacrylic acid) (PMMA), polyacrylic acid (PAA), poly(lactic-co-glycolicacid) (PLGA), hydroxyapatite, polyurethane (PU), a hyaluronic acid, and chitosan [35, 36].

3 Techniques for Antimicrobial Coatings

The surface modifications are performed through various techniques. The below listed techniques are currently used by researchers and industries in coating technology to develop antimicrobial surfaces. Coatings or surface modification techniques are selected based on the desired properties of developing surface. For instance, deposition techniques are used to develop antimicrobial thin films; coatings on biomedical devices are performed through implantation technique, etc. (Table 2).

Table 2 Use of numerous techniques for antimicrobial coatings and surfaces

Techniques	Applications	Literature
Deposition • Dip • Spin • Spray 	Thin-film coatings with AB properties Reservoir or platform for AB compounds Diffusion barrier coatings	[4, 5, 11, 37–45]
Sputtering 	Surface cleaning Adhesion optimization Nanopatterning Nanostructuring	[11, 38, 41–44, 46]
Functionalization 	Surface activation Surface amination Formation of polar groups Immobilization of molecules	[11, 37, 41–44, 47]
Implantation • Electrospinning • Electrodeposition (Electrolytic and electrophoretic deposition) • Layer by layer self assembly • Physical vapor deposition • Chemical vapor deposition • Micro-arc oxidation 	Introduction of different elements into the materials, providing control over: Bioactive properties Corrosion resistance Mechanical properties Crosslinking and densification of polymers	[11, 41–44, 48, 49]

4 Antimicrobial Evaluation Methods

A methodology for screening antimicrobial activity is needed from a material safety point of view as well as to verify the effectiveness of the antimicrobial modification process. Historically, the fundamentals of antimicrobial testing methodology were established for textile industry. As a result, the criteria for fabric antimicrobial screening are well developed. Increasing interest in polymer materials and modifying their properties for more complex applications led to the need to change existing standards in order to make them also applicable to polymers. Methods for antimicrobial activity evaluation can be divided into two groups in principle: (a) static methods and (b) dynamic methods [50–52] (Fig. 4).

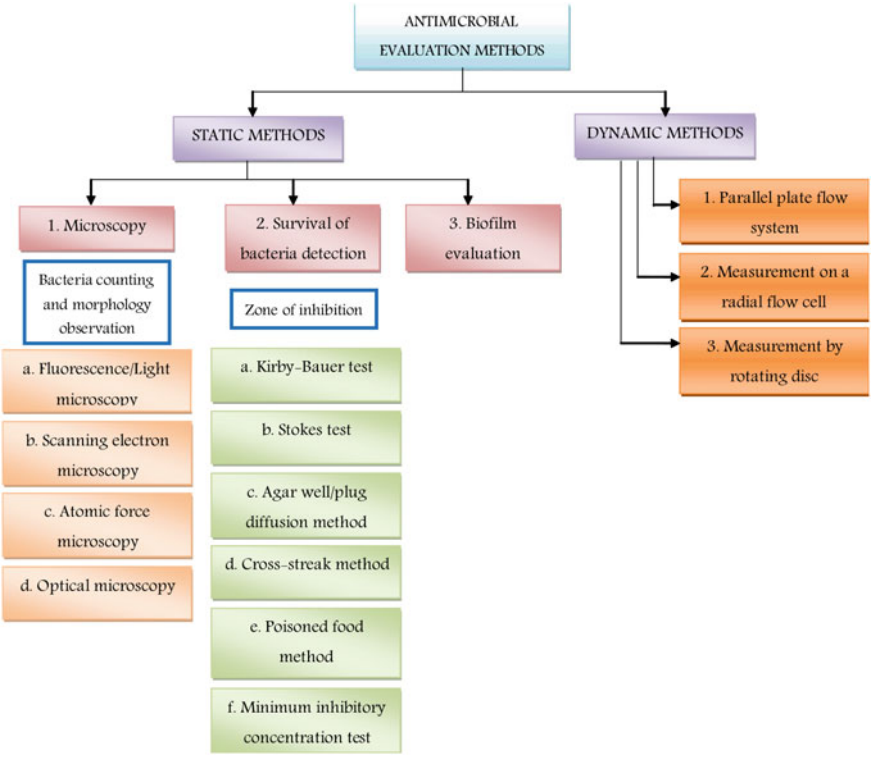


Fig. 4 Flowchart representing the various methods involved in antimicrobial evaluation

5 Applications of Antimicrobial Coatings

The polymers with antimicrobial property are used in numerous sectors due to their enhanced properties like safety and quality benefits compared to biocides of low molecular weight which are toxic in nature and exhibit short-term activity. The problems concerned with the use of conventional/traditional antimicrobial agents can be reduced by the use of antimicrobial polymers. Due to the advantages of polymers over low molecular weight active agents, the use of polymeric materials has gained demand in various sectors like textile industry, food packaging and storage, pharmaceutical and biomedical industries, water purification systems, fibers, etc., [53]. The spread of infections through contaminated surfaces was once limited to particular group of people which included astronauts who are exposed to restricted living spaces [54], people undergoing surgery/implantable devices [55]. Recently, the concern is about the spread of severe infections like acute respiratory syndrome (SARS) [56, 57] and methicillin-resistant Staphylococcus aureus (MSRA) which are originated from the contaminated surfaces. Therefore, the antimicrobial surfaces are not only restricted to use in defense, aerospace, medical industry, etc., but are also scattered in

solid–air interfaces which includes tables, door handles, healthcare units, computer keyboards, textiles and solid–liquid interface which include showers, drains, taps where biofilms occur frequently [13].

5.1 Antimicrobial Textile

One of the most vigorous and ongoing research areas in recent years is focused in the development of antimicrobial textiles, involving activities in the discovery and application of new antimicrobial agents, novel functional fibers, and new chemical finishes antimicrobial textiles will be able to address many challenges, bacteria, viruses, spores, and fungi to concern about daily hygienic issues such as odor producing microbes on clothing and sportswear as well as conservation needs of textile artefacts and the life of geotextiles. In recent years, substantial progress has been made in the development of new antimicrobial agents and technologies due to the wide range of antimicrobial textile applications. As illustrated by Varesano et al. [58], antimicrobial-property textiles may be used to manufacture goods such as towels, undergarments, outdoor clothing, footwear, hygienic uses, furnishings, medical uses, hospital linens, wound care wraps, upholstery, or wipes. It has also become widely used to impart anti-odor or biostatic properties in sportswear [59–61].

Antimicrobial textiles are fabricated using wide range of natural and synthetic active agents. Natural active agents include piper betel [62], red pepper seed oil [63], aloe vera [64], limonene [65], Mexican daisy [66], turmeric, tulsi [67], vanillin [68], lavender, rosemary and sage essential oils [69, 70], herbs and spices [71], whereas synthetic active agents include *N*-halamines [72–77], quaternary ammonium compounds [74, 78–82], triclosan [83], biguanides [84–87], and metal ions [88–93]. These active agents are incorporated into the fibers at different stages of fabrication using chemical and physical treatments.

5.2 Food Packaging

Due to the increase in consumer demand for minimally processed, preservative-free products, antimicrobial packaging has gained significant interest from the food industry in recent years. Several natural polymer-based coatings have been used to monitor common food-borne microorganisms and continuous development of new antimicrobial packaging materials [7]. Food packaging from polymeric films is one of the most widely used films because they are easy to produce and have excellent performance [94]. Polymer food packaging can safeguard food from microbial attacks and has properties such as flexibility, strength, stiffness, and an oxygen and moisture barrier [95, 96].

Numerous natural and synthetic antimicrobial agents are incorporated into a polymer matrix to produce efficient active food packaging materials. As per the literature

reported so far on developing antimicrobial food packing are discussed in this section. Antimicrobial agents origanum vulgare and thymus vulgaris are incorporated into low-density polyethylene polymer matrix [97], cinnamon oil was combined with solid wax paraffin to produce active food packaging [98], nisin or pediocin was added to polyethylene [99, 100], plantaricin BM-1 [101] was incorporated into polyethylene, low-density polyethylene and high-density polyethylene polymer matrices. Sodium benzoate [102] was doped with poly(butylene adipate-co-terephthalate) to develop nanocomposite film. Active films were developed using mixture of potassium sorbate and oregano essential oil, poly(butylene adipate-co-terephthalate) to preserve chicken steaks for 150 days [103]. Chitosan thin film was developed to extend the shelf life of butter cake using potassium sorbate and vanillin as active agents [104]. Fish gelatin films were prepared using chitosan and citric acid to develop active food packaging [105]. Gallic acid was grafted to chitosan to produce novel active packaging material to preserve white button mushroom [106]. The extended shelf life of apple and guava was investigated using binary-grated chitosan films [107]. Antifogging packaging films were developed by ternary blend hydrogel films induced with silver nanoparticles and grape fruit seed extract as active agents [108].

5.3 Biomedical Field

This section highlights the literature using several polymers in modern medicinal sphere which includes coronary stents, vascular grafts, heart valves, blood bags, blood oxygenators, renal dialyzers, catheters, hip prostheses, knee prostheses, intraocular lenses, contact lenses, cochlear implants, and dental implants and soforth [109]. Polyethylene is used in orthopedic implants, containers, catheters, and non-woven textiles. Polypropylene polymers are used in disposable items (e.g., syringes), non-woven textiles, membranes, sutures. In devices like films, tubing, catheters polymers like polyurethanes, polyvinylchloride, and polyether ether ketone are used. Polyamides, polyethylene terephthalate, and polytetrafluoroethylene are found using in sutures, packaging, dental implants, and artificial vascular grafts. Polycarbonates are used in containers and construction material. Poly(methyl methacrylate) is used in membranes, implants, and part of bone cement. Polylactide is used as resorbable implants [110–113].

6 Conclusion

The antimicrobial coatings have developed with leaps and bounds in recent years across the globe owing to their budding importance in preventing pathogenic infections. In this chapter, we have highlighted the commercial antimicrobial coatings and also the use of natural and synthetic active agents across the world by academic researchers. Despite a large number of antibacterial methods published in the

literature, however, to date very few mechanisms have made their way to clinical studies, and even less to clinical practice. While companies offer promises to give the costumers desired functional attributes, the effectiveness of the currently available products has been limited due to insufficient time spent testing and evaluating the active ingredients in the coating material. The design of universal antimicrobial coating for indoor and outdoor use remains a daunting task due to the health and safety issues involved. The discovery and integration of a safer antimicrobial agent into the long-lasting coating matrix is need of the hour.

References

1. Abe A, Genzer J, De Jeu WH, Kobayashi S; Leibler L, Long TE, Manners I, Terentjev EM, Vicent M, Voit B, Wegner G, Wiesner U (2011) *Bioactive Surfaces*
2. Nagaraja A, Jalageri MD, Puttaiahgowda YM, Raghava K, Raghu AV (2019) A review on various maleic anhydride antimicrobial polymers. J Microbiol Methods 163(2019):105650. https://doi.org/10.1016/j.mimet.2019.105650
3. Xue Y, Xiao H, Zhang Y (2015) Antimicrobial polymeric materials with quaternary ammonium and phosphonium salts. Int J Mol Sci 16(2):3626–3655. https://doi.org/10.3390/ijms16023626
4. Nagaraja A, Puttaiahgowda YM, Kulal A, Parambil AM, Varadavenkatesan T (2019) Synthesis, characterization, and fabrication of hydrophilic antimicrobial polymer thin film coatings. Macromol Res. 27(3):301–309. https://doi.org/10.1007/s13233-019-7040-5
5. Nagaraja A, Puttaiahgowda YM (2019) Synthesis and fabrication of high-potent antimicrobial polymeric ultrathin coatings. J Appl Polym Sci 136(34), 47893(1–10). https://doi.org/10.1002/app.47893
6. Jalageri MD, Puttaiahgowda MY, Parambil MA, Kulal A (2019) Design of multifunctionalized piperazine polymer and its activity toward pathogenic microorganisms. J Appl Polym Sci 136(19), 47521. https://doi.org/10.1002/app.47521
7. Jain A, Duvvuri LS, Farah S, Beyth N, Domb AJ, Khan W (2014) Antimicrobial polymers. Adv Healthc Mater 3(12):1969–1985. https://doi.org/10.1002/adhm.201400418
8. Chitanu GC, Zaharia LI, Carpov A (1997) Review: analysis and characterization of maleic copolymers. Int J Polym Anal Charact 4(1):1–20. https://doi.org/10.1080/10236669708033933
9. World Health Organization (2014) Antimicrobial resistance: global report on surveillance 2014. World Health Organization, pp 1–257. ISBN 9789241564748
10. WHO (2014) Antimicrobial resistance. Global report on surveillance. Bull World Health Organ 61(3), 383–394 https://doi.org/10.1007/s13312-014-0374-3
11. Cloutier M, Mantovani D, Rosei F (2015) Antibacterial coatings : Challenges, perspectives, and opportunities. Trends Biotechnol xx, 1–16 https://doi.org/10.1016/j.tibtech.2015.09.002
12. Rosenberg M, Ili K, Kahru A (2019) Potential ecotoxicological effects of antimicrobial surface coatings: a literature survey backed up by analysis of market reports. PeerJ 1–34. https://doi.org/10.7717/peerj.6315
13. Adlhart C, Gouveia I, Melo LF, Crijns F (208) Surface modifications for antimicrobial effects in the healthcare setting : a critical overview. J Hosp Infect 99:239–249. https://doi.org/10.1016/j.jhin.2018.01.018
14. Ajithkumar MP, Yashoda MP, Prasannakumar S, Sruth TV (2017) Poly(*N*-vinyl-2-pyrrolidone-maleic anhydride- styrene) grafted terpolymer: synthesis, characterization, and bactericidal property evaluation against *E. Coli* and *S. Epidermidis*. J Macromol Sci Part A Pure Appl Chem 54(7):480–488. https://doi.org/10.1080/10601325.2017.1320753

15. Tiwari A, Chaturvedi A, Coatings F, States U (2018) Antimicrobial coatings D technology advancement or scientific myth. In: Handbook of Antimicrobial Coatings, pp 1–5
16. Knetsch MLW, Koole LH (2011) New strategies in the development of antimicrobial coatings: the example of increasing usage of silver and silver nanoparticles. Polymers (Basel) 3(1):340–366. https://doi.org/10.3390/polym3010340
17. Roosjen A, Van Der Mei HC, Busscher HJ, Norde W (2004) Microbial adhesion to poly (ethylene oxide) brushes: influence of polymer chain length and temperature. Langmuir 20:10949–10955. https://doi.org/10.1021/la048469l
18. Bridges AW (2008) Anti-inflammatory polymeric coatings for implantable biomaterials and devices. J Diabetes Sci Technol 2(6):984–994
19. Hasan J, Crawford RJ, Ivanova EP (2013) Antibacterial surfaces: the quest for a new generation of biomaterials. Trends Biotechnol 31(5):295–304. https://doi.org/10.1016/j.tibtech.2013.01.017
20. Friedlander RS, Vlamakis H, Kim P, Khan M, Kolter R, Aizenberg J (2013) Bacterial Fl agella explore microscale hummocks and hollows to increase adhesion. Proc Natl Acad Sci 110(14):1–6. https://doi.org/10.1073/pnas.1219662110
21. Variola F, Zalzal SF, Leduc A, Barbeau J, Nanci A (2014) Oxidative nanopatterning of titanium generates mesoporous surfaces with antimicrobial properties. Int J Nanomed 2319–2325
22. Linnea KISTA, Victor H, Perez-luna GPL (1999) Surface-grafted, environmentally sensitive polymers for biofilm release. Appl Environ Microbiol 65(4):1603–1609
23. Tiller JC (2008) Coatings for prevention or deactivation of biological contaminants, 2nd edn., vol 1. Elsevier, Amsterdam
24. Pratt-Terpstra IH, Weerkamp AH, Busscher HJ (1987) Adhesion of oral streptococci from a flowing suspension to uncoated and albumin-coated surfaces. J Gen Microbiol 133:3199–3206
25. Lichter JA, Thompson MT, Delgadillo M, Nishikawa T, Rubner MF, Van Vliet K J (2008) Substrata mechanical stiffness can regulate adhesion of viable bacteria. Biomacromlocules 9:1571–1578. https://doi.org/10.1021/bm701430y
26. Tiller JC, Liao C, Lewis K, Klibanov AM (2001) Designing surfaces that kill bacteria on contact. Proc Natl Acad Sci USA 98(11):5981–5985. https://doi.org/10.1073/pnas.111143098
27. Lewis K, Klibanov AM (2005) Surpassing nature: rational design of sterile-surface materials. Trends Biotechnol 23(7):343–348. https://doi.org/10.1016/j.tibtech.2005.05.004
28. Green JD, Fulghum T, Nordhaus MA (2011) Review of immobilized antimicrobial agents and methods for testing. Biointerphases 6(4):13–28. https://doi.org/10.1116/1.3645195
29. Tiller JC, Lee SB, Lewis K, Klibanov AM (2002) Polymer surfaces derivatized with poly (vinyl-*N*-hexylpyridinium) kill airborne and waterborne bacteria. Biotechnol Bioeng. https://doi.org/10.1002/bit.10299
30. Lin J, Qiu S, Lewis K, Klibanov AM (2002) Bactericidal properties of flat surfaces and nanoparticles derivatized with alkylated polyethylenimines. Biotechnol Prog 18:1082–1086. https://doi.org/10.1021/bp025597w
31. Haldar J, An D, Alvarez L, Cienfuegos D, Chen J, Klibanov AM (2006) Polymeric coatings that inactivate both influenza. Proc Natl Acad Sci USA 103(47):17667–17671. https://doi.org/10.1073/pnas.0608803103
32. Kurt P, Wood L, Ohman DE, Wynne KJ, Commonwealth V, Uni V, Veterans M, Medical A (2007) Highly effective contact antimicrobial surfaces via polymer surface modifiers. Langmuir 23:4719–4723. https://doi.org/10.1021/la063718m
33. Waschinski BCJ, Zimmermann J, Salz U, Hutzler R, Sadowski G, Tiller JC (2008) Design of contact-active antimicrobial acrylate-based materials using biocidal macromers**. Adv Mater 20(1):104–108. https://doi.org/10.1002/adma.200701095
34. Kang S, Pinault M, Pfefferle LD, Elimelech M (2007) Single-walled carbon nanotubes exhibit strong antimicrobial activity. Langmuir 14:8670–8673
35. Campoccia D, Montanaro L, Renata C (2013) Biomaterials: a review of the biomaterials technologies for infection-resistant surfaces. Biomaterials 34(34):8533–8554. https://doi.org/10.1016/j.biomaterials.2013.07.089

36. Hetrick EM, Schoenfisch MH, Hetrick EM, Hetrick EM (2006) Reducing implant-related infections: active release strategies. Chem Soc Rev 780–789. https://doi.org/10.1039/b515219b
37. Vasilev K, Griesser SS, Griesser HJ (2011) Antibacterial surfaces and coatings produced by plasma techniques. Plasma Process Polym 1010–1023. https://doi.org/10.1002/ppap.201100097
38. Cloutier M, Tolouei R, Lesage O, Lévesque L, Turgeon S, Tatoulian M, Cloutier M, Lesage O (2014) Deposited via a hybrid plasma process on the long term antibacterial features of silver-doped diamond like carbon coatings deposited via a hybrid plasma process. Biointerphases 029013. https://doi.org/10.1116/1.4871435
39. Michl TD, Coad BR, Doran M, Osiecki M (2015) With bacteriostatic properties and no cytotoxic side effects 7058–7060. https://doi.org/10.1039/c5cc01722j
40. Zimmermann R, Pfuch A, Horn K, Heft A, Ro M, Linke R, Schnabelrauch M (2011) An approach to create silver containing antibacterial coatings by use of atmospheric pressure plasma chemical vapour deposition (APCVD) and combustion chemical vapour deposition (ccvd) in an economic way. Plasma Process Polym 8(4):295–304. https://doi.org/10.1002/ppap.201000113
41. Processing M, Fotovvati B, Namdari N, Dehghanghadikolaei A (2019) On coating techniques for surface protection: a review. J Manuf Mater Process. https://doi.org/10.3390/jmmp3010028
42. Kausar A (2018) Pure and applied chemistry polymer coating technology for high performance applications: fundamentals and advances. J Macromol Sci Part A 1325 https://doi.org/10.1080/10601325.2018.1453266
43. Fan Y, Li X, Yang R (2018) The surface modification methods for constructing polymer-coated stents. Int J Polym Sci 2018:1–8. https://doi.org/10.1155/2018/3891686
44. Vasilev K (2019) Nanoengineered antibacterial coatings. Coatings 654(9):1–12. https://doi.org/10.3390/coatings9100654
45. Jalageri MD, Yashoda MP, Ajithkumar MP, Varadavenkatesan T (2019) Synthesis and fabrication of highly functionalized Jeffamine antimicrobial polymeric coating. Polym Adv Technol 1–12. https://doi.org/10.1002/pat.4592
46. Pedro J, Serrano C, García-fern L, Barbeck M, Ghanaati S, Unger R, Kirkpatrick J, Arzt E, Funk L, Tur P (2015) Biomaterials nanostructured medical sutures with antibacterial properties. Biomaterials 52. https://doi.org/10.1016/j.biomaterials.2015.02.039
47. Duday D, Vreuls C, Moreno M, Frache G, Boscher ND, Zocchi G, Archambeau C, Van De Weerdt C, Martial J, Choquet P (2013) Surface & coatings technology atmospheric pressure plasma modified surfaces for immobilization of antimicrobial nisin peptides. Surf Coat Technol 218:152–161. https://doi.org/10.1016/j.surfcoat.2012.12.045
48. Jin G, Qin H, Cao H, Qian S, Zhao Y, Peng X, Zhang X, Liu X, Chu PK (2014) Biomaterials synergistic effects of dual Zn/Ag ion implantation in osteogenic activity and antibacterial ability of titanium. Biomaterials 35(27):7699–7713. https://doi.org/10.1016/j.biomaterials.2014.05.074
49. Zhao Y, Ibrahim M, Kan W, Wu G, Wang C, Zheng Y, Yeung KWK, Chu PK (2014) Acta biomaterialia enhanced antimicrobial properties, cytocompatibility, and corrosion resistance of plasma-modified biodegradable magnesium alloys. Acta Biomater 10(1):544–556. https://doi.org/10.1016/j.actbio.2013.10.012
50. Sedlarik V (2013) Antimicrobial modifications of polymers. Biodegradation - Life of Science 187–204. https://doi.org/10.5772/56222
51. Singh A, Tiwari A, Bajpai J, Bajpai AK (2018) 3-polymer-based antimicrobial coatings as potential biomaterials: from action to application. Elsevier, Amsterdam
52. An YH, Friedman RJ (1997) Methods laboratory methods for studies of bacterial adhesion. J Microbiol Methods 30:141–152. https://doi.org/10.1016/S0167-7012(97)00058-4
53. Rodríguez-hernández J (2017) Polymers against microorganisms. Springer Nature, Madrid, Spain

54. Wilson JW, Ott CM, Honer Zu Bentrup K, Ramamurthy R, Quick L, Porwollik S, Cheng P, Mcclelland M, Wilson JW, Ott CM, Ho K, Tsaprailis G, Radabaugh T, Hunt A, Fernandez D, Richter E, Shah M, Kilcoyne M, Joshi L, Hing S, Parra M, Dumars P, Norwood K, Bober R, Devich J, Ruggles A, Goulart C, Rupert M, Stodieck L, Stafford P, Catella L, Schurr MJ, Buchanan K, Morici L, Mccracken J, Allen P, Hammond T, Vogel J, Nelson R, Pierson DL, Nickerson CA (2007) Space flight alters bacterial gene expression and virulence and reveals a role for global regulator Hfq. Proc Natl Acad Sci USA 104(41):16299–16304. https://doi.org/10.1073/pnas.0707155104
55. Darouiche RO (2004) Treatment of infections associated with surgical implants. N Engl J Med 350(14):1422–1429. https://doi.org/10.1056/NEJMra035415
56. Chan JFW, Lau SKP, To KKW, Cheng VCC, Woo PCY, Yuen K (2015) Middle east respiratory syndrome coronavirus: another Zoonotic Betacoronavirus causing SARS-like disease. Clin Microbiol Rev 28(2):465–522. https://doi.org/10.1128/CMR.00102-14
57. Chu C, Cheng VCC, Hung IFN, Chan K, Tang BSF, Tsang THF, Chan K, Yuen K (2005) Viral load distribution in sars outbreak. Emerg Infect Dis 11(12):1882–1886. https://doi.org/10.3201/eid1112.040949
58. Varesano A, Vineis C, Aluigi A, Rombaldoni F (2011) Antimicrobial polymers for textile products. Sci Against Microb Pathog Commun Curr Res Technol Adv 99–110
59. Shin Y, Yoo DI, Jang J (2001) Molecular weight effect on antimicrobial activity of chitosan treated cotton fabrics. J Appl Polym Sci 80(13):2495–2501. https://doi.org/10.1002/app.1357
60. Son Y, Sun G (2003) Durable antimicrobial nylon 66 fabrics: ionic interactions with quaternary ammonium salts. J Appl Polym Sci 90(8):2194–2199. https://doi.org/10.1002/app.12876
61. Callewaert C, De Maeseneire E, Kerckhof F, Verliefde A, Van De Wiele T, Boon N (2014) Microbial odor profile of polyester and cotton clothes after a fitness session. Appl Environ Microbiol 80(21):6611–6619. https://doi.org/10.1128/aem.01422-14
62. Ramya K, Maheshwari V (2015) Antiseptic treatment for human foot wounds using piper betel extract finished bamboo/cotton fabrics. Indian J Fibre Text Res 40(June):213–216
63. Özyildiz F, Karagönlü S, Basal G, Uzel A, Bayraktar O (2013) Micro-encapsulation of ozonated red pepper seed oil with antimicrobial activity and application to nonwoven fabric. Lett Appl Microbiol 56(3):168–179. https://doi.org/10.1111/lam.12028
64. Krishnaveni V, Aparna B (2014) Microencapsulation of copper enriched aloe gel curative garment for atopic dermatitis. Indian J Fibre Text Res 13(October):795–803
65. Sundrarajan M, Rukmani A (2013) Durable antibacterial finishing on cotton by impregnation of limonene microcapsules. Adv Chem Lett 1(1):40–43. https://doi.org/10.1166/acl.2013.1003
66. Thilagavathi G, Bala SK (2007) Microencapsulation of herbal extracts for microbial resistance in healthcare textiles. Indian J Fibre Text Res 32(September):351–354
67. Saraswathi R, Krishnan PN, Dilip C (2010) Antimicrobial activity of cotton and silk fabric with herbal extract by micro encapsulation. Asian Pac J Trop Med 3(2):128–132. https://doi.org/10.1016/S1995-7645(10)60051-X
68. Panisello C, Peña B, Gilabert Oriol G, Constantí M, Gumí T, Garcia-Valls R (2013) Polysulfone/vanillin microcapsules for antibacterial and aromatic finishing of fabrics. Ind Eng Chem Res 52(29):9995–10003. https://doi.org/10.1021/ie3036096
69. Golja B, Šumiga B, Forte Tavčer P (2013) Fragrant finishing of cotton with microcapsules: comparison between printing and impregnation. Color Technol 5(129):338–346. https://doi.org/10.1111/cote.12044
70. Boh B, Knez E (2006) Microencapsulation of essential oils and phase change materials for applications in textile products. Indian J Fibre Text Res 1(31):72–82
71. Thilagavathi G, Rajendrakumar K, Rajendran R (2005) Development of ecofriendly antimicrobial textile finishes using herbs. Indian J Fibre Text Res 30(4):431–436
72. Ren X, Kocer HB, Worley SD, Broughton RM, Huang TS (2009) Rechargeable biocidal cellulose: synthesis and application of 3-(2,3-dihydroxypropyl)-5,5-dimethylimidazolidine-2,4-dione. Carbohydr Polym 75(4):683–687. https://doi.org/10.1016/j.carbpol.2008.09.012

73. Ren X, Kou L, Liang J, Worley SD, Tzou YM, Huang TS (2008) Antimicrobial efficacy and light stability of n-halamine siloxanes bound to cotton. Cellulose 15(4):593–598. https://doi.org/10.1007/s10570-008-9205-9
74. Yang Z, Peng H, Wang W, Liu T (2010) Crystallization behavior of poly(ε-caprolactone)/layered double hydroxide nanocomposites. J Appl Polym Sci 116(5):2658–2667. https://doi.org/10.1002/app
75. Barnes K, Liang J, Wu R, Worley SD, Lee J, Broughton RM, Huang TS (2006) Synthesis and antimicrobial applications of 5,5′-ethylenebis[5-methyl-3-(3-triethoxysilylpropyl)hydantoin]. Biomaterials 27(27):4825–4830. https://doi.org/10.1016/j.biomaterials.2006.05.023
76. Ren X, Kou L, Kocer HB, Zhu C, Worley SD, Broughton RM, Huang TS (2008) Antimicrobial coating of an *N*-halamine biocidal monomer on cotton fibers via admicellar polymerization. Colloids Surf A 317:711–716. https://doi.org/10.1016/j.colsurfa.2007.12.007
77. Liang J, Chen Y, Barnes K, Wu R, Worley SD, Huang T (2006) N-halamine/quat siloxane copolymers for use in biocidal coatings. Biomaterials 27:2495–2501. https://doi.org/10.1016/j.biomaterials.2005.11.020
78. Yu M, Gu G, Meng WD, Qing FL (2007) Superhydrophobic cotton fabric coating based on a complex layer of silica nanoparticles and perfluorooctylated quaternary ammonium silane coupling agent. Appl Surf Sci 253(7):3669–3673. https://doi.org/10.1016/j.apsusc.2006.07.086
79. Marini M, Bondi M, Iseppi R, Toselli M, Pilati F (2007) Preparation and antibacterial activity of hybrid materials containing quaternary ammonium salts via sol–gel process. Eur Polym J 43:3621–3628. https://doi.org/10.1016/j.eurpolymj.2007.06.002
80. Yao C, Li X, Neoh KG, Shi Z, Kang ET (2008) Surface modification and antibacterial activity of electrospun polyurethane fibrous membranes with quaternary ammonium moieties. J Memb Sci 320:259–267. https://doi.org/10.1016/j.memsci.2008.04.012
81. Shao H, Jiang L, Meng WD, Qing FL (2003) Synthesis and antimicrobial activity of a perfluoroalkyl-containing quaternary ammonium salt. J Fluor Chem 124(1):89–91. https://doi.org/10.1016/S0022-1139(03)00193-3
82. Kumar V, Bhardwaj YKÃ, Rawat KP, Sabharwal S (2005) Radiation-induced grafting of vinylbenzyltrimethylammonium chloride (VBT) onto cotton fabric and study of its antibacterial activities. Radiat Phys Chem 73:175–182. https://doi.org/10.1016/j.radphyschem.2004.08.011
83. Allmyr M, Adolfsson-erici M, Mclachlan MS, Sandborgh-englund G (2006) Triclosan in plasma and milk from swedish nursing mothers and their exposure via personal care products. Sci Total Environ 372:87–93. https://doi.org/10.1016/j.scitotenv.2006.08.007
84. Chen-Yu JH, Eberhardt DM, Kincade DH (2007) Antibacterial and laundering properties of AMS and PHMB as finishing agents on fabric for health care workers' uniforms. Cloth Text Res J 25(3):258–272. https://doi.org/10.1177/0887302X07303625
85. Blackburn RS, Harvey A, Kettle LL, Manian AP, Payne JD, Russell SJ (2007) Sorption of chlorhexidine on cellulose: mechanism of binding and molecular recognition. J Phys Chem B 111:8775–8784. https://doi.org/10.1021/jp070856r
86. Kawabata A, Taylor JA (2004) Effect of reactive dyes upon the uptake and antibacterial action of poly(hexamethylene biguanide) on cotton. Part 1: Effect of bis(monochlorotriazinyl) dyes. Color Technol 120(5):213–219. https://doi.org/10.1111/j.1478-4408.2004.tb00120.x
87. Krebs FC, Miller SR, Lee M, Labib M, Rando RF, Wigdahl B (2005) Polybiguanides, particularly polyethylene hexamethylene biguanide, have activity against human immunodeficiency virus type 1. Biomed Pharmacother 59:438–445. https://doi.org/10.1016/j.biopha.2005.07.007
88. Anita S, Ramachandran T, Rajendran R, Koushik CV, Mahalakshmi M (2011) A study of the antimicrobial property of encapsulated copper oxide nanoparticles on cotton fabric. Text Res J 81(10):1081–1088. https://doi.org/10.1177/0040517510397577
89. Fisher L, Ostovapour S, Kelly P, Whitehead KA, Cooke K, Storgårds E, Verran J (2014) Molybdenum doped titanium dioxide photocatalytic coatings for use as hygienic surfaces:

the effect of soiling on antimicrobial activity. Biofouling 30(8):911–919. https://doi.org/10.1080/08927014.2014.939959
90. Rai M, Yadav A, Gade A (2009) Silver nanoparticles as a new generation of antimicrobials. Biotechnol Adv 27(1):76–83. https://doi.org/10.1016/j.biotechadv.2008.09.002
91. Roberson M, Rangari V, Jeelani S, Samuel T, Yates C (2014) Synthesis and characterization silver, zinc oxide and hybrid silver/zinc oxide nanoparticles for antimicrobial applications. Nano Life 04(01):1440003. https://doi.org/10.1142/s1793984414400030
92. Romero L, Piccirillo C, Castro PML, Bowman C, Warwick MEA, Binions R (2015) Titanium dioxide thin films deposited by electric field-assisted CVD: effect on antimicrobial and photocatalytic properties. Chem Vap Depos 21(1–3):63–70. https://doi.org/10.1002/cvde.201407145
93. Swathy JR, Udhaya Sankar M, Chaudhary A, Aigal S, Anshup, Pradeep T (2014) Antimicrobial silver: an unprecedented anion effect. Sci Rep 11. https://doi.org/10.1038/srep07161
94. Silvestre C, Duraccio D, Cimmino S (2011) Food packaging based on polymer nanomaterials. Prog Polym Sci 36(12):1766–1782. https://doi.org/10.1016/j.progpolymsci.2011.02.003
95. Huang T, Qian Y, Wei J, Zhou C (2019) Polymeric antimicrobial food packaging and its applications. Polymers (Basel) 11(3). https://doi.org/10.3390/polym11030560
96. Caner C, Vergano PJ, Wiles JL (1998) Chitosan film mechanical and permeation properties as affected by acid, plasticizer, and storage. J Food Sci 63(6):1049–1053. https://doi.org/10.1111/j.1365-2621.1998.tb15852.x
97. Solano ACV, de Gante CR (2012) Two different processes to obtain antimicrobial packaging containing natural oils. Food Bioprocess Technol 5(6):2522–2528. https://doi.org/10.1007/s11947-011-0626-3
98. Rodríguez A, Nerín C, Batlle R (2008) New cinnamon-based active paper packaging against rhizopusstolonifer food spoilage. J Agric Food Chem 56(15):6364–6369. https://doi.org/10.1021/jf800699q
99. Meira SMM, Zehetmeyer G, Werner JO, Brandelli A (2017) A novel active packaging material based on starch-halloysite nanocomposites incorporating antimicrobial peptides. Food Hydrocoll 63:561–570. https://doi.org/10.1016/j.foodhyd.2016.10.013
100. La Storia A, Ercolini D, Marinello F, Mauriello G (2008) Characterization of bacteriocin-coated antimicrobial polyethylene films by atomic force microscopy. J Food Sci 73(4):48–54. https://doi.org/10.1111/j.1750-3841.2008.00713.x
101. Zhang M, Gao X, Zhang H, Liu H, Jin J, Yang W, Xie Y (2017) Development and antilisterial activity of PE-based biological preservative films incorporating plantaricin BM-1. FEMS Microbiol Lett 364(7). https://doi.org/10.1093/femsle/fnw283
102. Mondal D, Bhowmick B, Maity D, Mollick MR, Rana D, Rangarajan V, Sen R (2015) Investigation on sodium benzoate release from poly (butylene adipate-co-terephthalate)/ organoclay/sodium benzoate based nanocomposite film and their antimicrobial activity. J Food Sci 80(3). https://doi.org/10.1111/1750-3841.12745
103. Cestari LA, Gaiotto RC, Antigo JL, Scapim MRS, Madrona GS, Yamashita F, Pozza MSS, Prado IN (2015) Effect of active packaging on low-sodium restructured chicken steaks. J Food Sci Technol 52(6):3376–3382. https://doi.org/10.1007/s13197-014-1357-z
104. Sangsuwan J, Rattanapanone N, Pongsirikul I (2015) Development of active chitosan films incorporating potassium sorbate or vanillin to extend the shelf life of butter cake. Int J Food Sci Technol 50(2):323–330. https://doi.org/10.1111/ijfs.12631
105. Uranga J, Puertas AI, Etxabide A, Dueñas MT, Guerrero P, de la Caba K (2019) Citric acid-incorporated fish gelatin/chitosan composite films. Food Hydrocoll 86:95–103. https://doi.org/10.1016/j.foodhyd.2018.02.018
106. Liu J, Liu S, Zhang X, Kan J, Jin C (2018) Effect of gallic acid grafted chitosan film packaging on the postharvest quality of white button mushroom (agaricus bisporus). Postharvest Biol Technol 2019(147):39–47. https://doi.org/10.1016/j.postharvbio.2018.09.004
107. Kumar D, Kumar P, Pandey J (2018) Binary grafted chitosan film: synthesis, characterization, antibacterial activity and prospects for food packaging. Int J Biol Macromol 115(2017):341–348. https://doi.org/10.1016/j.ijbiomac.2018.04.084

108. Kanmani P, Rhim JW (2014) Development and characterization of carrageenan/grapefruit seed extract composite films for active packaging. Int J Biol Macromol 68:258–266. https://doi.org/10.1016/j.ijbiomac.2014.05.011
109. Vendra VK, Wu L, Krishnan S (2011) Polymer thin films for biomedical applications. In: Nanotechnologies for the life sciences, vol. 5. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, pp 1–54
110. Xu X, Chen X, Jing X (2006) Biodegradable electrospun poly (*L*-lactide) fibers containing antibacterial silver nanoparticles. Eur Polym J 42:2081–2087. https://doi.org/10.1016/j.eurpolymj.2006.03.032
111. Woo GLY, Mittelman MW, Santerre JP (2000) Synthesis and characterization of a novel biodegradable antimicrobial polymer. Biomaterials 21:1235–1246. https://doi.org/10.1016/S0142-9612(00)00003-X
112. Zhang W, Ji J, Zhang Y, Yan Q, Kurmaev EZ (2007) Effects of NH_3, O_2, and N_2 co-implantation on cu out-diffusion and antimicrobial properties of copper plasma-implanted polyethylene. Appl Surf Sci 253:8981–8985. https://doi.org/10.1016/j.apsusc.2007.05.019
113. Buschow KHJ, Cahn RW, Flemings MC, Ilschner B, Kramer EJ, Subhash Mahajan A, Veyssière P (2008) Encyclopedia of materials: science and technology

Chapter 3
Potential Target Sites that Are Affected by Antimicrobial Surfaces

M. I. Abou-Dobara and N. F. Omar

1 Introduction

Potential target sites affected by antimicrobials may generally involve all essential biosynthetic pathways and cellular structures. Bacterial targets and their antimicrobials, traditionally, are grouped in six categories: cell wall biosynthesis (β-lactams, glycopeptides), cell membranes (colistin, daptomycin), protein biosynthesis (aminoglycosides, macrolides, tetracyclines, oxazolidinones, streptogramines), DNA replication (fluoroquinolones), RNA synthesis (ansamycins), and folate biosynthesis (sulfonamides, antifolates) [1]. The structures of these traditional bacterial targets are unique to bacteria, hence help to avoid side effects in mammals.

Antibacterials that affect a single target would develop high-level resistances due to single-step mutations in the target molecule, like the case of vancomycin [2]. Under the stress of antimicrobials, bacteria can easily adapt by mutation or by horizontal gene transfer [3, 4]; these bacteria develop enzymes that degrade or modify antibiotics, structural modifications or mechanisms that block antibiotics from reaching their targets, and new biosynthesis pathways that sometimes induced by the antibiotic [5]. Then, evolutionary pressure will select for antibiotic-resistant bacteria. Combating resistance depends on ecological strategies (as using probiotics) or developing new antibiotics; new antibiotics are designed based on prodrugs or by rational design of new molecules.

To design any structure-based drug, a suitable target is firstly identified [6]. Targets can be comprehensively defined as products of essential and conserved bacterial genes; these include different RNA species, DNA, new proteins, or macromolecules of these constituents. Rational design, for example, aims to design molecules that

M. I. Abou-Dobara · N. F. Omar (✉)
Botany and Microbiology Department, Faculty of Science, Damietta University, Damietta, Egypt
e-mail: noha_omar15@yahoo.com

M. I. Abou-Dobara
e-mail: aboudobara@gmail.com

S. Snigdha et al. (eds.), *Engineered Antimicrobial Surfaces*, Materials Horizons: From Nature to Nanomaterials, https://doi.org/10.1007/978-981-15-4630-3_3

can bind a specific pocket in the target enzyme to inhibit it, which blocks an essential metabolic pathway. Thus, selecting and evaluating this target requires a deep understanding of both the biology of the bacteria and the structure of the target. This chapter reviews the potential target sites and steps to evaluate the efficiency of a target site. Then, the crystal structure of targets can be obtained from the Protein Data Bank or constructed by making a homology model.

2 Bacterial Cell Division Proteins as Targets

The bacterial cell cycle involved a DNA cycle (replication and segregation), then a cell division cycle [7]. During the cell division, the divisome protein complexes mediate the synthesis, hydrolysis, and modification of cell wall components [8]. Cell division is initiated by the formation of a polymerized ring (Z ring), at midcell division site, of the FtsZ protein [7]. Then, the first macromolecular complex (divisome or proteo-ring) is formed by the interaction of the Z-ring with other regulatory proteins (ZipA, FtsA, ZapA, FtsK, FtsQ, FtsL, FtsB, FtsW, FtsI, FtsN, and AmiC in *E. coli* or their homologs in other bacteria) [9, 10]; this divisome drives the division process through positioning the new peptidoglycan and regulating synthesis of two new cell poles [11] to form the septal ring [10].

Any imbalance in these activities kills the cell, so inhibiting any of the divisome proteins would kill bacteria. The main divisome proteins represent antimicrobial targets due to their conservation in most bacterial species [12]; however, some proteins are unique to their genera which may represent species-specific targets.

2.1 FtsZ Protein

FtsZ represents a promised target for the design of antibacterial drugs, especially broad-spectrum antibiotics; it is the most conserved and critical protein for the cell division.

Few exceptions were recorded to lack FtsZ, as Chlamydiaceae, since they are obligate intracellular that may use the host cell for division [13] while the FtsZ modulators are less conserved among bacterial species [14].

The polymerization of FtsZ into the Z-ring is tightly regulated, which controls the timing and position of the division septum [15–17]. The Z-ring has a dynamic nature; its diameter must continuously be limited so its half-life is always about 10 s [18]; its inhibition interrupts cell division leading to filamentation and death of the cell [19]; it must be disassembly during septation [10]. Thus, different FtsZ inhibitors can be predicated: inhibitors that prevent the association between two FtsZ subunits: inhibitors that stabilize the protofilaments of Z ring; and inhibitors that interfere with sites of interactions between FtsZ and other essential division proteins.

In nature, under stress conditions, the bacteria synthesize the SulA protein (an inhibiting protein to FtsZ polymerization) to postpone cell division; SulA binds to the T7 loop of FtsZ which inhibit the addition of new FtsZ molecules to form the Z-ring [20], hence inhibit cell division. *B. subtilis* also naturally uses the peptide MciZ to inhibit Z-ring formation [21]. Now, both natural and synthetic inhibitors that target FtsZ have been described.

On searching for FtsZ inhibitors, detection of FtsZ polymerization either in vivo or in vitro is available; crystal structures of FtsZ from several bacterial species are available [22–25]; FtsZ polymerization state can be in vitro assayed by light scattering [26] or colorimetry [27]; the localization of FtsZ in cell can be monitored by FtsZ-fluorescent protein fusions [28]; details of the formed filaments can be examined by electron microscopy [29].

Both inhibitors that prevent the association between two FtsZ subunits and that stabilize the protofilaments act through blocking GTP-binding site in FtsZ; the behavior of FtsZ polymers in the cell is driven by guanosine triphosphate (GTP) binding and hydrolysis [12]. The GTP-binding site is shared by the two FtsZ subunits so GTP molecule couples the FtsZ monomers head to tail; the nucleotide is bound by the T2, T3, and T4 loops of the first FtsZ molecule, and the c-phosphate is bound by the T7 loop of the incoming FtsZ molecule; hydrolysis of GTP dissociates the polymers. Thus, blocking the GTP-binding site in FtsZ reduces the available FtsZ molecules under critical concentration for polymerization [30]; and it also prevents GTPase activity to dissociate FtsZ polymer [31]. Most of these inhibitors are GTP analogs with bulky substitutions at the C8 position [22, 32, 33]. The GTP analogs bind FtsZ, but their bulky substitutions prevent the association of a second FtsZ subunit and consequently inhibit the GTPase activity due to the absence of the second molecule. Despite the homology between FtsZ of the various species, some variations were reported in the inhibitory mode of the GTP analogs among species [32]. Such GTP analogs are not suitable antibiotics, but it is informative for further design of FtsZ inhibitors.

For the last type of inhibitors (that interfere with sites of interactions between FtsZ and other essential division proteins), the C-terminal domain of FtsZ is a promising target. The C-terminal linker (50–60 residues in most bacterial FtsZ but over 200 residues in alpha-proteobacteria) is critical for FtsZ assembly [34, 35]; it also mediates interactions between FtsZ and the cell wall synthesis machinery [36]. The amino acid residues of this region lack conservative sequences among species [37]. Thus, it may be used for a species-selective inhibition of these interactions.

2.2 Divisome Modulating Proteins: FtsA, ZipA and ClpP

The Z-ring interacts with ZipA, FtsA, and other regulatory proteins to anchor its position to the inner surface of the cytoplasmic membrane and to recruit other proteins of the divisome [38, 39]; either the overproduction or depletion of these proteins interrupts FtsZ-ring and blocks septation.

FtsA is the second most conserved cell division protein after FtsZ; it has an essential role in the contraction of the ring [40]. FtsA to FtsZ molecules ratio in both *E. coli* and *B. subtilis* should be 1:5 as a determinant ratio [41, 42]. FtsA polymerizes and attaches to the membrane through its ATP binding site by a conformational switch of its helix located at the C-terminus [43]. Peptides that inhibit the ATPase activity of FtsA affect the interaction of FtsZ with FtsA [44]. Thus, blocking the ATP binding site of FtsA will be a means to design future antibiotics [45].

ZipA is a less conserved integral membrane protein; it found only in Gamma-proteobacteria [46]. Its functions overlap those of FtsA; it interacts also to the Z-ring with the conserved C-terminus of FtsZ [40], via its cytoplasmic C-terminal domain [47]. This hydrophobic cleft of ZipA represents a target antibiotic site since it blocks the FtsZ–ZipA interaction [48]; several small aromatic derivatives inhibited the FtsZ–ZipA interaction by binding to the hydrophobic pocket of ZipA, as the FtsZ peptide [49, 50]. Thus, the *in silico* approach to screen for additional inhibitors is based on that chemical structures [51].

ClpP regulated the degradation of FtsZ, via ClpXP ATP-dependent protease [52]. Deregulating ClpP (overactivation or inhibition) inhibits cell division through uncontrolled FtsZ degradation [53]. A new class of antibiotics, cyclic acyldepsipeptides, was reported to kill bacteria by activating ClpP independent of ClpX [54]; the uncontrolled protease activity degrades FtsZ, so Z-ring formation is inhibited. It induces filamentation of rod-shaped, like *Bacillus subtilis*, and swelling of cocci, as *S. aureus* and *Streptococcus pneumonia*e [55].

2.3 FtsEX Complex

FtsEX belongs to a small subclass of ABC transporters that uses mechano-transmission to perform roles in the periplasm; FtsE corresponds to the ATP binding subunit and FtsX to the integral membrane subunit [56]. FtsEX regulates periplasmic peptidoglycan hydrolase activities during the separation of daughter cells [57].

FtsE and FtsX are essential in the pathogenic *Streptococcus pneumoniae* [58] and in *E. coli* only at low ionic strength growth conditions [56]. The inhibition of these regulatory complexes or even the ATPase activity of FtsE could be a target for developing new antibiotics [12].

2.4 FtsW Protein

FtsW is an essential member of the divisome complex for septal cell wall assembly [8]. It translocates the lipid II (a peptidoglycan precursor) across the cytoplasmic membrane [59] then forms a complex with class B penicillin-binding proteins to polymerize lipid II into peptidoglycan [60].

Bacterial mutants lacked FtsW protein showed a dominant-negative lethal phenotype [61]. Thus, FtsW is a suitable target for new antibiotics. Lipid II analogs would be competitive inhibitors for FtsW [12].

2.5 FtsQLB Complex

The trimeric FtsQ, FtsL, and FtsB complex (or their homologs) form a core component of the divisome and are conserved among bacterial species [62]. This functions as a part of a sensing mechanism that induces the cell wall remodeling during the assembly of the divisome [63].

FtsQ is the determinant for the complexes formation because it is present in only 25–50 copies per cell [12]. The crystal structure of the periplasmic domain of FtsQ in complex with the C-terminal fragment of FtsB showed that the C-terminal region of FtsB is a key binding region of FtsQ; it indicates the regions to be targeted with inhibitors [64]. It has been also proposed that the FtsL instability could be an antimicrobial target in the divisome formation [65].

3 DNA as a Target

DNA-binding compounds inhibit the growth of both bacteria and eukaryotes; however, these disadvantages can be overcome by specific options (will be discussed). On the market, few DNA-targeting antimicrobials are available, such as metronidazole [66] and nitrofurantoin [67]. Binding of compounds to DNA interacts with DNA either covalently (through alkylation) or non-covalently (to the major or minor groove of the helix or between bases) [68–70]. All of these interactions may damage DNA strands or interfere with enzymes of DNA replication, transcription, or repair mechanisms, which kill the cell.

Targeting DNA by its intercalation is found in nature; *Streptomyces* spp. produce several antimicrobials that intercalate DNA, such as actinomycins [71]. Actinomycin D contains a planar tricyclic phenoxazone ring that intercalates double-stranded DNA and two cyclic pentapeptide lactone rings that interact with the minor groove around the intercalated phenoxazone ring [72]. Bis-intercalators (as echinomycin, triostin A and sandramycin), produced by bacteria, consist of a peptide core surrounded by two intercalating planar aromatic groups [73]. Several synthetic intercalators, including coordination complexes, also inhibit bacteria, via DNA binding [74].

The minor groove of DNA is another site for DNA binding. The inhibitors bind to the edges of the base pairs of the DNA, via reversible non-covalent interactions [75]. The isohelical shape, as a compatible shape, of the inhibitor molecule is thought to be important for the minor groove binder [76]; metals can also enhance their antibacterial activity [77]. The activity of these compounds can depend on the concentration; they bind DNA either in a 1:1 stoichiometry, or in a 2:1 stoichiometry (bind to DNA in an

antiparallel orientation) [78]. Some minor groove binders (as pentamidine, netropsin and distamycin A) have specific target sequences [79]; this enhances the selectivity of DNA binders to bacterial DNA and even to specific genes. This sequence specificity can be manipulated based on specific rules in lexitropsins, which bind antiparallel in the minor groove with pyrrole (Py)–imidazole (Im), Py-Im targets C–G, Im–Py targets G–C, and Py/Py targets A–T or T–A [80]. However, the available target sequences are still relatively short (6-7 nucleotides) [81].

Major groove binders are relatively less researched than minor groove binders. The major groove binders usually have similar size and compatible shape to α-helices [74]. Several natural and synthetic major groove binders showed antimicrobial activity. Dinuclear iron(II) supramolecular helicate $[Fe_2L_3]^{4+}$, a synthetic major groove binder, showed activity against both Gram-negative as Gram-positive bacteria [82]. Some aminoglycosides were modified as reversible major groove binders; these chemical modifications design antimicrobials bind to a specific sequence to block access to various transcription factors [83].

Resistances to DNA-binding compounds arise through DNA-repair mechanisms such as overexpression of DNA-repair protein RecA to resist metronidazole in *Bacteroides fragilis* [84]; in nature, *Streptomyces* spp. also produce UvrA-like excision repair proteins for their self-resistance against daunorubicin and doxorubicin [85] and echinomycin [86]. That resistance may also arise through general mechanisms by reducing the intracellular accumulation.

The toxicity of DNA-binding compounds is faced by increasing their selectivity through designing sequence-specific binders (as mentioned before), using prodrugs, and enhancing selective microbial uptake. An example of prodrugs is metronidazole that only activated by reductases of anaerobes [66] to a DNA damaging nitro radical; thus, mutations in these reductases [87] or production of alternative reductases [88] cause metronidazole resistance. For enhancing selective uptake, conjugation to specific molecules could be a useful approach [89].

4 Peptidoglycan Biosynthesis Enzymes as Targets

Peptidoglycan is the main component of bacterial cell walls. It is composed of N-acetylglucosamine (GlcNAc) and *N*-acetylmuramic acid (MurNAc) moieties that are cross-linked through stems of peptides [90]. Peptidoglycan is biosynthesized through stages, which are all targets for antibacterial agents [91]. The cytoplasmic step involves the synthesis of uridine diphosphate-*N*-acetylmuramyl-pentapeptide (UDP-MurNAc-pentapeptide) from uridine diphosphate-*N*-acetylglucosamine (UDP-GlcNAc), via the Mur enzymes MurA-F [92]. At the inner face of the cytoplasmic membrane, the integral membrane protein MraY transfers the phospho-MurNAc-pentapeptide motif of UDP-MurNAc-pentapeptide, via a lipid carrier (undecaprenyl phosphate) to form lipid I [93]; lipid I is linked to a GlcNAc by the membrane-associated enzyme MurG to generate lipid II [94], which is translocated to periplasm by flippases [61]. In the periplasm, lipid II is polymerized by glycosyltransferases

(including class A penicillin-binding proteins; PBPs) to peptides then cross-linked, via transpeptidases (class A and B PBPs) to produce the peptidoglycan structure [95].

The peptidoglycan has no counterpart in eukaryotic cells, so its targeting by antimicrobials has minimum potential drug side effects [96]. Peptidoglycan biosynthesis enzymes, as antimicrobial targets, are well conserved across bacterial species [97]. For several years, the transpeptidases that catalyzed the late stages of peptidoglycan biosynthesis are inhibited by β-lactam antibiotics, such as penicillins and cephalosporins [95, 98, 99]. However, bacterial resistance against β-lactam has raised through several routes: inactivation of the β-lactam ring by lactamase enzymes, efflux by outer membrane pumps, the acquisition of β-lactam-insensitive transpeptidase PBPs as seen in methicillin-resistant *Staphylococcus aureus* (MRSA) [100], and transfer of resistant genes and recombination as seen in *Streptococcus pneumoniae* [101] and *Neisseria gonorrhoeae* [102].

Other steps of peptidoglycan biosynthesis have been now considered as antimicrobial targets. Crystal structures and in vitro assays of those proteins are available [103–105]. Thus, several compounds have been synthesized to target these proteins or even transition state of these proteins [106]; however, those compounds target Mur enzymes showed weak or no antibacterial activity; perhaps due to their failure to pass the bacterial membrane or formation of multi-protein complexes of Mur proteins [107] which making their active sites inaccessible for inhibitors. Thus, regarding the peptidoglycan biosynthesis proteins, we focused on MraY, MurG, and PBPs.

4.1 MraY Protein

MraY is an integral membrane protein, so it is accessible from the periplasmic side of the cytoplasmic membrane [93]. MraY has been investigated as a potential target for new antibiotics [12]; its inhibition would prevent synthesis of lipid I (the precursor of lipid II); it was isolated and biochemically characterized [108–111] with high throughput screening assays [112].

In nature, the bacteriophage UX174 [113] produces E-peptide that inserts between MraY TM domains to prevent its association with other membrane proteins (MurG or/and FtsW), hence inhibit MraY protein [114]. MraY is also a target for several classes of nucleoside natural inhibitors, produced by Streptomycetes, including peptidyl nucleosides class (mureidomycins, pacidamycins, napsamycins, and sansanmycins), fatty acyl nucleosides (liposidomycins and caprazamycins), lipopeptidyl nucleosides (muraymycins), nucleoside disaccharides (tunicamycins, streptovirudines) and glycosyl nucleosides (capuramycins) [115]. Although, these compounds are non-suitable for clinical use due to their toxicity to eukaryotic membrane-associated glycosyltransferases [116].

Several natural product analogs have inhibited MraY of Gram-positive bacteria, including MRSA [117]. Combined MraY/MurG screens have also yielded inhibitors

to Gram-negative MraY [118]. MraY-aimed drug design could target either its catalytic region or its interaction regions with other proteins. MraY is a dimeric molecule whose active site cleft is located within the inner leaflet of the membrane and faces the cytoplasm [119]. This region represents a target; it also thought to recognize other proteins such as MurF and MurG [120].

4.2 MurG Protein

MurG is a soluble membrane-associated glycosyltransferase that transfers GlcNAc from UDP-GlcNAc to the C4 hydroxyl of the membrane-anchored lipid I to form lipid II [121]. MurG is composed of two domains (N- and C-domains folded with α/β open-sheet motif) linked through a hinge region [122]. Based on structural insights (substrate binding and catalytic mechanism), the target amino acid residues are determined; UDP-GlcNAc binds to the C-terminal domain of MurG, while lipid I binds to the N-terminal domain [121]; through its N-terminal hydrophobic residues (conserved glycine-rich stretch termed the G-loops), MurG also electrostatically interacts with the negatively charged lipid membrane [123]. Sequence alignment of MurG homologs showed conserved residues located near the cleft between the two domains [121, 123, 124]. The critical residues (T16, H19, Y106-numbers according to *E. coli* MurG) to bind lipid I are invariant in MurG homologs across bacterial species [123].

Known inhibitors to glysosyltransferases typically possess the nucleoside moiety [125]. Trunkfield et al. reported 10 of 18 compounds that inhibited *E. coli* MurG in vitro [126]. A steroid-like compound (murgocil) also inhibited *S. aureus* by locking the binding site of UDP-GlcNAc in MurG [127]; however, stable murgocil-resistant mutants raised, via single non-synonymous mutations near the uracil binding pocket of MurG. But, murgocil bioactivity is restricted to staphylococci rather than other bacterial species [127].

4.3 Penicillin-Binding Proteins (PBPs)

Penicillin-binding proteins (PBPs) catalyze the late stages of peptidoglycan biosynthesis. They come in several variants that perform different functions; some PBPs catalyze the polymerization of the glycan strand (transglycosylation) and the cross-linking between glycan chains (transpeptidation); other PBPs hydrolyze the last D-alanine of stem pentapeptides (DD-carboxypeptidation) or the peptide bond between two glycan strands (endopeptidation). They are classified based on the similarity of their amino acid sequence and their structural features [95]. Low molecular mass PBPs are described as class C PBPs. High molecular mass PBPs are classified as class A or class B PBPs; they are multimodular PBPs responsible for peptidoglycan polymerization and insertion into pre-existing cell wall [128, 129]; their C-terminal penicillin-binding domain has a transpeptidase activity. In class A, the N-terminal

domain has a glycosyltransferase activity; in class B, the N-terminal domain mediates the cell morphogenesis by interacting with other division proteins [130, 131].

Each bacteria has several types of PBPs that were historically numbered according to their migration on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) [95]; for example, *S. aureus* PBP2 is a class A PBP similar to *E. coli* PBP1a and *S. aureus* PBP1 is similar to *E. coli* PBP3. *E. coli* possesses seven class C PBPs, three class A PBPs, and two class B PBPs; the deletion of any of them is not lethal for the bacteria [132]. *Neisseria gonorrhoeae* has only four PBPs [133]. *B. subtilis* produces 16 PBPs [134]. Sensitive strains of *S. aureus* to β-lactam antibiotics have two class B PBPs [135], but resistant strains have two additional insensitive PBPs to β-lactams [136]. Due to their complex life cycle and production of β-lactam molecules, *Streptomyces* spp. produce several PBPs (e.g., 21 PBPs in *S. coelicolor*) at different stages [95]. Thus, identifying which PBPs are essential in bacterial pathogens represents a key step to develop PBPs inhibitors.

β-lactam antibiotics are structurally similar to the natural substrate of transpeptidase (the D-Ala-D-Ala end of the stem pentapeptide precursors), so they bind to PBPs forming inhibited acyl-enzyme [137]. The sensitivity of PBPs to β-lactam antibiotics varies according to the stability of the formed stable acyl-enzyme. The acylation rates of benzylpenicillin with PBPs range from 20 M^{-1} s^{-1} for the penicillin-resistant class B PBPs [138] to 300.000 M^{-1} s^{-1} for class C type-4 PBPs [139]. β-lactam antibiotics covalently linked to the serine active site of the acyl-enzyme PBP4a: the amide group of the side chain is inserted between the asparagine of the second motif and the backbone of β3 strand, the carboxylate of the thiazolidine or dihydrothiazine ring form a hydrogen bond to hydroxyl groups of the KTGT motif, and the carbonyl oxygen lies in the oxyanion hole [140].

Three families of bacterial enzymes recognize β-lactam antibiotics [141]: transpeptidase enzymes (PBPs), which are the targets for antibiotics; β-lactam synthases, which biosynthesize penicillin, cephalosporins and monobactams; and β-lactamases, the degrading enzymes of drug-resistant bacteria. Class B PBPs play a role in the resistance to β-lactams; *S. aureus* PBP2a and *E. faecium* PBP5 can take over the transpeptidase function of all other PBPs [142]. To overcome the resistance to β-lactams, alternative drugs are designed to inhibit the same reaction such as the γ-lactam Lactivicin and its derivatives [143, 144] or boronic acid compounds that mimic the transition state of the enzyme [145].

5 Quorum Sensing as a Target

Quorum sensing (QS) regulates the bacterial gene expression in response to cell density in the bacterial population. In all QS systems, the bacterial cell secretes a signal molecule into the surrounding environment; the concentration of these molecules increases with the growth of the population until a threshold concentration at which it activates a receptor protein to regulate several physiological processes [146]. These processes include antibiotic susceptibility by controlling biofilms formation [147]

and/or transfer of resistance genes as in methicillin resistance *S. aureus* [148] and *S. pneumoniae* [149]. Thus, inhibiting QS would expand the drug targets to several processes, restore susceptibility to conventional antimicrobials, and also preserve the host-microbiome [150]. Strategies to disrupt QS are referred to as quorum quenching (QQ); QQ disrupts QS through inactivating the signal molecule, inhibiting the signal molecule biosynthesis or blocking of the signal transduction.

In Gram-negative bacteria, QS systems commonly employ *N*-acyl-homoserine lactones (AHLs) as a signal molecule [146]. AHL biosynthesis is mediated by LuxI-synthases and bind to a receptor protein belonging to the LuxR-family [146, 151]. AHL-dependent QS can be disrupted by inactivation of AHL either by lactonases or acylases (amidases), which are common in microbes [152]. Lactonases target the conserved homoserine lactone ring of AHLs so it is a broad-spectrum in-activator of AHLs molecules, but acylases target the AHL amide bond so it has substrate specificity [153]. Thus, for example, the expression of a lactonase gene from *Bacillus* in transgenic potato enhanced its resistance against the soft-rot pathogen *Erwinia carotovora* [154]. AHL-dependent QS can also be disrupted by AHL analogs that competitively inhibit the receptors (LuxR proteins) [155, 156]. AHL synthases inhibitors have also been developed; three inhibitors were active against two different acyl-HSL [151].

In addition to the AHLs, *P. aeruginosa* employs 2-alkyl-4-quinolones (AQs) including 2-heptyl-4(1H)-quinolone (HHQ) and 2-heptyl-3-hydroxy-4(1H)-quinoline (PQS) as QS signal molecules [157, 158]. These QS systems regulate virulence genes; hence, they are potential targets. AQ is biosynthesized by PqsABCD enzymes [159]; solving the structure of PqsD [160] allowed the rational design of AQ biosynthesis inhibitors, such as the 2-benzamidobenzoic acid derivatives [161]. In a plant infection of *P. aeruginosa*, AQ-signalling has also inactivated by a recombinant dioxygenase that converts PQS to N-octanoyl anthranilic acid and carbon monoxide. Hence disrupt PQS-controlled genes including virulence genes [162].

In Gram-positive bacteria, QS employs autoinducing peptides (AIPs) as signal molecules to regulate virulence genes [163, 164]. AIPs expressed as pro-peptides that later generate the active QS signal [164]. AIPs signals are transduced by sensor kinases from the membrane to receptors inside the cell, via a phosphorylation cascade [165]. AIP sequences are variable; they consist of seven to nine amino acids with a central cysteine that is covalently linked to the C-terminal amino acid carboxylate. AIP-dependent QS is driven by *agrACBD* operon [165] that activated by *AgrA*. *AgrA* also up-regulates secreted virulence factors and down-regulate surface proteins involved in host cell adhesion and biofilm formation [164, 165]. AIP sequestration allows controlling *S. aureus* virulence [166]; antibodies with high-affinity for AIP-IV reduced virulence factor production and prevented skin abscess in a mouse skin infection model [167]. AIP biosynthesis inhibitors have also been identified including the fungal cyclohexenone metabolite (ambuic acid) [168]. Regarding inhibition of AIP reception, *S. aureus* strains produce four groups of AIP; each AIP activates its specific AgrC receptor [164]. Several studies aimed to develop cross-group inhibitors of all four agr groups; a non-native AIP (*N*-acetylated trAIP-I D2A) inhibits all four agr groups at nanomolar concentrations [169]. Similar approaches have also inhibited

the *fsr* system (similar *agr* system of *E. faecalis*) that induced two pathogenicity-related extracellular proteases [170]. Although AHL- and AIP-dependent QS systems are widespread in bacteria, QS systems controlling conserved virulence across all pathogens have not yet been identified. Thus, designing broad-spectrum QSIs is still unlikely.

6 Other Miscellany Targets

To overcome the current antibiotics crisis, non-essential bacterial processes (like host-pathogen interactions, cell attachment, or immunosuppression) are alternatively targeted; it will provide a new generation of drugs with a long-lasting life. The development of anti-virulence compounds requires a well understanding of the molecular mechanisms involved in host colonization (attachments, invasions, or biofilm formations) and virulence factors production. Anti-virulence compounds (as bicyclic 2-pyridones) target bacterial attachment (an essential stage in urinary tract infections by *E. coli*), via selectively disrupting the biogenesis of P-pili [150]. Other anti-virulence compounds inhibit type III secretion (a virulence strategy to inject proteins into human cells) in *Yersinia* species and *Pseudomonas aeruginosa* [171].

The bacterial ribosome is the target of several classes of antibiotics that block protein synthesis. The RNA-based drug has several advantages; RNA is more accessible than DNA; its structural diversity may provide better selective drugs. Small interfering RNAs (siRNAs) or microRNAs (miRNAs) have been designed for modulating gene expression [172]. However, these strategies would be limited against bacteria by instability in vivo and pharmacokinetic properties.

The folate biosynthesis pathway from GTP to tetrahydrofolate represents potential targets for selective drugs. All organisms require folate cofactors for essential processes such as the synthesis of purines, thymidine, and some amino acids; only bacteria synthesize folate cofactors. Inhibitors of dihydropteroate synthase (sulfonamides and sulfones), and selective inhibitors of dihydrofolate reductase (DHFR, trimethoprim, and analogs) have been used in several infections, either alone or in combinations [173].

Potential antimicrobial targets also include essential precursors and structural components. Lipid II is a specific bacterial membrane-anchored that is essential for PG synthesis. Lipid II is blocked by several antimicrobial agents including glycopeptides (e.g., vancomycin), nisin, ramoplanin, and mannopeptimycins [174], hence inhibit PG biosynthesis.

Teichoic acid represents also a potential target, due to its key roles in bacterial resistance to antimicrobials and host defenses, cell division, maintaining cell shape. Inhibiting teichoic acids synthesis restores the sensitivity of methicillin-resistant *S. aureus* to β-lactams [175]. To inhibit teichoic acids synthesis, the D-alanylation pathway is targeted [122].

7 Evaluation of New Potential Targets (D-Alanyl Carrier Protein Ligase as a Model)

Antimicrobial potential targets should be essential to either the life or pathogenicity of bacteria; it should also be conserved across a range of pathogens; hence, the drug would have a range of applicability.

The D-alanylation of teichoic acid represents a potential antimicrobial target in Gram-positive bacteria [176]; its deficiency enhances cell wall autolysis [177], and bacterial sensitivity to both immune defenses [178, 179] and antibiotics [180, 181]. D-Alanyl carrier protein ligase (DltA) is the key enzyme in the pathway of teichoic acid D-alanylation [182]. Since the first DltA inhibitor suppressed *Bacillus subtilis* [180], DltA has suggested as a potential antimicrobial target of Gram-positive bacteria [183].

Regarding its conservation across Gram-positive pathogenic bacteria, it has a functional [176] and relative structural conservation (Fig. 1). BLAST searches indicated that DltA mainly present in firmicutes (including *Bacillus*, *Staphylococcus*, *Listeria*, *Lactobacillus*, *Streptococcus,* and *Enterococcus*).

The built phylogenetic tree of 65 DltA sequences (retrieved from UniProt database) of Gram-positive pathogens (Fig. 1) divides DltA into two clades (one containing those of spore-forming pathogens and the other containing those of non-spore-forming pathogens), while DltA proteins of *Enterococcus* are distributed between the two clades. The dissimilarity distance between the two clades is less than 0.2, which reflects relative structural conservation. Within the clade of non-spore forming pathogens, streptococci of the pyogenic group (*Streptococcus pyogenes*, Lancefield group A) clustered together with staphylococci, while different serotypes of *Streptococcus pneumonia*e clustered with *Listeria*.

Amino acids alignments of DltA from Gram-positive pathogens showed mainly seven conserved regions; these regions contain intra-genus and sometimes intra-species characteristic conserved residues. Figures 2, 3, 4, 5 and 6 which show the seven conserved regions and indicated the taxa characteristic residues. Regions I-V was located in the N-terminal major domain of DltA, while region VII was located in the C-terminal minor domain (crystal structure of DltA, PDB codes: 3fce and 3fcc); the region VI begins at the inter-domain hinge [188, 189]. Although region III has the most conservation, it has not any active catalytic residues. Except for region III, these conserved regions contained the catalytic binding sites of DltA.

Targets usually have more than one binding site according to their molecular complexity; the blocking of these sites would inhibit the target molecules. Pre-adenylated DltA uses ATP to activate D-alanine and forms the D-alanyl-AMP intermediate then transfers it onto D-alanyl carrier protein [188, 190]. Thus, ATP and D-alanine binding sites represent target sites to inhibit DltA. These sites are conserved within conservative regions I, II, IV, V, VI, VII, as shown by the amino acid sequence logo (Fig. 7).

In region I, T152 and S153 residues catalyze the adenylation step, via their binding to ATP [191]. The D-alanine binding sites (D197 and V301) [189] were located in the

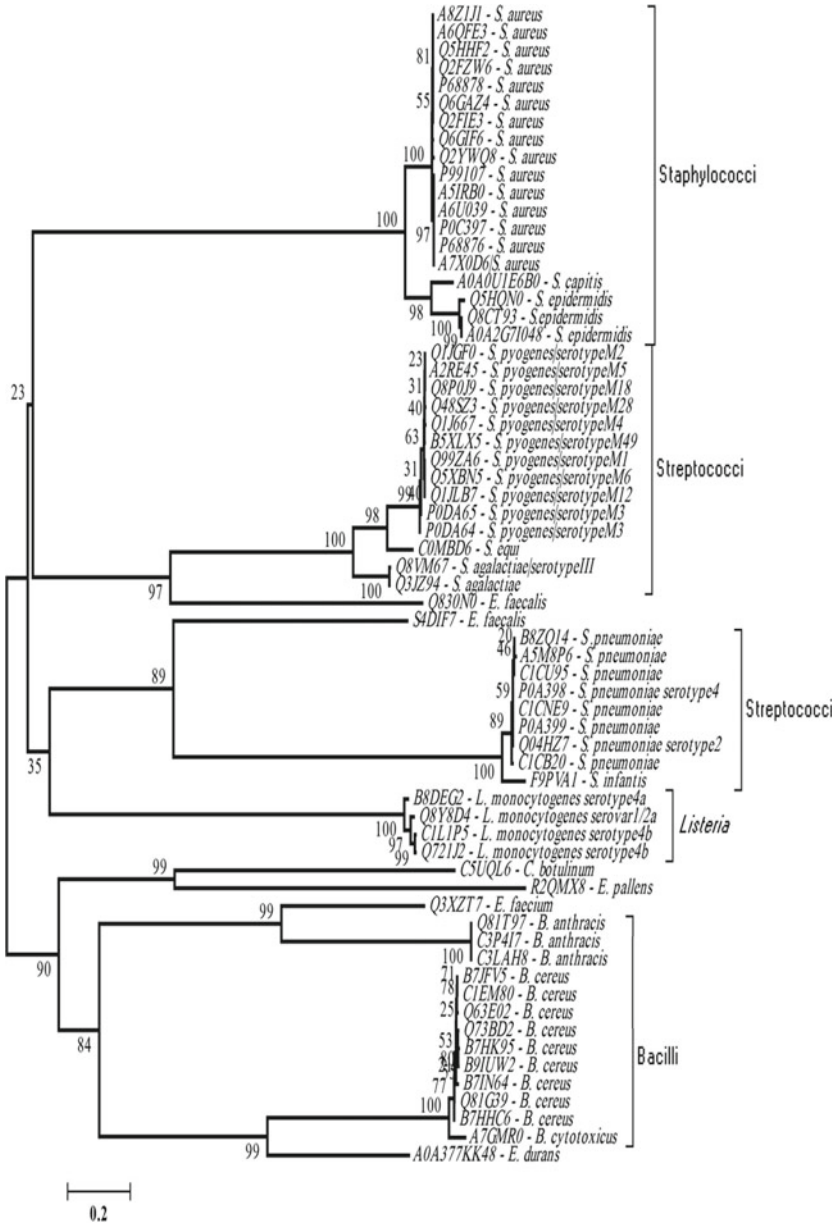


Fig. 1 Phylogenetic tree based on D-alanyl carrier protein ligase (DltA) sequence from 65 taxa of Gram-positive pathogens. The tree was inferred using the neighbor-joining method [184]. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches [185]. The scale represents the dissimilarity distance; distances were computed using the Poisson correction method [186] and are in the units of the number of amino acid substitutions per site. All positions containing gaps were eliminated from the dataset. There were a total of 485 positions in the final dataset. Phylogenetic analyses were conducted in MEGA4 [187]

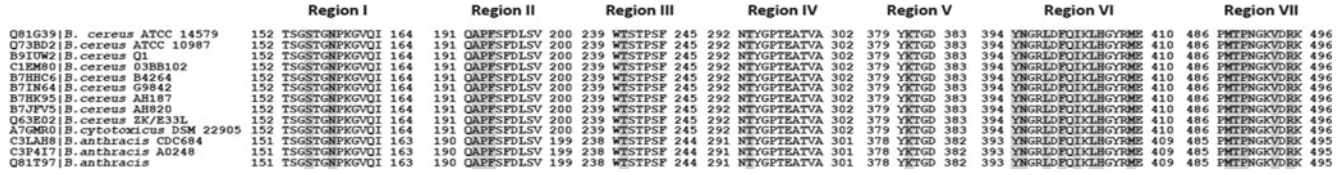


Fig. 2 Conserved sequence regions in D-alanyl carrier protein ligase (DltA) of *Bacillus* spp.; shaded regions indicate intra-genus characteristic conserved residues. The first column denotes the accession numbers of DltA sequences, in the UniProt database, and their bacterial sources

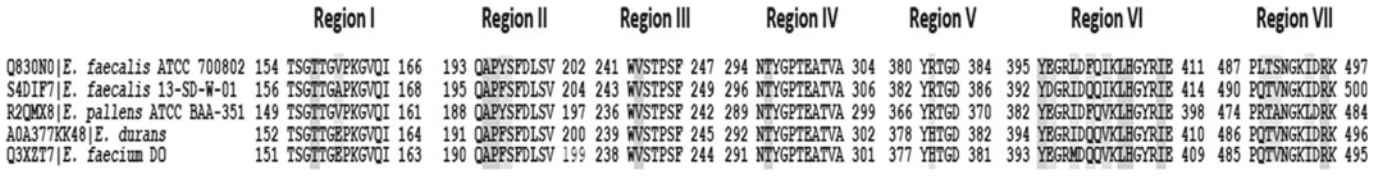


Fig. 3 Conserved sequence regions in D-alanyl carrier protein ligase (DltA) of *Enterococcus* spp.; dark and light gray shaded regions indicate intra-genus characteristic highly and relatively conserved residues, respectively. The first column denotes the accession numbers of DltA sequences, in the UniProt database, and their bacterial sources

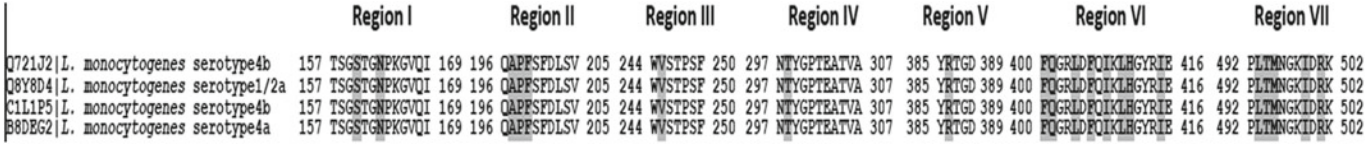


Fig. 4 Conserved sequence regions in D-alanyl carrier protein ligase (DltA) of *Listeria monocytogenes*; shaded regions indicate intra-genus characteristic conserved residues. The first column denotes the accession numbers of DltA sequences, in the UniProt database, and their bacterial sources

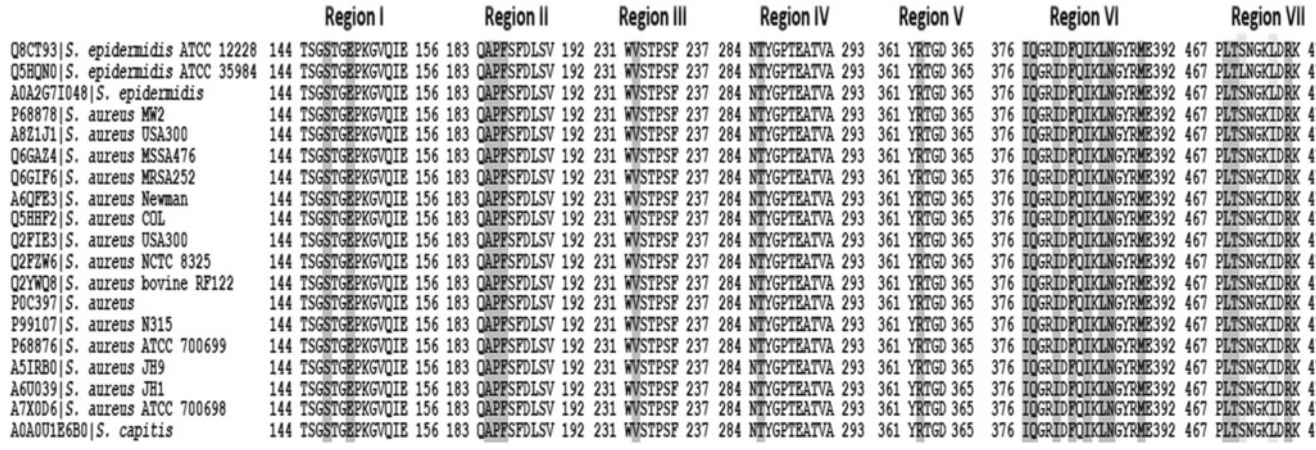


Fig. 5 Conserved sequence regions in D-alanyl carrier protein ligase (DltA) of *Staphylococcus* spp.; dark and light gray shaded regions indicate intra-genus and intra-species characteristic conserved residues, respectively. The first column denotes the accession numbers of DltA sequences, in the UniProt database, and their bacterial sources

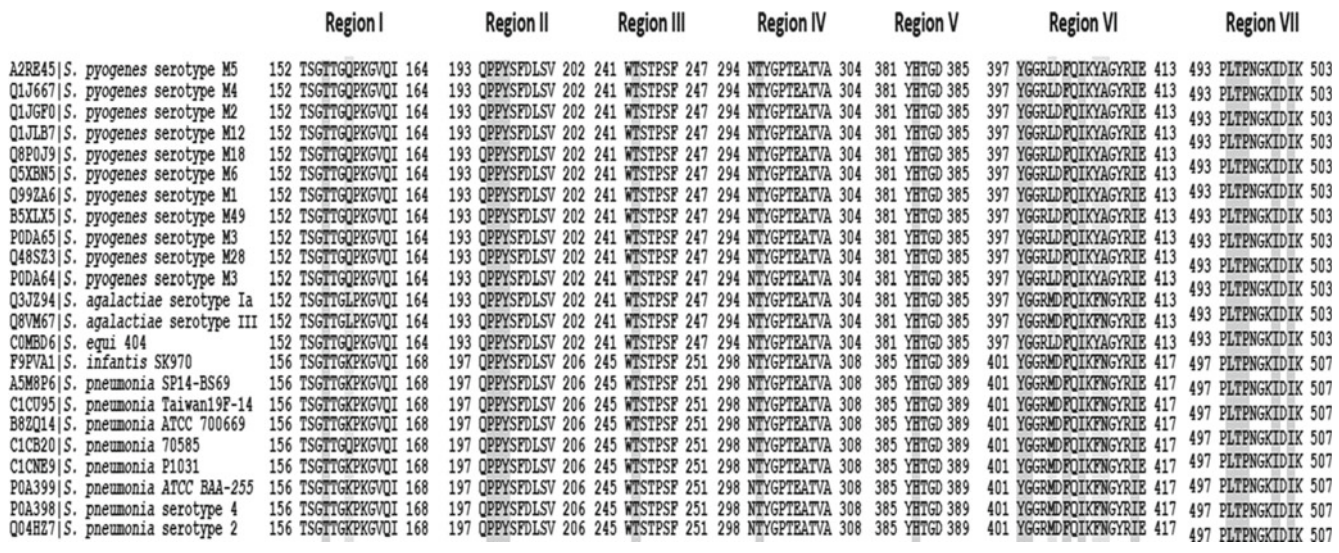


Fig. 6 Conserved sequence regions in D-alanyl carrier protein ligase (DltA) of *Streptococcus* spp.; dark and light gray shaded regions indicate intra-genus and intra-species characteristic conserved residues, respectively. The first column denotes the accession numbers of DltA sequences, in the UniProt database, and their bacterial sources

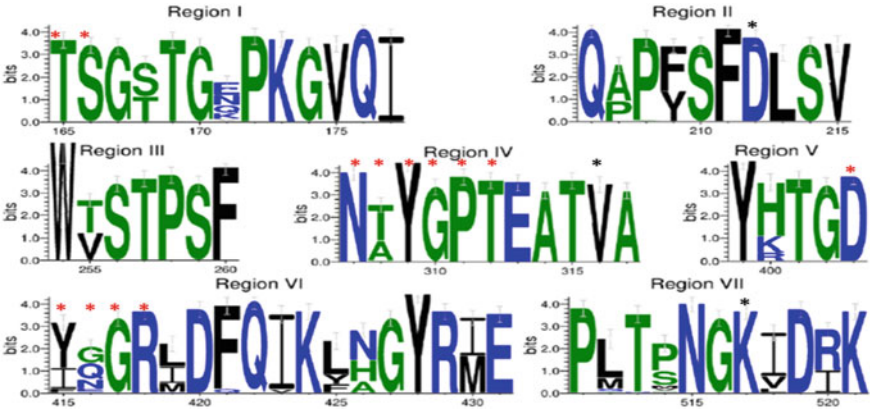


Fig. 7 Sequence logos of D-alanyl carrier protein ligase (DltA) from different Gram-positive pathogens (*Bacillus*, *Staphylococcus*, *Listeria*, *Streptococcus,* and *Enterococcus*). Conserved sequence regions (corresponding to residues of *B. cereus* DltA [189]): CSR-1, residues 152–164; CSR-2, residues 191–200; CSR-3, residues 239–245; CSR-4, residues 292–302; CSR-5, residues 379–383; CSR-6, residues 394–410; CSR-7, residues 486–496. Red and black asterisks signify ATP and D-alanine binding sites, respectively. Sequence logos were created using the WebLogo 3.0 server [195]

conserved regions II and IV, respectively. The region IV also contains the segment N292 to T297 that mediated ATP-DltA binding [192]; this is essential in the pre-adenylation state [182]; following to that segment, Glu298 also stabilizes the DltA conformation for efficient adenylation [189]. In regions V and VI, D383 and the segment Y394-R397 also represent two essential ATP binding sites [188]. In the region VII, only one essential residue (K492) binds to both ATP and D-alanine in the catalysis [189].

These conserved binding sites (Fig. 7) would be preferred sites for rational approaches; these are essential for DltA activity and conserved across the target pathogens, so resistant mutations in these sites are non-expected.

In terms of toxicity, targets for an antibacterial agent are chosen rationally on the basis of differences between the biochemical pathways in bacteria and eukaryotic cells [193]; it should be specific for the prokaryotes without any structural homology in mammalian cells. Structural homologs to DltA, in humans, are limited to beta-alanine-activating enzyme and acyl-CoA synthetase family with less than 27% percent identity (Table 1). However, different isoforms of the human beta-alanine-activating enzyme showed high total scores; thus, the interference of DltA inhibitors to the human beta-alanine-activating enzymes should be practically investigated. That specificity and conservation of DltA active site structure across Gram-positive bacteria suggest that DltA would be a promising target for new Gram-positive bacterial antibiotics. Nevertheless, a practical investigation of DltA homogeneity to the human beta-alanine-activating enzymes is required.

Table 1 The homologous human proteins to D-Alanyl carrier protein ligase (DltA); only experimentally confirmed hits (prefix NM) with alignment score higher than 50 have been listed

Homologous proteins from humans (Homo sapiens)	Total score	*E*-value	Percent identity	Accession number
Beta-alanine-activating enzyme isoform 5	99.0	1e−20	26.32%	NP_001273600.1
Beta-alanine-activating enzyme isoform 6	98.6	1e−20	26.32%	NP_001273601.1
Beta-alanine-activating enzyme isoform 9	97.8	3e−20	26.04%	NP_001310822.1
Beta-alanine-activating enzyme isoform 7	97.8	3e−20	26.32%	NP_001310819.1
Beta-alanine-activating enzyme isoform 1	97.8	3e−20	26.32%	NP_861522.2
Beta-alanine-activating enzyme isoform 8	97.8	3e−20	26.32%	NP_001310821.1
Beta-alanine-activating enzyme isoform 3	97.4	3e−20	26.04%	NP_001273598.1
Beta-alanine-activating enzyme isoform 2	97.4	4e−20	26.32%	NP_001273597.1
Acyl-CoA synthetase family member 3, mitochondrial isoform 1 precursor	85.5	1e−16	26.77%	NP_001120686.1
Acyl-CoA synthetase family member 2, mitochondrial isoform 2 precursor	83.6	8e−16	22.57%	NP_079425.3
Acyl-CoA synthetase family member 2, mitochondrial isoform 1	82.8	1e−15	22.75%	NP_001275897.1
Acyl-CoA synthetase family member 2, mitochondrial isoform 5	82.0	2e−15	25.62%	NP_001275900.1
Acyl-CoA synthetase family member 2, mitochondrial isoform 3	82.4	2e−15	23.19%	NP_001275898.1
Acyl-CoA synthetase family member 2, mitochondrial isoform 4	81.3	3e−15	25.62%	NP_001275899.1
Very long-chain acyl-CoA synthetase isoform 1	58.9	5e−08	21.53%	NP_003636.2

(continued)

Table 1 (continued)

Homologous proteins from humans (Homo sapiens)	Total score	*E*-value	Percent identity	Accession number
Acetyl-coenzyme A synthetase 2-like, mitochondrial isoform 1 precursor	57.4	1e−07	19.52%	NP_115890.2
Acetyl-coenzyme A synthetase 2-like, mitochondrial isoform 2 precursor	54.3	1e−06	20.00%	NP_001239604.1
Acyl-CoA synthetase short-chain family member 3, mitochondrial isoform 2 precursor	54.3	1e−06	22.93%	NP_001317171.1
Acyl-CoA synthetase short-chain family member 3, mitochondrial isoform 1 precursor	53.9	2e−06	22.93%	NP_078836.1

DltA homologs were identified by BLASTP 2.9.0+ searches [194] limited to include homo sapiens (taxid:9606) and exclude bacteria (taxid:2) using *B. cereus* DltA (accession number: Q81G39) as queries, default algorithm parameters of BLOSUM62 Matrix, and filtering low complexity regions

8 Conclusions

Traditional antimicrobial targets are related to essential bacterial processes, so targeting antibiotics to them creates a strong adaptation pressure. Bacteria resist these antibiotics through mutations in the target site or removing the antibiotic out of the cell by efflux pumps; this complicates the problem because any new analogs would also be resisted by the same mechanism; this is the case of second-, third-, and fourth-generation antibiotics that modified after its first-generation with the same mechanism of action.

New essential antimicrobial targets sites (like cell division proteins and some peptidoglycan biosynthesis enzymes), or even non-essential bacterial processes (like host-pathogen interactions and systems of quorum sensing), would allow improving a new generation of drugs with a long-lasting life. These drugs may target those constituents either, via blocking their proteins or macromolecular complexes or, via interacting with specific sequences of their mediated genes. New potential targets (as D-alanyl carrier protein ligase) should accept criteria of presence in a specific spectrum of bacteria, possessing of conservative binding targets, and absence in humans even in any homolog form.

References

1. Lange RP, Locher HH, Wyss PC, Then RL (2007) The targets of currently used antibacterial agents: lessons for drug discovery. Curr Pharm Des 13(30):3140–3154
2. Okano A, Isley NA, Boger DL (2017) Peripheral modifications of [Ψ [CH2NH] Tpg4] vancomycin with added synergistic mechanisms of action provide durable and potent antibiotics. Proc Natl Acad Sci 114(26):E5052–E5061
3. Bromham L (2009) Why do species vary in their rate of molecular evolution? Biol Let 5(3):401–404
4. Dunning Hotopp JC (2011) Horizontal gene transfer between bacteria and animals. Trends Genet (Regular ed) 27(4):157–163
5. Fernandes P (2006) Antibacterial discovery and development—the failure of success? Nat Biotechnol 24(12):1497
6. Cain R, Narramore S, McPhillie M, Simmons K, Fishwick CW (2014) Applications of structure-based design to antibacterial drug discovery. Bioorg Chem 55:69–76
7. Rothfield LI, Justice SS (1997) Bacterial cell division: the cycle of the ring. Cell 88(5):581–584
8. Egan AJ, Vollmer W (2013) The physiology of bacterial cell division. Ann NY Acad Sci 1277(1):8–28
9. Harry E, Monahan L, Thompson L (2006) Bacterial cell division: the mechanism and its precison. Int Rev Cytol 253:27–94
10. Aarsman ME, Piette A, Fraipont C, Vinkenvleugel TM, Nguyen-Distèche M, den Blaauwen T (2005) Maturation of the *Escherichia coli* divisome occurs in two steps. Mol Microbiol 55(6):1631–1645
11. van der Ploeg R, Verheul J, Vischer NO, Alexeeva S, Hoogendoorn E, Postma M, Banzhaf M, Vollmer W, den Blaauwen T (2013) Colocalization and interaction between elongasome and divisome during a preparative cell division phase in *Escherichia coli*. Mol Microbiol 87(5):1074–1087
12. Blaauwen Td, Andreu JM, Monasterio O (2014) Bacterial cell division proteins as antibiotic targets. Bioorg Chem 55:27–38
13. Vaughan S, Wickstead B, Gull K, Addinall SG (2004) Molecular evolution of FtsZ protein sequences encoded within the genomes of archaea, bacteria, and eukaryota. J Mol Evol 58(1):19–29
14. Romberg L, Levin PA (2003) Assembly dynamics of the bacterial cell division protein FtsZ: poised at the edge of stability. Annu Rev Microbiol 57(1):125–154
15. Goehring NW, Gueiros-Filho F, Beckwith J (2005) Premature targeting of a cell division protein to midcell allows dissection of divisome assembly in *Escherichia coli*. Genes Dev 19(1):127–137
16. Margolin W (2005) FtsZ and the division of prokaryotic cells and organelles. Nat Rev Mol Cell Biol 6(11):862
17. Weiss DS (2004) Bacterial cell division and the septal ring. Mol Microbiol 54(3):588–597
18. Anderson DE, Gueiros-Filho FJ, Erickson HP (2004) Assembly dynamics of FtsZ rings in *Bacillus subtilis* and *Escherichia coli* and effects of FtsZ-regulating proteins. J Bacteriol 186(17):5775–5781
19. Meier EL, Goley ED (2014) Form and function of the bacterial cytokinetic ring. Curr Opin Cell Biol 26:19–27
20. Chen Y, Milam SL, Erickson HP (2012) SulA inhibits assembly of FtsZ by a simple sequestration mechanism. Biochemistry 51(14):3100–3109
21. Handler AA, Lim JE, Losick R (2008) Peptide inhibitor of cytokinesis during sporulation in *Bacillus subtilis*. Mol Microbiol 68(3):588–599
22. Läppchen T, Pinas VA, Hartog AF, Koomen G-J, Schaffner-Barbero C, Andreu JM, Trambaiolo D, Löwe J, Juhem A, Popov AV (2008) Probing FtsZ and tubulin with C8-substituted GTP analogs reveals differences in their nucleotide binding sites. Chem Biol 15(2):189–199

23. Matsui T, Yamane J, Mogi N, Yamaguchi H, Takemoto H, Yao M, Tanaka I (2012) Structural reorganization of the bacterial cell-division protein FtsZ from *Staphylococcus aureus*. Acta Crystallogr D Biol Crystallogr 68(9):1175–1188
24. Leung AK, White EL, Ross LJ, Reynolds RC, DeVito JA, Borhani DW (2004) Structure of *Mycobacterium tuberculosis* FtsZ reveals unexpected, G protein-like conformational switches. J Mol Biol 342(3):953–970
25. Oliva MA, Trambaiolo D, Löwe J (2007) Structural insights into the conformational variability of FtsZ. J Mol Biol 373(5):1229–1242
26. Mendieta J, Rico AI, López-Viñas E, Vicente M, Mingorance J, Gómez-Puertas P (2009) Structural and functional model for ionic (K^+/Na^+) and pH dependence of GTPase activity and polymerization of FtsZ, the prokaryotic ortholog of tubulin. J Mol Biol 390(1):17–25
27. Scheffers D-J, de Wit JG, den Blaauwen T, Driessen AJ (2002) GTP hydrolysis of cell division protein FtsZ: evidence that the active site is formed by the association of monomers. Biochemistry 41(2):521–529
28. Thanedar S, Margolin W (2004) FtsZ exhibits rapid movement and oscillation waves in helix-like patterns in *Escherichia coli*. Curr Biol 14(13):1167–1173
29. Popp D, Iwasa M, Narita A, Erickson HP, Maéda Y (2009) FtsZ condensates: an in vitro electron microscopy study. Biopolym: Original Res Biomol 91(5):340–350
30. González JM, Vélez M, Jiménez M, Alfonso C, Schuck P, Mingorance J, Vicente M, Minton AP, Rivas G (2005) Cooperative behavior of *Escherichia coli* cell-division protein FtsZ assembly involves the preferential cyclization of long single-stranded fibrils. Proc Natl Acad Sci 102(6):1895–1900
31. Plaza A, Keffer JL, Bifulco G, Lloyd JR, Bewley CA (2010) Chrysophaentins A–H, antibacterial bisdiarylbutene macrocycles that inhibit the bacterial cell division protein FtsZ. J Am Chem Soc 132(26):9069–9077
32. Marcelo F, Huecas S, Ruiz-Ávila LB, Cañada FJ, Perona A, Poveda A, Martín-Santamaría S, Morreale A, Jiménez-Barbero JS, Andreu JM (2013) Interactions of bacterial cell division protein FtsZ with C8-substituted guanine nucleotide inhibitors. A combined NMR, biochemical and molecular modeling perspective. J Am Chem Soc 135(44):16418–16428
33. Schaffner-Barbero C, Gil-Redondo R, Ruiz-Avila LB, Huecas S, Läppchen T, den Blaauwen T, Diaz JF, Morreale A, Andreu JM (2010) Insights into nucleotide recognition by cell division protein FtsZ from a mant-GTP competition assay and molecular dynamics. Biochemistry 49(49):10458–10472
34. Buske P, Levin PA (2013) A flexible C-terminal linker is required for proper FtsZ assembly in vitro and cytokinetic ring formation in vivo. Mol Microbiol 89(2):249–263
35. Gardner KAA, Moore DA, Erickson HP (2013) The C-terminal linker of *Escherichia col*i FtsZ functions as an intrinsically disordered peptide. Mol Microbiol 89(2):264–275
36. Sundararajan K, Miguel A, Desmarais SM, Meier EL, Huang KC, Goley ED (2015) The bacterial tubulin FtsZ requires its intrinsically disordered linker to direct robust cell wall construction. Nat Commun 6:7281
37. Haney SA, Glasfeld E, Hale C, Keeney D, He Z, de Boer P (2001) Genetic Analysis of the *Escherichia coli* FtsZ·ZipA Interaction in the Yeast Two-hybrid System characterization of ftsz residues essential for the interactions with zipa AND WITH FtsA. J Biol Chem 276(15):11980–11987
38. den Blaauwen T (2013) Prokaryotic cell division: flexible and diverse. Curr Opin Microbiol 16(6):738–744
39. Vicente M, Rico AI (2006) The order of the ring: assembly of *Escherichia coli* cell division components. Mol Microbiol 61(1):5–8
40. Loose M, Mitchison TJ (2014) The bacterial cell division proteins FtsA and FtsZ self-organize into dynamic cytoskeletal patterns. Nat Cell Biol 16(1):38
41. Feucht A, Lucet I, Yudkin MD, Errington J (2001) Cytological and biochemical characterization of the FtsA cell division protein of *Bacillus subtilis*. Mol Microbiol 40(1):115–125
42. Rueda S, Vicente M, Mingorance J (2003) Concentration and assembly of the division ring proteins FtsZ, FtsA, and ZipA during the *Escherichia coli* cell cycle. J Bacteriol 185(11):3344–3351

43. Krupka M, Cabré EJ, Jiménez M, Rivas G, Rico AI, Vicente M (2014) Role of the FtsA C terminus as a switch for polymerization and membrane association. MBio 5(6):e02221–02214
44. Paradis-Bleau C, Sanschagrin F, Levesque RC (2005) Peptide inhibitors of the essential cell division protein FtsA. Protein Eng Des Sel 18(2):85–91
45. Lara B, Rico AI, Petruzzelli S, Santona A, Dumas J, Biton J, Vicente M, Mingorance J, Massidda O (2005) Cell division in cocci: localization and properties of the *Streptococcus pneumoniae* FtsA protein. Mol Microbiol 55(3):699–711
46. Margolin W (2000) Themes and variations in prokaryotic cell division. FEMS Microbiol Rev 24(4):531–548
47. Hale CA, Rhee AC, De Boer PA (2000) ZipA-induced bundling of FtsZ polymers mediated by an interaction between C-terminal domains. J Bacteriol 182(18):5153–5166
48. Mosyak L, Zhang Y, Glasfeld E, Haney S, Stahl M, Seehra J, Somers WS (2000) The bacterial cell division protein ZipA and its interaction with an FtsZ fragment revealed by X-ray crystallography. EMBO J 19(13):3179–3191
49. Kenny CH, Ding W, Kelleher K, Benard S, Dushin EG, Sutherland AG, Mosyak L, Kriz R, Ellestad G (2003) Development of a fluorescence polarization assay to screen for inhibitors of the FtsZ/ZipA interaction. Anal Biochem 323(2):224–233
50. Jennings LD, Foreman KW, Rush TS III, Tsao DH, Mosyak L, Kincaid SL, Sukhdeo MN, Sutherland AG, Ding W, Kenny CH (2004) Combinatorial synthesis of substituted 3-(2-indolyl) piperidines and 2-phenyl indoles as inhibitors of ZipA–FtsZ interaction. Bioorg Med Chem 12(19):5115–5131
51. Rush TS, Grant JA, Mosyak L, Nicholls A (2005) A shape-based 3-D scaffold hopping method and its application to a bacterial protein–protein interaction. J Med Chem 48(5):1489–1495
52. Camberg JL, Hoskins JR, Wickner S (2009) ClpXP protease degrades the cytoskeletal protein, FtsZ, and modulates FtsZ polymer dynamics. Proc Natl Acad Sci 106(26):10614–10619
53. Ye F, Li J, Yang C-G (2017) The development of small-molecule modulators for ClpP protease activity. Mol BioSyst 13(1):23–31
54. Lee B-G, Park EY, Lee K-E, Jeon H, Sung KH, Paulsen H, Rübsamen-Schaeff H, Brötz-Oesterhelt H, Song HK (2010) Structures of ClpP in complex with acyldepsipeptide antibiotics reveal its activation mechanism. Nat Struct Mol Biol 17(4):471
55. Sass P, Josten M, Famulla K, Schiffer G, Sahl H-G, Hamoen L, Brötz-Oesterhelt H (2011) Antibiotic acyldepsipeptides activate ClpP peptidase to degrade the cell division protein FtsZ. Proc Natl Acad Sci 108(42):17474–17479
56. Schmidt KL, Peterson ND, Kustusch RJ, Wissel MC, Graham B, Phillips GJ, Weiss DS (2004) A predicted ABC transporter, FtsEX, is needed for cell division in *Escherichia coli*. J Bacteriol 186(3):785–793
57. Pichoff S, Du S, Lutkenhaus J (2019) Roles of FtsEX in cell division. Res Microbiol (in press)
58. Sham LT, Barendt SM, Kopecky KE, Winkler ME (2011) Essential PcsB putative peptidoglycan hydrolase interacts with the essential FtsXSpn cell division protein in *Streptococcus pneumoniae* D39. Proc Natl Acad Sci 108(45):E1061–E1069
59. Mohamed M, Awad S, Ahmed N (2011) Synthesis and antimicrobial evaluation of some 6-aryl-5-cyano-2-thiouracil derivatives. Acta Pharm 61(2):171–185
60. Taguchi A, Welsh MA, Marmont LS, Lee W, Sjodt M, Kruse AC, Kahne D, Bernhardt TG, Walker S (2019) FtsW is a peptidoglycan polymerase that is functional only in complex with its cognate penicillin-binding protein. Nat Microbiol 4(4):587
61. Mohammadi T, Sijbrandi R, Lutters M, Verheul J, Martin NI, den Blaauwen T, de Kruijff B, Breukink E (2014) Specificity of the transport of lipid II by FtsW in *Escherichia coli*. J Biol Chem 289(21):14707–14718
62. Gonzalez MD, Akbay EA, Boyd D, Beckwith J (2010) Multiple interaction domains in FtsL, a protein component of the widely conserved bacterial FtsLBQ cell division complex. J Bacteriol 192(11):2757–2768
63. Tsang MJ, Bernhardt TG (2015) A role for the FtsQLB complex in cytokinetic ring activation revealed by an ftsL allele that accelerates division. Mol Microbiol 95(6):925–944

64. Choi Y, Kim J, Yoon HJ, Jin KS, Ryu S, Lee HH (2018) Structural insights into the FtsQ/FtsB/FtsL complex, a key component of the divisome. Sci Rep 8(1):18061
65. Bramkamp M, Weston L, Daniel RA, Errington J (2006) Regulated intramembrane proteolysis of FtsL protein and the control of cell division in *Bacillus subtilis*. Mol Microbiol 62(2):580–591
66. Löfmark S, Edlund C, Nord CE (2010) Metronidazole is still the drug of choice for treatment of anaerobic infections. Clin Infect Dis 50(Supplement_1):S16–S23
67. Wagenlehner FM, Wullt B, Perletti G (2011) Antimicrobials in urogenital infections. Int J Antimicrob Agents 38:3–10
68. Baraldi PG, Bovero A, Fruttarolo F, Preti D, Tabrizi MA, Pavani MG, Romagnoli R (2004) DNA minor groove binders as potential antitumor and antimicrobial agents. Med Res Rev 24(4):475–528
69. Pindur U, Jansen M, Lemster T (2005) Advances in DNA-ligands with groove binding, intercalating and/or alkylating activity: chemistry, DNA-binding and biology. Curr Med Chem 12(24):2805–2847
70. Richards AD, Rodger A (2007) Synthetic metallomolecules as agents for the control of DNA structure. Chem Soc Rev 36(3):471–483
71. Waksman SA, Woodruff HB (1940) The soil as a source of microorganisms antagonistic to disease-producing bacteria. J Bacteriol 40(4):581
72. Paramanathan T, Vladescu I, McCauley MJ, Rouzina I, Williams MC (2012) Force spectroscopy reveals the DNA structural dynamics that govern the slow binding of Actinomycin D. Nucleic Acids Res 40(11):4925–4932
73. Zolova OE, Mady AS, Garneau-Tsodikova S (2010) Recent developments in bisintercalator natural products. Biopolymers 93(9):777–790
74. Bolhuis A, Aldrich-Wright JR (2014) DNA as a target for antimicrobials. Bioorg Chem 55:51–59
75. Haq I, Ladbury JE, Chowdhry BZ, Jenkins TC, Chaires JB (1997) Specific binding of Hoechst 33258 to the d (CGCAAATTTGCG) 2 duplex: calorimetric and spectroscopic studies. J Mol Biol 271(2):244–257
76. Cory M, Tidwell RR, Fairley TA (1992) Structure and DNA binding activity of analogs of 1,5-bis(4-amidinophenoxy)pentane (pentamidine). J Med Chem 35(3):431–438
77. Montazerozohori M, Zahedi S, Naghiha A, Zohour MM (2014) Synthesis, characterization and thermal behavior of antibacterial and antifungal active zinc complexes of bis (3(4-dimethylaminophenyl)-allylidene-1,2-diaminoethane. Mater Sci Eng, C 35:195–204
78. Pelton JG, Wemmer DE (1989) Structural characterization of a 2: 1 distamycin Ad (CGCAAATTGGC) complex by two-dimensional NMR. Proc Natl Acad Sci 86(15):5723–5727
79. Ginsburg H, Nissani E, Krugliak M, Williamson DH (1993) Selective toxicity to malaria parasites by non-intercalating DNA-binding ligands. Mol Biochem Parasitol 58(1):7–15
80. Dervan PB, Bürli RW (1999) Sequence-specific DNA recognition by polyamides. Curr Opin Chem Biol 3(6):688–693
81. Morinaga H, Bando T, Takagaki T, Yamamoto M, Hashiya K, Sugiyama H (2011) Cysteine cyclic pyrrole–imidazole polyamide for sequence-specific recognition in the DNA minor groove. J Am Chem Soc 133(46):18924–18930
82. Howson SE, Bolhuis A, Brabec V, Clarkson GJ, Malina J, Rodger A, Scott P (2012) Optically pure, water-stable metallo-helical 'flexicate' assemblies with antibiotic activity. Nat Chem 4(1):31
83. Bhaduri S, Ranjan N, Arya DP (2018) An overview of recent advances in duplex DNA recognition by small molecules. Beilstein J Org Chem 14(1):1051–1086
84. Steffens LS, Nicholson S, Paul LV, Nord CE, Patrick S, Abratt VR (2010) *Bacteroides fragilis* RecA protein overexpression causes resistance to metronidazole. Res Microbiol 161(5):346–354
85. Lomovskaya N, Hong S-K, Kim S-U, Fonstein L, Furuya K, Hutchinson R (1996) The *Streptomyces peucetius drrC* gene encodes a UvrA-like protein involved in daunorubicin resistance and production. J Bacteriol 178(11):3238–3245

86. Watanabe K, Hotta K, Praseuth AP, Koketsu K, Migita A, Boddy CN, Wang CC, Oguri H, Oikawa H (2006) Total biosynthesis of antitumor nonribosomal peptides in *Escherichia coli*. Nat Chem Biol 2(8):423
87. Moore JM, Salama NR (2005) Mutational analysis of metronidazole resistance in *Helicobacter pylori*. Antimicrob Agents Chemother 49(3):1236–1237
88. Reysset G (1996) Genetics of 5-Nitroimidazole resistance in *Bacteroides* species. Anaerobe 2(2):59–69
89. Sparr C, Purkayastha N, Kolesinska B, Gengenbacher M, Amulic B, Matuschewski K, Seebach D, Kamena F (2013) Improved efficacy of fosmidomycin against *Plasmodium* and *Mycobacterium* species by combination with the cell-penetrating peptide octaarginine. Antimicrob Agents Chemother 57(10):4689–4698
90. Matteï PJ, Neves D, Dessen A (2010) Bridging cell wall biosynthesis and bacterial morphogenesis. Curr Opin Struct Biol 20(6):749–755
91. Gautam A, Vyas R, Tewari R (2011) Peptidoglycan biosynthesis machinery: a rich source of drug targets. Crit Rev Biotechnol 31(4):295–336
92. Barreteau H, Kovač A, Boniface A, Sova M, Gobec S, Blanot D (2008) Cytoplasmic steps of peptidoglycan biosynthesis. FEMS Microbiol Rev 32(2):168–207
93. Bouhss A, Trunkfield AE, Bugg TD, Mengin-Lecreulx D (2007) The biosynthesis of peptidoglycan lipid-linked intermediates. FEMS Microbiol Rev 32(2):208–233
94. Crouvoisier M, Mengin-Lecreulx D, van Heijenoort J (1999) UDP-*N*-acetylglucosamine: *N*-acetylmuramoyl-(pentapeptide) pyrophosphoryl undecaprenol *N*-acetylglucosamine transferase from *Escherichia coli*: overproduction, solubilization, and purification. FEBS Lett 449(2–3):289–292
95. Sauvage E, Kerff F, Terrak M, Ayala JA, Charlier P (2008) The penicillin-binding proteins: structure and role in peptidoglycan biosynthesis. FEMS Microbiol Rev 32(2):234–258
96. Bugg TD, Braddick D, Dowson CG, Roper DI (2011) Bacterial cell wall assembly: still an attractive antibacterial target. Trends Biotechnol 29(4):167–173
97. Silver LL (2011) Challenges of antibacterial discovery. Clin Microbiol Rev 24(1):71–109
98. Kong KF, Schneper L, Mathee K (2010) Beta-lactam antibiotics: from antibiosis to resistance and bacteriology. APMIS 118(1):1–36
99. Schneider T, Sahl H-G (2010) An oldie but a goodie–cell wall biosynthesis as antibiotic target pathway. Int J Med Microbiol 300(2–3):161–169
100. Fuda C, Suvorov M, Vakulenko SB, Mobashery S (2004) The basis for resistance to β-lactam antibiotics by penicillin-binding protein 2a of methicillin-resistant *Staphylococcus aureus*. J Biol Chem 279(39):40802–40806
101. Hakenbeck R, Brückner R, Denapaite D, Maurer P (2012) Molecular mechanisms of β-lactam resistance in *Streptococcus pneumoniae*. Future Microbiol 7(3):395–410
102. Tomberg J, Unemo M, Davies C, Nicholas RA (2010) Molecular and structural analysis of mosaic variants of penicillin-binding protein 2 conferring decreased susceptibility to expanded-spectrum cephalosporins in *Neisseria gonorrhoeae*: role of epistatic mutations. Biochemistry 49(37):8062–8070
103. Hu Y, Helm JS, Chen L, Ginsberg C, Gross B, Kraybill B, Tiyanont K, Fang X, Wu T, Walker S (2004) Identification of selective inhibitors for the glycosyltransferase MurG via high-throughput screening. Chem Biol 11(5):703–711
104. Barbosa MD, Ross HO, Hillman MC, Meade RP, Kurilla MG, Pompliano DL (2002) A multitarget assay for inhibitors of membrane-associated steps of peptidoglycan biosynthesis. Anal Biochem 306(1):17–22
105. Branstrom AA, Midha S, Longley CB, Han K, Baizman ER, Axelrod HR (2000) Assay for identification of inhibitors for bacterial MraY translocase or MurG transferase. Anal Biochem 280(2):315–319
106. Hrast M, Sosič I, Šink R, Gobec S (2014) Inhibitors of the peptidoglycan biosynthesis enzymes MurA-F. Bioorg Chem 55:2–15
107. Norris V, Den Blaauwen T, Cabin-Flaman A, Doi RH, Harshey R, Janniere L, Jimenez-Sanchez A, Jin DJ, Levin PA, Mileykovskaya E (2007) Functional taxonomy of bacterial hyperstructures. Microbiol Mol Biol Rev 71 (1):230–253

108. Roos C, Zocher M, Müller D, Münch D, Schneider T, Sahl H-G, Scholz F, Wachtveitl J, Ma Y, Proverbio D (2012) Characterization of co-translationally formed nanodisc complexes with small multidrug transporters, proteorhodopsin and with the *E. coli* MraY translocase. Biochimica et Biophysica Acta (BBA)-Biomembr 1818(12):3098–3106
109. Ma Y, Münch D, Schneider T, Sahl H-G, Bouhss A, Ghoshdastider U, Wang J, Dötsch V, Wang X, Bernhard F (2011) Preparative scale cell-free production and quality optimization of MraY homologues in different expression modes. J Biol Chem 286(45):38844–38853
110. Al-Dabbagh B, Henry X, Ghachi ME, Auger G, Blanot D, Parquet C, Mengin-Lecreulx D, Bouhss A (2008) Active site mapping of MraY, a member of the polyprenyl-phosphate *N*-acetylhexosamine 1-phosphate transferase superfamily, catalyzing the first membrane step of peptidoglycan biosynthesis. Biochemistry 47(34):8919–8928
111. Bouhss A, Crouvoisier M, Blanot D, Mengin-Lecreulx D (2004) Purification and characterization of the bacterial MraY translocase catalyzing the first membrane step of peptidoglycan biosynthesis. J Biol Chem 279(29):29974–29980
112. Shapiro AB, Jahić H, Gao N, Hajec L, Rivin O (2012) A high-throughput, homogeneous, fluorescence resonance energy transfer-based assay for phospho-*N*-acetylmuramoyl-pentapeptide translocase (MraY). J Biomol Screen 17(5):662–672
113. Bernhardt TG, Struck DK, Young R (2001) The lysis protein E of φX174 is a specific inhibitor of the MraY-catalyzed step in peptidoglycan synthesis. J Biol Chem 276(9):6093–6097
114. Tanaka S, Clemons WM Jr (2012) Minimal requirements for inhibition of MraY by lysis protein E from bacteriophage ΦX174. Mol Microbiol 85(5):975–985
115. Winn M, Goss RJ, Kimura K-i, Bugg TD (2010) Antimicrobial nucleoside antibiotics targeting cell wall assembly: Recent advances in structure–function studies and nucleoside biosynthesis. Nat Prod Rep 27(2):279–304
116. Walsh CT, Zhang W (2011) Chemical logic and enzymatic machinery for biological assembly of peptidyl nucleoside antibiotics. ACS Publications
117. Tanino T, Al-Dabbagh B, Mengin-Lecreulx D, Bouhss A, Oyama H, Ichikawa S, Matsuda A (2011) Mechanistic analysis of muraymycin analogues: a guide to the design of MraY inhibitors. J Med Chem 54(24):8421–8439
118. Mihalyi A, Jamshidi S, Slikas J, Bugg TD (2014) Identification of novel inhibitors of phospho-MurNAc-pentapeptide translocase MraY from library screening: isoquinoline alkaloid michellamine B and xanthene dye phloxine B. Bioorg Med Chem 22(17):4566–4571
119. Chung BC, Zhao J, Gillespie RA, Kwon D-Y, Guan Z, Hong J, Zhou P, Lee S-Y (2013) Crystal structure of MraY, an essential membrane enzyme for bacterial cell wall synthesis. Science 341(6149):1012–1016
120. White CL, Kitich A, Gober JW (2010) Positioning cell wall synthetic complexes by the bacterial morphogenetic proteins MreB and MreD. Mol Microbiol 76(3):616–633
121. Ha S, Walker D, Shi Y, Walker S (2000) The 1.9 Å crystal structure of *Escherichia coli* MurG, a membrane-associated glycosyltransferase involved in peptidoglycan biosynthesis. Protein Sc 9(6):1045–1052
122. Hu Y, Chen L, Ha S, Gross B, Falcone B, Walker D, Mokhtarzadeh M, Walker S (2003) Crystal structure of the MurG: UDP-GlcNAc complex reveals common structural principles of a superfamily of glycosyltransferases. Proc Natl Acad Sci 100(3):845–849
123. Brown K, CM Vial S, Dedi N, Westcott J, Scally S, DH Bugg T, A Charlton P, MT Cheetham G (2013) Crystal structure of the *Pseudomonas aeruginosa* MurG: UDP-GlcNAc substrate complex. Protein Peptide Lett 20(9):1002–1008
124. Crouvoisier M, Auger G, Blanot D, Mengin-Lecreulx D (2007) Role of the amino acid invariants in the active site of MurG as evaluated by site-directed mutagenesis. Biochimie 89(12):1498–1508
125. Teo AC, Roper DI (2015) Core steps of membrane-bound peptidoglycan biosynthesis: recent advances, insight and opportunities. Antibiotics 4(4):495–520
126. Trunkfield AE, Gurcha SS, Besra GS, Bugg TD (2010) Inhibition of *Escherichia coli* glycosyltransferase MurG and *Mycobacterium tuberculosis* Gal transferase by uridine-linked transition state mimics. Bioorg Med Chem 18(7):2651–2663

127. Mann PA, Müller A, Xiao L, Pereira PM, Yang C, Ho Lee S, Wang H, Trzeciak J, Schneeweis J, dos Santos MM (2013) Murgocil is a highly bioactive staphylococcal-specific inhibitor of the peptidoglycan glycosyltransferase enzyme MurG. ACS Chem Biol 8(11):2442–2451
128. Born P, Breukink E, Vollmer W (2006) In vitro synthesis of cross-linked murein and its attachment to sacculi by PBP1A from *Escherichia coli*. J Biol Chem 281(37):26985–26993
129. Goffin C, Ghuysen J-M (1998) Multimodular penicillin-binding proteins: an enigmatic family of orthologs and paralogs. Microbiol Mol Biol Rev 62(4):1079–1093
130. Nguyen-Distèche M, Ayala J (2008) Morphogenesis of rod-shaped sacculi. FEMS Microbiol Rev 32(2):321–344
131. Zapun A, Vernet T, Pinho MG (2008) The different shapes of cocci. FEMS Microbiol Rev 32(2):345–360
132. Meberg BM, Sailer FC, Nelson DE, Young KD (2001) Reconstruction of *Escherichia coli* mrcA (PBP 1a) mutants lacking multiple combinations of penicillin binding proteins. J Bacteriol 183(20):6148–6149
133. Stefanova ME, Tomberg J, Davies C, Nicholas RA, Gutheil WG (2004) Overexpression and enzymatic characterization of *Neisseria gonorrhoeae* penicillin-binding protein 4. Eur J Biochem 271(1):23–32
134. Scheffers D-J, Errington J (2004) PBP1 is a component of the *Bacillus subtilis* cell division machinery. J Bacteriol 186(15):5153–5156
135. Pereira S, Henriques A, Pinho M, De Lencastre H, Tomasz A (2007) Role of PBP1 in cell division of *Staphylococcus aureus*. J Bacteriol 189(9):3525–3531
136. Zapun A, Contreras-Martel C, Vernet T (2008) Penicillin-binding proteins and β-lactam resistance. FEMS Microbiol Rev 32(2):361–385
137. Tipper DJ, Strominger JL (1965) Mechanism of action of penicillins: a proposal based on their structural similarity to acyl-D-alanyl-D-alanine. Proc Natl Acad Sci USA 54(4):1133
138. Hujer AM, Kania M, Gerken T, Anderson VE, Buynak JD, Ge X, Caspers P, Page MG, Rice LB, Bonomo RA (2005) Structure-activity relationships of different β-lactam antibiotics against a soluble form of *Enterococcus faecium* PBP5, a type II bacterial transpeptidase. Antimicrob Agents Chemother 49(2):612–618
139. Stefanova ME, Tomberg J, Olesky M, Höltje J-V, Gutheil WG, Nicholas RA (2003) *Neisseria gonorrhoeae* penicillin-binding protein 3 exhibits exceptionally high carboxypeptidase and β-lactam binding activities. Biochemistry 42(49):14614–14625
140. Lim D, Strynadka NC (2002) Structural basis for the β-lactam resistance of PBP2a from methicillin-resistant *Staphylococcus aureus*. Nat Struct Mol Biol 9(11):870
141. Konaklieva MI (2014) Molecular targets of β-lactam-based antimicrobials: beyond the usual suspects. Antibiotics 3(2):128–142
142. Guinane CM, Cotter PD, Ross RP, Hill C (2006) Contribution of penicillin-binding protein homologs to antibiotic resistance, cell morphology, and virulence of *Listeria monocytogenes* EGDe. Antimicrob Agents Chemother 50(8):2824–2828
143. Macheboeuf P, Fischer DS, Brown T Jr, Zervosen A, Luxen A, Joris B, Dessen A, Schofield CJ (2007) Structural and mechanistic basis of penicillin-binding protein inhibition by lactivicins. Nat Chem Biol 3(9):565
144. Brown T Jr, Charlier P, Herman R, Schofield CJ, Sauvage E (2010) Structural basis for the interaction of lactivicins with serine β-lactamases. J Med Chem 53(15):5890–5894
145. Zervosen A, Sauvage E, Frère J-M, Charlier P, Luxen A (2012) Development of new drugs for an old target—the penicillin binding proteins. Molecules 17(11):12478–12505
146. Atkinson S, Williams P (2009) Quorum sensing and social networking in the microbial world. J R Soc Interface 6(40):959–978
147. Hentzer M, Wu H, Andersen JB, Riedel K, Rasmussen TB, Bagge N, Kumar N, Schembri MA, Song Z, Kristoffersen P (2003) Attenuation of *Pseudomonas aeruginosa* virulence by quorum sensing inhibitors. EMBO J 22(15):3803–3815
148. Cheung GY, Wang R, Khan BA, Sturdevant DE, Otto M (2011) Role of the accessory gene regulator agr in community-associated methicillin-resistant *Staphylococcus aureus* pathogenesis. Infect Immun 79(5):1927–1935

149. Zhu L, Lau GW (2011) Inhibition of competence development, horizontal gene transfer and virulence in *Streptococcus pneumoniae* by a modified competence stimulating peptide. PLoS Pathog 7(9):e1002241
150. Rasko DA, Sperandio V (2010) Anti-virulence strategies to combat bacteria-mediated disease. Nat Rev Drug Discov 9(2):117
151. Christensen QH, Grove TL, Booker SJ, Greenberg EP (2013) A high-throughput screen for quorum-sensing inhibitors that target acyl-homoserine lactone synthases. Proc Natl Acad Sci 110(34):13815–13820
152. Chun CK, Ozer EA, Welsh MJ, Zabner J, Greenberg E (2004) Inactivation of a *Pseudomonas aeruginosa* quorum-sensing signal by human airway epithelia. Proc Natl Acad Sci 101(10):3587–3590
153. Hong K-W, Koh C-L, Sam C-K, Yin W-F, Chan K-G (2012) Quorum quenching revisited—from signal decays to signalling confusion. Sensors 12(4):4661–4696
154. Dong Y-H, Wang L-H, Xu J-L, Zhang H-B, Zhang X-F, Zhang L-H (2001) Quenching quorum-sensing-dependent bacterial infection by an *N*-acyl homoserine lactonase. Nature 411(6839):813
155. Mattmann ME, Shipway PM, Heth NJ, Blackwell HE (2011) Potent and selective synthetic modulators of a quorum sensing repressor in *Pseudomonas aeruginosa* identified from second-generation libraries of *N*-acylated L-homoserine lactones. Chem Bio Chem 12(6):942–949
156. O'Loughlin CT, Miller LC, Siryaporn A, Drescher K, Semmelhack MF, Bassler BL (2013) A quorum-sensing inhibitor blocks *Pseudomonas aeruginosa* virulence and biofilm formation. Proc Natl Acad Sci 110(44):17981–17986
157. Heeb S, Fletcher MP, Chhabra SR, Diggle SP, Williams P, Cámara M (2011) Quinolones: from antibiotics to autoinducers. FEMS Microbiol Rev 35(2):247–274
158. Lee J, Wu J, Deng Y, Wang J, Wang C, Wang J, Chang C, Dong Y, Williams P, Zhang L-H (2013) A cell-cell communication signal integrates quorum sensing and stress response. Nat Chem Biol 9(5):339
159. Dulcey CE, Dekimpe V, Fauvelle D-A, Milot S, Groleau M-C, Doucet N, Rahme LG, Lépine F, Déziel E (2013) The end of an old hypothesis: the *Pseudomonas* signaling molecules 4-hydroxy-2-alkylquinolines derive from fatty acids, not 3-ketofatty acids. Chem Biol 20(12):1481–1491
160. Bera AK, Atanasova V, Robinson H, Eisenstein E, Coleman JP, Pesci EC, Parsons JF (2009) Structure of PqsD, a *Pseudomonas* quinolone signal biosynthetic enzyme, in complex with anthranilate. Biochemistry 48(36):8644–8655
161. Weidel E, de Jong JC, Brengel C, Storz MP, Braunshausen A, Negri M, Plaza A, Steinbach A, Müller R, Hartmann RW (2013) Structure optimization of 2-benzamidobenzoic acids as PqsD inhibitors for *Pseudomonas aeruginosa* infections and elucidation of binding mode by SPR, STD NMR, and molecular docking. J Med Chem 56(15):6146–6155
162. Pustelny C, Albers A, Büldt-Karentzopoulos K, Parschat K, Chhabra SR, Cámara M, Williams P, Fetzner S (2009) Dioxygenase-mediated quenching of quinolone-dependent quorum sensing in *Pseudomonas aeruginosa*. Chem Biol 16(12):1259–1267
163. LaSarre B, Federle MJ (2013) Exploiting quorum sensing to confuse bacterial pathogens. Microbiol Mol Biol Rev 77(1):73–111
164. Thoendel M, Kavanaugh JS, Flack CE, Horswill AR (2010) Peptide signaling in the staphylococci. Chem Rev 111(1):117–151
165. Gordon CP, Williams P, Chan WC (2013) Attenuating *Staphylococcus aureus* virulence gene regulation: a medicinal chemistry perspective. J Med Chem 56(4):1389–1404
166. Peterson MM, Mack JL, Hall PR, Alsup AA, Alexander SM, Sully EK, Sawires YS, Cheung AL, Otto M, Gresham HD (2008) Apolipoprotein B is an innate barrier against invasive *Staphylococcus aureus* infection. Cell Host Microbe 4(6):555–566
167. Park J, Jagasia R, Kaufmann GF, Mathison JC, Ruiz DI, Moss JA, Meijler MM, Ulevitch RJ, Janda KD (2007) Infection control by antibody disruption of bacterial quorum sensing signaling. Chem Biol 14(10):1119–1127

168. Nakayama J, Uemura Y, Nishiguchi K, Yoshimura N, Igarashi Y, Sonomoto K (2009) Ambuic acid inhibits the biosynthesis of cyclic peptide quormones in gram-positive bacteria. Antimicrob Agents Chemother 53(2):580–586
169. Lyon GJ, Wright JS, Muir TW, Novick RP (2002) Key determinants of receptor activation in the agr autoinducing peptides of *Staphylococcus aureus*. Biochemistry 41(31):10095–10104
170. Nakayama J, Yokohata R, Sato M, Suzuki T, Matsufuji T, Nishiguchi K, Kawai T, Yamanaka Y, Nagata K, Tanokura M (2013) Development of a peptide antagonist against fsr quorum sensing of *Enterococcus faecalis*. ACS Chem Biol 8(4):804–811
171. Jessen DL, Bradley DS, Nilles ML (2014) A type III secretion system inhibitor targets YopD while revealing differential regulation of secretion in calcium-blind mutants of *Yersinia pestis*. Antimicrob Agents Chemother 58(2):839–850
172. Heidenreich O (2003) RNA siRNAs: magic bullets for functional genomics and therapy? Eur Pharm Rev 8(4):19–28
173. Then R (2004) Antimicrobial dihydrofolate reductase inhibitors-achievements and future options. J Chemother 16(1):3–12
174. Ng V, Chan WC (2016) New found hope for antibiotic discovery: lipid II inhibitors. Chem Eur J 22(36):12606–12616
175. Healy VL, Lessard IA, Roper DI, Knox JR, Walsh CT (2000) Vancomycin resistance in enterococci: reprogramming of the D-Ala–D-Ala ligases in bacterial peptidoglycan biosynthesis. Chem Biol 7(5):R109–R119
176. Percy MG, Gründling A (2014) Lipoteichoic acid synthesis and function in gram-positive bacteria. Annu Rev Microbiol 68:81–100
177. Wecke J, Perego M, Fischer W (1996) D-alanine deprivation of *Bacillus subtilis* teichoic acids is without effect on cell growth and morphology but affects the autolytic activity. Microbial Drug Resist 2(1):123–129
178. Kristian SA, Datta V, Weidenmaier C, Kansal R, Fedtke I, Peschel A, Gallo RL, Nizet V (2005) D-alanylation of teichoic acids promotes group a *streptococcus* antimicrobial peptide resistance, neutrophil survival, and epithelial cell invasion. J Bacteriol 187(19):6719–6725
179. Walter J, Loach DM, Alqumber M, Rockel C, Hermann C, Pfitzenmaier M, Tannock GW (2007) D-Alanyl ester depletion of teichoic acids in *Lactobacillus reuteri* 100-23 results in impaired colonization of the mouse gastrointestinal tract. Environ Microbiol 9(7):1750–1760
180. May JJ, Finking R, Wiegeshoff F, Weber TT, Bandur N, Koert U, Marahiel MA (2005) Inhibition of the D-alanine: D-alanyl carrier protein ligase from *Bacillus subtilis* increases the bacterium's susceptibility to antibiotics that target the cell wall. FEBS J 272(12):2993–3003
181. McBride SM, Sonenshein AL (2011) The dlt operon confers resistance to cationic antimicrobial peptides in Clostridium difficile. Microbiology 157(5):1457–1465
182. Perego M, Glaser P, Minutello A, Strauch MA, Leopold K, Fischer W (1995) Incorporation of D-alanine into lipoteichoic acid and wall teichoic acid in *Bacillus subtilis* identification of genes and regulation. J Biol Chem 270(26):15598–15606
183. Abou-Dobara MI, Omar NF, Diab MA, El-Sonbati AZ, Morgan SM, El-Mogazy MA (2019) Allyl rhodanine azo dye derivatives: Potential antimicrobials target D-alanyl carrier protein ligase and nucleoside diphosphate kinase. J Cell Biochem 120(2):1667–1678
184. Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4(4):406–425
185. Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39(4):783–791
186. Zuckerkandl E, Pauling L (1965) Evolutionary divergence and convergence in proteins. In: Evolving genes and proteins. Elsevier, pp 97–166
187. Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol Biol Evol 24(8):1596–1599
188. Du L, He Y, Luo Y (2008) Crystal structure and enantiomer selection by D-alanyl carrier protein ligase DltA from *Bacillus cereus*. Biochemistry 47(44):11473–11480
189. Osman KT, Du L, He Y, Luo Y (2009) Crystal Structure of *Bacillus cereus* D-Alanyl Carrier Protein Ligase (DltA) in Complex with ATP. J Mol Biol 388(2):345–355

190. Reichmann NT, Cassona CP, Gründling A (2013) Revised mechanism of D-alanine incorporation into cell wall polymers in Gram-positive bacteria. Microbiology 159(9):1868–1877
191. Stuible H-P, Büttner D, Ehlting J, Hahlbrock K, Kombrink E (2000) Mutational analysis of 4-coumarate: CoA ligase identifies functionally important amino acids and verifies its close relationship to other adenylate-forming enzymes. FEBS Lett 467(1):117–122
192. Yonus H, Neumann P, Zimmermann S, May JJ, Marahiel MA, Stubbs MT (2008) Crystal structure of DltA implications for the reaction mechanism of non-ribosomal peptide synthetase adenylation domains. J Biol Chem 283(47):32484–32491
193. Gwynn MN, Portnoy A, Rittenhouse SF, Payne DJ (2010) Challenges of antibacterial discovery revisited. Ann NY Acad Sci 1213(1):5–19
194. Altschul SF, Wootton JC, Gertz EM, Agarwala R, Morgulis A, Schäffer AA, Yu YK (2005) Protein database searches using compositionally adjusted substitution matrices. FEBS J 272(20):5101–5109
195. Crooks GE, Hon G, Chandonia J-M, Brenner SE (2004) WebLogo: a sequence logo generator. Genome Res 14(6):1188–1190

Chapter 4
Carbon Nanotube-Based Antimicrobial and Antifouling Surfaces

R. Teixeira-Santos, M. Gomes, and F. J. Mergulhão

1 Introduction

Carbon nanotubes (CNTs) were first introduced in 1991 by Lijima [1]. These carbon nanomaterials have a small, thin, hollow, and concentric cylindrical structure which is closed at both ends [2]. Carbon nanotubes can be classified as single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs). Single-walled carbon nanotubes consist of a single graphene layer wrapped in a seamless cylinder, whereas MWCNTs are composed by multiple graphene layers wrapped to form concentric tubes [2, 3].

Carbon nanotubes are attractive nanomaterials because of their outstanding properties, such as excellent electrical and thermal conductivity, high tensile strength, high hydrophobicity, microbial immobilization potential, and ability to blend with other materials to form nanocomposites (NC) [2–7]. Therefore, because of their unusual properties, there has been a vast interest in exploiting CNTs for several applications (Fig. 1).

In the last decade, CNTs were introduced in pharmaceutical and medical fields. The chemical stability of CNTs enables them to adsorb or conjugate with a wide variety of therapeutic molecules (proteins, antibodies, DNA, enzymes, drugs) acting as vehicles for drug delivery [8]. CNTs have also been used for the construction of biosensors for the detection of biomolecules and biological cells, tissue engineering, and neuronal interfaces [2, 8]. In addition, due to their antimicrobial activity, CNTs have been used in the fabrication of biomedical devices and prosthetic implants [9, 10].

Recently, the combination of CNTs and antimicrobial drugs or other bioactive molecules appears to be a promising strategy to fight antimicrobial resistance and develop new options in antimicrobial therapy [11–13].

R. Teixeira-Santos · M. Gomes · F. J. Mergulhão (✉)
LEPABE - Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal
e-mail: filipem@fe.up.pt

S. Snigdha et al. (eds.), *Engineered Antimicrobial Surfaces*, Materials Horizons: From Nature to Nanomaterials, https://doi.org/10.1007/978-981-15-4630-3_4

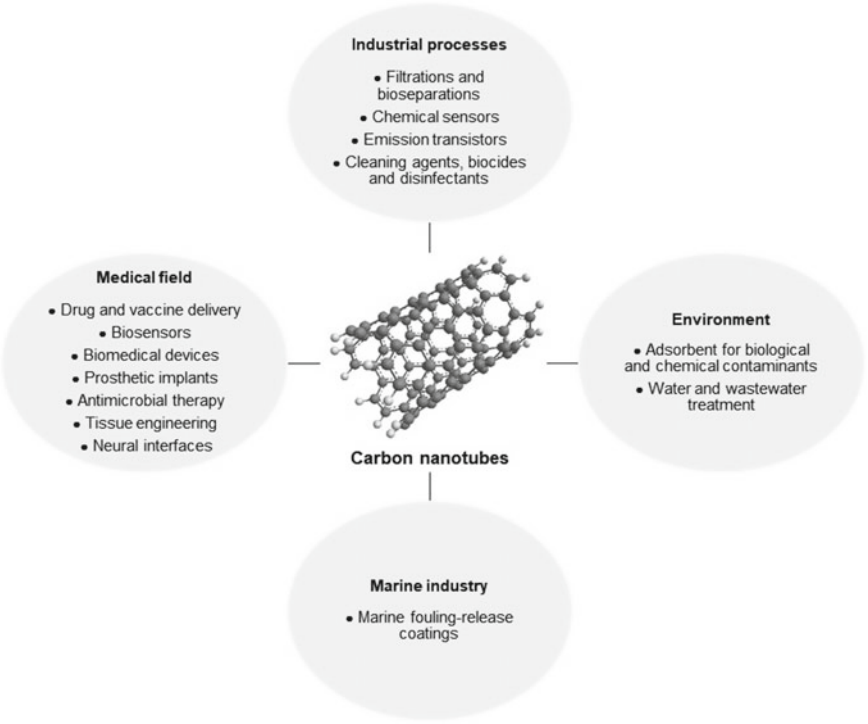


Fig. 1 Main applications of carbon nanotube-based surfaces

Prior to the scientific interest in the utilization of CNTs on biomedical applications, their mainstream use was in the industrial field. CNTs have been used to produce emission transistors and chemical sensors and to develop membranes for filtration and other separation processes [7, 14]. In addition, they have also been used to produce cleaning agents, biocides, and disinfectants for numerous industrial processes [15].

Moreover, the antibiofouling properties of CNTs allowed their application in the marine industry. Fouling on ship hulls decreases speed and increases fuel consumption. Up to date, several studies have proposed CNTs as good candidates for the development of fouling-release coatings against microalgae and barnacles [16–18].

Lastly, because of their antimicrobial and antifouling properties, CNTs have also been applied in water and wastewater treatment, and as absorbents for biological and chemical contaminants [19–22].

In this chapter, the antimicrobial and antifouling properties of CNTs will be reviewed using published studies. Additionally, based on collected data, the development of new CNT surfaces and their potential medical applications are discussed.

2 Antimicrobial and Antifouling Properties of Pristine CNTs

Carbon nanotubes are some of the most attractive nanomaterials for the development of antimicrobial and antifouling surfaces. The antimicrobial activity of CTNs depends on multiple factors related to their structure and composition such as (1) size and length; (2) physical disposition (aggregated or dispersed); and (3) the number of layers (single- or multi-walled) [6, 23, 24]. Table 1 lists several studies demonstrating the antimicrobial activity of pristine single- and multi-walled CNTs against different bacterial species.

In 2007, Kang et al. provided for the first time the evidence that pristine single-walled CNTs exhibit strong antimicrobial activity, inducing cell membrane damage by direct contact and, thus, reducing cell viability by 80% [25]. Since then, different mechanisms have been proposed to explain the toxicity of CNTs.

In 2008, a study involving gene expression analysis demonstrated that cell membrane damage is the main CNT-biocidal mechanism. According to the authors, bacteria exposed to CNTs suffer oxidative stress, followed by cell membrane damage and, ultimately, the release of intracellular content [6]. Nagai and Toyokuni considered that the cell membrane damage occurs through direct piercing of the bacterial surface [26]. Previously, Kang et al. reported that the length of CNTs plays a crucial role during their interactions with the cell membrane, where shorter tubes exhibit higher toxicity compared to longer tubes [6, 27]. Aslan et al. also demonstrated that shorter SWCNTs are more toxic due to higher density of open tube ends [9]. Similarly, smaller diameters were shown to induce accentuated cell membrane damage through the cell surface interaction [28]. On the other hand, studies have also postulated that bacterial death is caused by agglomerated nanotube networks trapping the cell surface, a phenomenon that triggers oxidative stress and inhibits bacterial growth [29, 30]. According to Arias and Yang, CNTs with large diameter (15–30 nm) mostly interact with bacteria through their sidewalls [4].

Likewise, several authors have demonstrated that SWCNTs exhibit more toxic effects in bacteria than MWCNTs [6, 24]. Indeed, SWCNTs showed a strong effectiveness in piercing the bacterial membrane [24]. In a study by Kang et al., it was shown with *Escherichia coli* that after incubation for 1 h with MWCNTs, most of the cells were still intact (Fig. 2a), whereas with SWCNTs, the majority of cells lost their integrity and became flattened (Fig. 2b) [6]. Additionally, the same authors also showed that in the presence of both MWCNTs and SWCNTs, *E. coli* expresses high levels of stress-related genes. Although most of the genes expressed in cells exposed to MWCNTs are also expressed in cells exposed to SWCNTs (Fig. 3), the quantity and magnitude of expression were much higher with SWCNTs [6].

Conversely, Young et al. described that MWCNTs have higher toxicity for bacteria than SWCNTs [31]. Despite the discrepant findings, the antimicrobial activity of single- and multi-walled CNTs have been demonstrated against a broad range of species including *Lactobacillus acidophilus*, *Bifidobacterium adolescentis*, *Enterococcus faecalis*, *Staphylococcus aureus* and *Escherichia coli* [6, 24, 25]. However,

Table 1 Studies demonstrating the antimicrobial and antifouling activities of pristine carbon nanotubes

Property	Wall type	Species	Main conclusions	Refs.
Antimicrobial	Single	*E. coli*	Bacteria exposed to CNTs for 1 h exhibited a substantial loss in their viability (80%).	[25]
	Single and multi	*E. coli*	The percentage of inactivated cells attached to SWNT (80%) was higher than MWNTs (24%).	[6]
	Single and multi	*L. acidophilus* *B. adolescentis* *E. coli* *E. faecalis* *S. aureus*	CNTs demonstrated a significant and dose-dependent antibacterial activity against Gram-positive or Gram-negative bacteria when compared to the control ($p < 0.01$ or $p < 0.05$).	[24]
	Multi	*E. coli*	The MIC values obtained for MWCNTs were very high, indicating low toxicity for bacteria.	[15]
	Multi	*E. coli* *P. aeruginosa* *B. subtilis*	The viability study showed significant MWCNT toxicity (2-log reduction in cell density) against *E. coli*, *P. aeruginosa* and *B. subtilis*.	[33]
Antimicrobial and antifouling	Multi	*P. fluorescens*	The percentage of inactivated bacteria exposed to MWCNTs was 44%. Results showed that CNTs have a significant effect on the inhibition of bacterial adhesion under electrochemical potential.	[32]

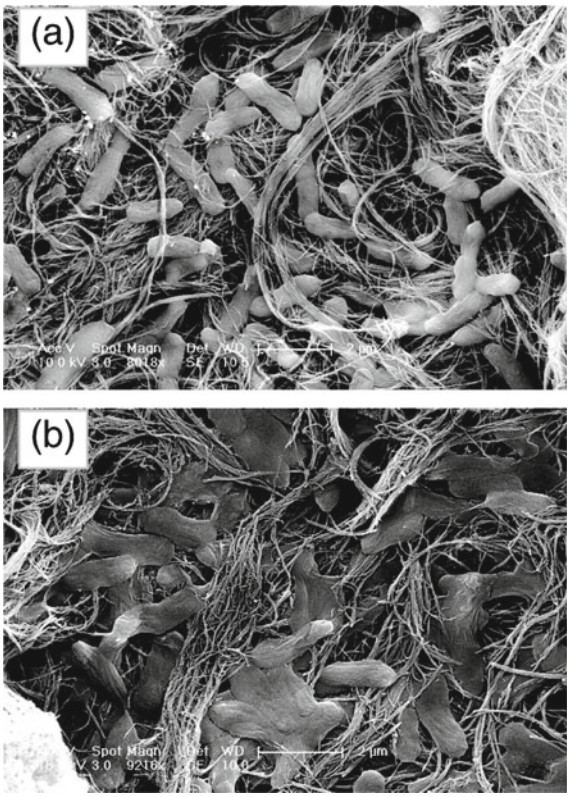


Fig. 2 Scanning electron microscopy (SEM) images of *E. coli* cells exposed to CNTs. **a** Cells incubated with MWCNTs for 60 min. **b** Cells incubated with SWCNTs for 60 min. The bars in both images represent 2 μm. Reprinted with permission from Kang et al. [6]. Copyright 2008 American Chemical Society

CNTs appear to display a selective activity, presenting lower toxicity against rod-like bacteria than spherical ones. This result suggests that their action may also depend on the shapes of bacteria [24].

Recently, the toxicity of MWCNTs was also evaluated for *Bacillus subtilis, Pseudomonas fluorescens* and *Pseudomonas aeruginosa* [32, 33]. The viability studies showed that the percentage of inactivated cells was significant and dependent on the concentration of CNTs [33]. In opposition, Vassallo et al. tested the antimicrobial activity of MWCNTs and obtained high MIC (minimal inhibitory concentration) values (> 100 mg/L), suggesting that MWCTNs display low toxicity for bacteria [15]. Thus, the antimicrobial activity of CNTs depends on a multiplicity of factors that may be modulated according to the desired application.

In the last decade, several studies have been developed with the purpose of improving the antimicrobial activity of CNTs. Their results will be further explored in the following sections.

The antibiofouling properties of CNTs also make them an attractive nanomaterial for a wide range of applications. Fouling can be inhibited by different mechanisms such as (1) increase of biocidal activity; (2) increase in resistance to protein adhesion; and (3) increase in resistance to other fouling components at the material surface [2].

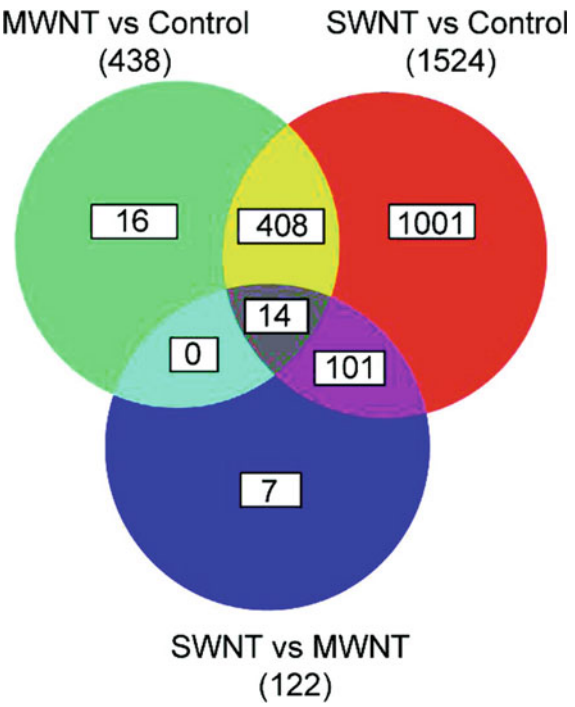


Fig. 3 Number of genes induced or repressed more than twofold for cells exposed to SWNTs and MWNTs compared to the culture without CNTs (control). Reprinted with permission from Kang et al. [6]. Copyright 2008 American Chemical Society

The effects of CNTs on biofilm formation have been addressed in many studies in order to evaluate their potential to inhibit microorganism attachment and proliferation at different stages (Table 1) [27]. Malek et al. reported that the biofilm inhibition increases with the increasing CNT length, proposing that longer CNTs are more flexible, which may prevent microbial attachment [34]. Zhang et al. showed that pristine multi-walled CNTs have a significant effect on the inhibition of *P. fluorescens* adhesion upon application of an electrochemical potential. However, this effect can be increased through surface modification of the MWCNTs [32]. Hence, the antifouling potential of CNTs may be improved by their modification or association with different materials.

3 Development of CNT-Based Antimicrobial and/or Antifouling Surfaces

Despite the promising antimicrobial and antifouling properties of pristine CNTs, their practical application is limited essentially due to their hydrophobic nature [2]. The modification of CNTs and/or their association with materials such as polymers, metals, or biomolecules results in a nanocomposite (NC) which may have improved activity. Simultaneously, the functionalization of CNTs can help in their dispersion

in different matrices, increase biocompatibility, and decrease toxicity for human cells [2].

3.1 Single-Walled CNT Surfaces

The section above clearly demonstrated that SWNTs interact with microorganisms and exhibit strong antimicrobial properties. Likewise, their potential to inhibit the adhesion of organisms and other molecules should be highlighted. These observations point to the use of SWCNTs as building blocks for the development of antimicrobial and/or antifouling surfaces. Thus, the present section intends to explore the effect of functionalized SWCNTs or their nanocomposites on the improvement of these properties. Table 2 provides a description of surface modifications made to SWCNTs in order to increase their blending capacity in different materials, their antimicrobial and antifouling potential against several species.

As previously mentioned, CNTs can be functionalized, for instance, with acid or carboxyl moieties in order to increase their interaction with bacterial cells and the formation of bacterial–CNT aggregates [4]. Studies have reported that bacterial binding is facilitated upon CNT functionalization [2, 35]. Arias and Yang investigated the effects of different SWCNT surface functional groups (–OH, –COOH, and $–NH_2$) on their antimicrobial activity against both Gram-negative (*Salmonella typhimurium*) and Gram-positive bacteria (*B. subtilis and S. aureus*). Results showed that SWCNTs functionalized with –OH and –COOH groups exhibited a strong antimicrobial activity (7-log reduction), whereas the SWCNTs with $-NH_2$ groups only displayed antimicrobial activity at higher concentrations. Although functionalization of the SWCNTs promoted bacteria–SWCNTs interactions regardless of the surface group, the antimicrobial activity occurred in a selective way [4].

Several studies have reported the antimicrobial activity of silver and other noble metals and their potential to prevent and control healthcare-associated infections [2, 36]. Chaudhari et al. evaluated the antimicrobial activity of silver-coated SWCNTs functionalized with antimicrobial peptides (AMPs) against *S. aureus* using a skin model. In the skin treated with functionalized silver-CNTs, the bacterial proliferation was significantly inhibited (10^5 cfu/g) compared to non-treated skin (10^8 cfu/g) [12]. Silver nanoparticles (NP) have the capability to bind to the bacterial cell wall and penetrate it, causing changes in membrane permeability and, consequently, cell death. The production of reactive oxygen species may also be a consequence of silver NP action. [37]. Simultaneously, it is known that AMPs display a broad-spectrum antimicrobial activity toward bacteria, fungi, and viruses [36]. Thus, the synergic association of silver NP with AMPs enhanced the toxicity of SWCNTs. These observations may be helpful to develop new antimicrobial therapies [12].

Carbon nanotubes can be also functionalized with natural antimicrobial enzymes such as lysozyme (LSZ), increasing their toxicity to bacteria [38]. The antimicrobial activity of LSZ has been previously described with its mechanism of action consisting of the lyse of the cell wall by hydrolyzing the β-1,4 linkage between *N*-acetylmuramic

Table 2 Studies reporting the development of SWCNT-based surfaces and their interaction with different bacterial species

Property	Material blend	Species	Main conclusions	Refs.
Antimicrobial	**Functionalized CNTs** CNTs with different surface groups (–OH, –COOH, and $–NH_2$)	*S. typhimurium* *B. subtilis* *S. aureus*	SWNTs with -OH and –COOH surface groups exhibited strong antimicrobial activity to both Gram-positive and Gram-negative bacteria (7 log reduction). SWNTs-NH_2 only exhibited antimicrobial activity at higher concentrations.	[4]
	Silver Silver-coated CNTs functionalized with antimicrobial peptides (TP359, TP226 and TP557)	*S. aureus*	The bacterial viability increased 4 log in the non-treated skin model, whereas skin treated with functionalized silver-coated CNTs exhibited an increase of only 1 log (from 10^4 to 10^5 cfu/g).	[12]
	Enzymes CNTs with lysozyme (LSZ) and DNA (layer-by-layer)	*M. lysodeikticus* *S. aureus*	Coating terminating in a LSZ-SWCNT layer exhibited high antimicrobial activity (84% reduction in cell density).	[38]
	Antimicrobial peptides Antimicrobial peptides (TP359, TP226 and TP557)-functionalized silver-coated CNTs	*S. aureus*	Functionalized silver-coated CNTs inhibited *S. aureus* proliferation on a skin model.	[12]
	Polymers CNTs incorporated within poly(lactic-co-glycolic acid)	*E. coli* *S. epidermidis*	The bacterial metabolic activity was significantly diminished in the presence of SWNT-PLGA. Up to 98% of bacteria die within 1 h on SWNT-PLGA versus 15–20% on pure PGLA.	[9]

(continued)

Table 2 (continued)

Property	Material blend	Species	Main conclusions	Refs.
	Polymers Polyvinyl-*N*-carbazole (PVK, 97 wt%)/CNTs (3 wt%) composite	*E. coli* *B. subtilis*	PVK-SWNT composite induced high bacterial inactivation (94% for *E. coli* and 90% for *B. subtilis*) in planktonic cells. PVK-SWNT-coated surfaces demonstrated a significant reduction of biofilm growth.	[41]
	CNTs layer-by-layer assembled with the polyelectrolytes poly(L-lysine) (PLL) and poly(L-glutamic acid) (PGA)	*E. coli* *S. epidermidis*	SWNT/PLL/PGA films demonstrated higher inhibition rates (up to 90%) for *E. coli* and *S. epidermidis* compared to control films (PLL/PGA, 20%).	[42]
	Oxidized-CNT/Poly(vinyl alcohol) (PVOH) composite	*P. aeruginosa*	The viability of cells deposited on O-SWCNT/PVOH surfaces decreased exponentially with increasing CNT loading.	[43]
	CNTs/Porphyrin nanocomposite	*S. aureus*	CNTs/porphyrin nanocomposite induced cell membrane damage in the presence of visible light.	[44]
	Functionalized CNT copolymer of star-shaped poly(ε-caprolactone) (stPCL) and poly (ethylene glycol) (PEG) composite	*P. aeruginosa* *S. aureus*	The CNT/stPCL-PEG copolymer inhibited the proliferation of *S. aureus* and *P. aeruginosa* but to a lower extent than the pure polymer matrix.	[45]
Antimicrobial and antifouling	**Polymers** CNTs covalently bound to polyamide membranes	*E. coli*	SWNT membranes achieved up to 60% inactivation of the attached bacteria after 1 h of contact time. Additionally, SWNTs delayed the onset of membrane biofouling during operation.	[46]

acid (NAM) and *N*-acetylglucosamine (NAG) on peptidoglycan [2, 39, 40]. Nepal et al. evaluated the antimicrobial activity of LSZ-functionalized SWCNTs against Gram-positive bacteria (*Micrococcus lysodeikticus* and *S. aureus*). It was observed that this SWCNT composite exhibited a high biocidal activity toward the tested bacteria [38].

The association of SWCNTs with polymers to form nanocomposites has been vastly explored. Aslan et al. incorporated SWCNTs within poly(lactic-co-glycolic acid) (PLGA) matrix and evaluated its activity against *E. coli* and *Staphylococcus epidermidis*. Bacteria exposed to the SWCNTs-PLGA decreased their metabolic activity and viability (98% of cell reduction compared to 15–20% obtained for pure PLGA) [9]. The association of polyvinyl-N-carbazole with SWCNTs resulted in higher bacterial inactivation of planktonic cells (94% for *E. coli* and 90% for *B. subtilis*), and surfaces coated with this NC also demonstrated a significant reduction of biofilm formation [41]. Likewise, poly(L-lysine) and poly(L-glutamic acid) used to form SWNT-NC presented high inactivation values (up to 90%) for *E. coli* and *S. epidermidis* [42]. Goodwin and co-workers prepared a SWCNT-poly(vinyl alcohol) composite and investigated its activity against *P. aeruginosa.* The viability of bacteria adhered to this surface decreased exponentially with increasing SWCNT concentrations [43].

Recently, Sah et al. explored the potential of photosensitive molecules like porphyrins to produce a SWCNT-NC with biocidal activity against *S. aureus.* The bacteria–NC interaction in the presence of visible light induced cell membrane damage [44]. Conversely, the functionalized SWCNTs/copolymer of poly(ε-caprolactone) (stPCL) and poly (ethyleneglycol) (PEG) composite did not show antimicrobial activity [45].

While some SWCNT-composites display antimicrobial activity, others show a combination of antimicrobial and antifouling properties. Tiraferri et al. demonstrated that SWCNTs covalently bound to polyamide membranes inactivated 66% of attached bacteria and delayed the onset of membrane biofouling, which may be helpful in the filtration process [46].

Although SWCNTs have demonstrated promising results in antimicrobial and antifouling surfaces, the number of published studies is limited when compared to MWCNTs.

3.2 Multi-walled CNT Surfaces

Multi-walled carbon nanotubes have been vastly explored and applied in various sectors due to their favorable properties. Up to date, several studies about their antimicrobial potential were published. Table 3 describes the studies carried out during the last decade regarding the biocidal effect of MWCNTs and their interaction with a wide range of bacterial and fungal species.

In order to improve the interactions between CNTs and microorganisms, the functionalization of MWCNTs is a common procedure. Several studies reported on

Table 3 Studies reporting the development of MWCNT-based antimicrobial surfaces and their interaction with different bacteria and fungi

Material blend	Species	Main conclusions	Refs.
Functionalized CNTs CNTs functionalized with –OH, –COOH, and $–NH_2$ surface groups	*S. typhimurium* *B. subtilis* *S. aureus*	MWCNTs functionalized with –OH, –COOH, and NH_2 did not display significant antimicrobial activity against all tested bacteria.	[4]
	E. coli *P. aeruginosa* *S. aureus*	The bacterial inactivation percentage of MWCNT-COOH was 34.1, 26.9, and 22.8% for *E. coli*, *P. aeruginosa*, and *S. aureus*, respectively.	[47]
	E. coli *S. aureus* *B. subtilis*	The bacterial inactivation percentage of MWCNT-COOH was 30, 50, and 40% for *B. subtilis*, *S. aureus*, and *E. coli*, respectively.	[3]
	E. coli *P. aeruginosa* *S. aureus*	The bacterial inactivation percentage of MWCNT –COOH was 20 ± 0.8, 26.8 ± 1.1, and 14.7 ± 0.5% for *E. coli*, *P. aeruginosa*, and *S. aureus*, respectively.	[48]
	L. acidophilus *B. adolescentis* *E. coli* *E. faecalis* *S. aureus*	MWCNT-OH and MWCNT-COOH induced significant and dose-dependent antibacterial activity against all tested bacteria.	[24]
	V. parahaemolyticus	The antimicrobial activity of f-MWCNTs was time dependent. f-MWCNTs mostly wound around surfaces of *V. parahaemolyticus* cells instead of piercing into the bacterial cells.	[49]

(continued)

Table 3 (continued)

Material blend	Species	Main conclusions	Refs.
CNTs functionalized with ethanolamine (MEA, DEA, and TEA)	*E. coli* *K. pneumoniae* *P. aeruginosa* *S. typhimurium* *B. subtilis* *S. aureus* *B. cereus* *S. pneumoniae*	The results based on minimal inhibitory concentration (MIC) and radial diffusion assay demonstrated that the antimicrobial activity of MWCNT-TEA > MWCNT-DEA > MWCNT-MEA > pristine MWCNT.	[50]
CNTs functionalized with oxygen groups		The results suggested that the reduction of surface carboxyl groups and the redox activity of carbonyl groups enhanced the antimicrobial activity of MWNT.	[51]
Silver and other noble metals CNTs coated with silver nanoparticles	*E. coli* *P. aeruginosa* *S. aureus*	The antimicrobial activity of f-MWCNTs-Ag was 93.7, 69.7, and 56.7% for *E. coli*, *P. aeruginosa,* and *S. aureus*, respectively.	[47]
	E. coli *S. epidermidis*	The viability percentage of bacteria exposed to Ag/CNTs-deposited filter was 0.1 and 0.9% for *E. coli* and *S. epidermidis*, respectively.	[52]
	Methylobacterium spp. *Sphingomonas* spp.	The inactivation percentage of Ag-MWCNTs (40 or 50 μg/mL) was 100% for all tested bacteria.	[54]
	E. coli	Ag-MWCNT inactivated 97% of bacteria.	[53]
Copper nanoparticles grafted on CNT surfaces	*E. coli*	Cu-MWCNT inactivated 75% of bacteria.	[53]
Silver nanoparticles (AgNPs)-deposited CNTs functionalized with an amphiphilic poly(propyleneimine) dendrimer (MWCNT-APPI-AgNPs)	*E. coli* *S. aureus* *B. subtilis*	The inactivation percentage of MWCNTs-APPI-AgNPs was 99.8, 99.7, and 93.1% for *B. subtilis*, *S. aureus*, and *E. coli*, respectively.	[3]

(continued)

Table 3 (continued)

Material blend	Species	Main conclusions	Refs.
Cadmium sulfide (CdS) and silver sulfide (Ag_2S) quantum dots immobilized on poly(amidoamine)-grafted carbon nanotubes	*E. coli* *P. aeruginosa* *S. aureus*	The antimicrobial activity of f-MWCNT-CdS was 87.2 ± 4.1, 68.9 ± 2.5 and 46.7 ± 1.4% for *E. coli*, *P. aeruginosa* and *S. aureus*, respectively. The efficacy of f-MWCNT-Ag_2S was of 97.8 ± 2.1, 78.5 ± 2.9 and 55.7 ± 1.5% for *E. coli*, *P. aeruginosa* and *S. aureus*.	[48]
Zinc Oxide (ZnO)-coated CNTs	*E. coli*	ZnO/MWCNTs exhibited a strong antibacterial activity for *E. coli* (3- to 6-log reduction) comparing with pristine MWCNTs.	[56]
Sandwiched type structure based on polymer colloids, CNTs, and silver nanoparticles	*E. coli* *S. aureus*	The polymer colloids/AgNPs/MWCNTs exhibited a good antimicrobial activity as demonstrated by disc inhibition zone (11.53 and 9.73 mm for *E. coli* and *S. aureus*, respectively, versus ≈7 mm obtained for the control).	[55]
CNTs coated with titanium alloy and impregnated with rifampicin	*S. epidermidis*	Coated surfaces induced a significant inhibition of biofilm formation for up to 5 days.	[10]
Carbon nanotubes/titanium oxide/gold nanocomposite (NC)	*S. dysenteriae* *P. vulgaris* *K. pneumoniae* *C. albicans* *B. subtilis* *S. pneumoniae* *S. aureus*	The new nanocomposite exhibited high antimicrobial activity when compared with controls.	[57]
Enzymes Laccase and chloroperoxidase (CPO) separately immobilized onto carbon nanotubes	*E. coli* *S. aureus* *B. cereus* *B. anthracis*	Laccase-CNTs displayed >99% bactericidal activity against *E. coli* and *S. aureus*, and >98% sporicidal activity against *B. cereus* and *B. anthracis*. The CPO-CNTs also showed >99% antimicrobial activity against *E. coli* and *S. aureus*.	[58]

(continued)

Table 3 (continued)

Material blend	Species	Main conclusions	Refs.
Polymers CNTs functionalized with amphiphilic poly(propyleneimine) dendrimer (APPI)	*E. coli* *S. aureus* *B. subtilis*	The inactivation percentages of MWCNT-APPI for *B. subtilis*, *S. aureus*, and *E. coli* were 96.6, 96.5, and 87%, respectively.	[3]
CNTs functionalized with aromatic polyamide dendrimer	*E. coli* *P. aeruginosa* *S. aureus*	The antimicrobial activity of f-MWCNTs was 72.6, 65.2, and 35.5% for *E. coli*, *P. aeruginosa,* and *S. aureus*, respectively.	[47]
Poly(amidoamine) (PAMAM)-grafted CNTs	*E. coli* *P. aeruginosa* *S. aureus*	The bacteria killing ability of PAMAM-grafted CNTs was 34.1 ± 1.2, 60 ± 1.8, and 22.8 ± 0.9% for *E. coli*, *P. aeruginosa,* and *S. aureus*, respectively.	[48]
Oxidized CNTs/poly(vinyl alcohol) (PVOH) nanocomposite	*P. aeruginosa*	The percentage of viable cells deposited on O-MWCNT/PVOH surfaces decreased with increasing CNT concentration.	[43]
Hydrogels Chitosan-CNTs hydrogels	*E. coli* *S. aureus* *C. tropicalis*	Chitosan-CNT hydrogel exhibited strong antimicrobial activity toward *S. aureus* and *C. tropicalis* when compared to *E. coli*.	[59]
	B. subtilis *S. pneumoniae* *E. coli* *P. aeruginosa* *G. candidium* *C. albicans* *A. fumigatus* *S. racemosum*	CNT composites displayed a higher potency against Gram-positive than Gram-negative bacteria. Chitosan/MWCNT composites have equally or even higher activities than the reference bactericides or fungicides against some of the tested microbes.	[60]

the functionalization of MWCNTs with -COOH surface groups. MWCNT-COOH reduced the bacterial viability by 20–40% for *E. coli*, 27% for *P. aeruginosa*, 15–50% for *S. aureus*, and 30% for *B. subtilis* [3, 47, 48]. Chen et al. also demonstrated that MWCNT-COOH and MWCNT-OH showed a significant and dose-dependent antimicrobial activity against *L. acidophilus*, *B. adolescentis*, *E. coli*, *E. faecalis*, and *S. aureus* [24]. The same effect was detected against *Vibrio parahaemolyticus* [49]. However, Arias and Yang [4] observed that MWCNTs functionalized (f-MWCNTs) with –OH, –COOH, and $–HN_2$ did not have significant antimicrobial activity, contrary to what was found by the same authors for SWCNTs (Fig. 4).

Zardini et al. tested MWCNTs functionalized with ethanolamine against a broad range of species and verified that f-MWCNTs exhibited a higher antimicrobial activity than pristine MWCNTs [50]. Finally, it was shown that MWCNTs functionalized with oxygen groups can have enhanced antimicrobial activity [51].

Similarly to SWCNTs, MWCNTs coated with silver nanoparticles (AgNPs) exhibited excellent biocidal activity. Studies reported that the bacterial inactivation percentage of MWCNT-AgNPs was 93.7–99% for *E. coli* and *S. epidermidis*, 69.7%

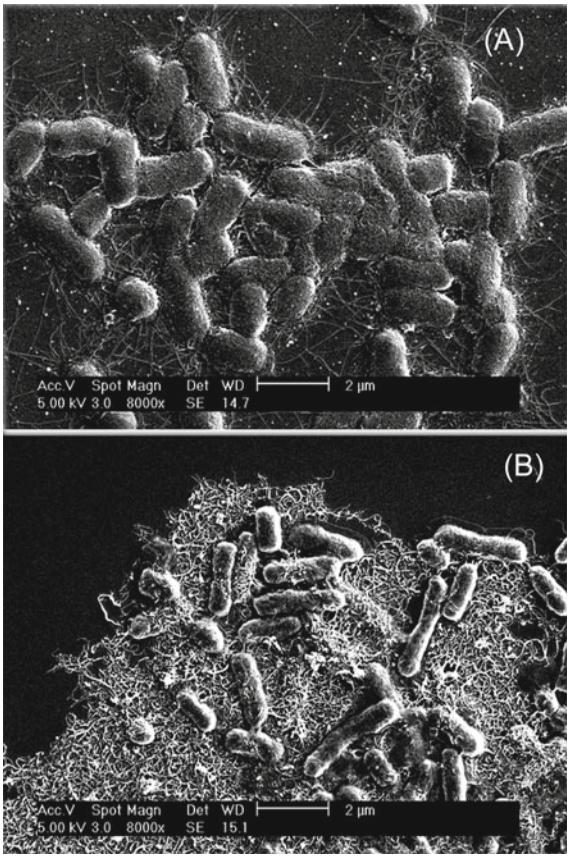


Fig. 4 SEM images of cell aggregates formed between *Salmonella* spp. cells and (**a**) SWNTs-COOH and (**b**) MWNTs-COOH. Reprinted with permission from Arias and Yang [4]. Copyright 2009 American Chemical Society

for *P. aeruginosa*, 56.7% for *S. aureus* and 100% for *Methylobacterium* spp. and *Sphingomonas* spp. [47, 52–54]. The association of MWCNT-AgNPs with amphiphilic poly(propyleneimine) dendrimers kept the inactivation percentages high for *E. coli*, *S. aureus,* and *B. subtilis* (>90%) [3]. Likewise, the immobilization of MWCNT-AgNPs with polymer colloids revealed a good antimicrobial activity against *E. coli* and *S. aureus* [55], and silver sulfide (Ag_2S) quantum dots immobilized on poly(amidoamine)-grafted MWCNTs were shown to reduce bacterial viability by 97.8, 78.5, and 55.7% for *E. coli*, *P. aeruginosa,* and *S. aureus*, respectively. Moreover, Ag_2S-MWCNTs displayed better biocidal activity than MWCNTs coated with cadmium sulfide quantum dots [48].

The use of MWCNTs blended to other noble metals has also shown promising results. Bacterial cells exposed to MWCNTs coated with copper nanoparticles had their viability reduced by 75% [53]. Similarly, zinc oxide-coated MWCNTs showed strong antimicrobial activity against *E. coli* [56]. A nanocomposite constituted by MWCNTs, titanium, and gold exhibited high antibacterial activity against several species including *Shigella dysenteriae*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, *B. subtilis*, *S. aureus*, and *Candida albicans* [57]. Lastly, multi-walled CNTs coated with a titanium alloy and impregnated with rifampicin were able to prevent biofilm formation for up five days [10].

Enzymes like laccase and chloroperoxidase (CPO) were immobilized onto MWCNTs. Laccase- and CPO-MWCNTs reduced more than 99% of bacterial viability for *E. coli* and *S. aureus*. MWCNTs combined with laccase also inhibited the *Bacillus cereus* and *Bacillus anthracis* spore formation by more than 98% [58].

The antimicrobial activity of nanocomposites formed by MWCNTs and different polymers has also been investigated. Murugan and Vimala evaluated the biocidal effect of MWCNTs functionalized with amphiphilic poly(propyleneimine) dendrimer (APPI). This NC was able to inactivate by 96.6% *B. subtilis*, 96.5% *S. aureus*, and 87% *E. coli* [3]. In another study conducted by Neelgund and Oki, MWCNTs functionalized with aromatic polyamide dendrimer presented a good antimicrobial activity against *E. coli* (72.6%) and *P. aeruginosa* (65.2%) [47]. On the other hand, poly(amidoamine)-grafted MWCNTs showed a reduced effect against all tested bacteria [48].

It is also possible that the antimicrobial activity of nanocomposites improves with increasing concentrations of CNTs. Goodwin et al. reported that the viability of bacteria deposited on MWCNT- poly(vinyl alcohol) surfaces decreased with increasing MWCNT concentration [43].

Recently, the interest in hydrogel-based materials has increased due to their physiological nature [59]. Some authors have explored MWCNT nanocomposites based on chitosan hydrogels. Venkatesan et al. reported the strong antimicrobial activity of chitosan-MWCNT hydrogel against *E. coli*, *S. aureus,* and *Candida tropicalis* [59]. Mohamed et al. also evaluated the biocidal activity of this kind of nanocomposites. Chitosan-MWCNT hydrogels showed a broad-spectrum antimicrobial activity [60]. Indeed, the use of chitosan as antimicrobial agent was previously reported by several authors [61, 62].

As described above, there are various material blends that may be applied aiming to develop effective antimicrobial surfaces.

Concerning the antifouling properties of MWCNTs, most studies relate to MWCNT-polymers nanocomposites. Indeed, the dispersion of MWCNTs can be increased by their addition to polymers. Thus, the bulk properties of CNTs may be extended through the nanocomposite improving their antifouling properties. Simultaneously, the addition of MWCNTs to polymers confers them certain properties such as resistance to protein interaction and higher biocompatibility [2].

Table 4 describes MWCNT modifications and/or associations with different polymers and their performance in increasing the fouling resistance. Only one study addressed the activity of oxidized MWCNTs on decreasing bacterial adhesion by eightfold to tenfold. [32].

The activity of MWCNTs incorporated into polydimethylsiloxane (PDMS) was reported in numerous studies. In all of them, the antifouling properties of PDMS were increased by the addition of MWCNTs, thus, decreasing biofouling [16–18, 63]. Moreover, MWCNT-PDMS coatings were applied to inhibit the microalga *Ulva linza* adhesion and decrease the removal stress for barnacles from ship hulls [16, 17].

Other studies have described the performance of MWCNT-polymer NCs to avoid bacterial adhesion and biofilm formation. Kim et al. showed that MWCNTs incorporated into poly(methyl methacrylate) inhibited *S. aureus*, *Streptococcus mutans,* and *C. albicans* adhesion by 35–95% [64]. Likewise, the biofilm growth on MWCNT-polyethylene composites decreased by 89.3 and 29% for *P. fluorescens* and *Mycobacterium smegmatis*, respectively [65]. *P. fluorescens* adhesion was also inhibited by the incorporation of MWCNTs into polyvinylidene fluoride membranes [66]. Lastly, Lin et al. demonstrate that tetraaniline covalently bonded to MWCNTs reduced the surface coverage percentage of *S. epidermidis* by more than 50% [67].

Protein adhesion to membranes during filtration processes can be reduced by the addition of MWCNTs. Liu et al. prepared a membrane composed of poly(sulfone), poly(sulfobetaine methacrylate), and MWCNTs that exhibited fouling resistance for bovine serum albumin (BSA) and fibrinogen in ultrafiltration processes [68]. The incorporation of MWCNTs into polyethersulfone (PES)-based membranes displayed higher flux and slower fouling rates than the usual PES membranes [69, 70]. Similar results were obtained for a study where PES membranes were incorporated with poly(citric acid)-grafted MWCNTs [71]. In another study performed by Takizawa et al., the presence of MWCNTs on polyamide reverse-osmosis membranes resulted in weaker interactions between the BSA molecules and membrane surface [72].

Polymer membranes are frequently used for water and wastewater treatments. In this context, several studies have been carried out to produce membranes with high fouling resistance and, consequently, high water flux. The combination of polyethyleneimine, MWCNTs, and trimesoyl chloride conferred to membranes high hydrophilicity, increasing their antifouling properties [73]. Also, the incorporation of CNTs into a polypropylene matrix showed high resistance to fouling deposition [74].

The polyamide membranes containing MWCNTs also demonstrated high fouling resistance rates against humic acid [20].

Table 4 Studies reporting the development of MWCNT-based antifouling surfaces

Material blend	Species	Main conclusions	Refs.
Functionalized CNTs Oxidized CNTs (O-CNT), oxidized-annealed CNTs (OA-CNT)	*P. fluorescens*	The rate of bacterial adhesion decreased eightfold to tenfold when an electric potential was applied.	[32]
Polymers CNTs incorporated into polydimethylsiloxane	*Zoospores* of *U. linza* *Barnacle cyprid*	Between 45 and 65% of the settled spores were removed from all coatings by exposure to a wall shear stress of 52 Pa. Adding 0.2% MWCNTs to the PDMS decreased the critical removal stress for barnacles significantly (70% compared to the control).	[16]
	U. linza	Addition of CNTs to amphiphilic block copolymers in PDMS caused a small reduction in the percentage of biomass released compared to the block copolymer without CNT (87% vs 76%).	[17]
		The antifouling properties of the PDMS matrix were improved with the incorporation of cMWCNT fillers, preventing biofouling for more than 14 week in marine environments.	[18]
		Nanocomposite surfaces only demonstrated weak modulating effects on the biological colonization.	[63]
CNTs with poly(sulfone) (PSF) and poly(sulfobetaine methacrylate) (PSBMA) (MWCNT-PSF/PSBMA)		The membrane made of PSF/MWCNT-PSF/PSBMA nanocomposite exhibited antifouling properties in BSA and fibrinogen ultrafiltration experiments.	[68]
CNTs incorporated into poly(methyl methacrylate) (PMMA)	*S. aureus* *S. mutans* *C. albicans*	Significant antiadhesive effects (35–95%) against all tested bacteria were verified for the 1% CNT/PMMA compared to the PMMA control group.	[64]

(continued)

Table 4 (continued)

Material blend	Species	Main conclusions	Refs.
CNTs with polyethersulfone (PES) blend membrane		CNT/PES blend membranes displayed a 15% higher flux and 7-fold slower fouling rate than the PES membranes.	[69]
CNTs incorporated into alumina/polyethersulfone hollow fiber membranes		CNT/alumina/PES membranes showed higher antifouling ability with the flux recoveries rates increasing by 84.1% for BSA and 53.2% for humic acid compared to the samples without CNTs.	[70]
Poly (citric acid)-grafted CNTs (PCA-g-MWCNT) incorporated as nanofiller in polyethersulfone (PES)		Compared to commercial PES hemodialysis membranes, the PES/PCA-g-MWCNT MMMs showed a lower flux decline (5-fold) and higher water flux recovery ratio (from 15.8$Lm^{-2}h^{-1}$ to 95.36$Lm^{-2}h^{-1}$)..	[71]
Polyethyleneimine/carbon nanotubes/trimesoyl chloride (PEI/CNT/TMC)		The high hydrophilicity and negatively charged PEI/CNT/TMC surface render membranes with good antifouling properties (90% more than PEI/CNT surface).	[73]
CNTs-Polyethylene (PE) composites	*P. fluorescens* *M. smegmatis*	Biofilm growth on PE-CNTs composites surface compared to PE decreased by 89.3% and 29% for *P. fluorescens* and *Mycobacterium smegmatis*, respectively.	[65]
CNTs with polypropylene (PP)		The present CNTs/PP nanocomposite showed a high resistance for fouling deposition in comparison with the typical PP matrix. After 24 h, the fluorescence intensity associated with the deposition of foulant was tenfold higher for the PP matrix.	[74]

(continued)

Table 4 (continued)

Material blend	Species	Main conclusions	Refs.
CNTs-polyamide nanocomposite (MWCNT-PA) reverse-osmosis (RO) membranes		MWCNTS-PA nanocomposite membranes had a flux reduction of 15% compared to 34–50% obtained for commercial membranes.	[72]
Carbon nanotube polyamide (CNT-PA) nanocomposite membrane		The fouling resistance against humic acid was constant for CNT-PA membranes. Conversely, the flow in commercial membranes decreased by 5%.	[20]
Interlaced CNT electrodes (ICE) on a polyvinylidene fluoride (PVDF) microfiltration membrane	*P. fluorescens*	The optimal operating conditions (2V alternating current) reduced the fouling rateby 75% versus the control and achieved up to 96% fouling resistance recovery.	[66]
Tetraaniline (TANI) covalently bonded to carbon nanotubes	*S. epidermidis*	Results revealed that the surface coverage percentage of *S. epidermidis* drops more than 50% from the unmodified to the modified film.	[67]
Polypyrrole (PPy)-coated CNTs nanocomposites		Results showed that the pure water flux increased from 152.8 L/m^2 h to 378.8 and 399.3 L/m^2 h for 0.1 and 1 wt% of PPy-coated raw and oxidized MWCNTs hybrid membranes, respectively.	[81]
Thermo-responsive *N*-isopropyle acryleamide (NIPAAm) polymerized on the surface of CNTs		The MWCNT-NIPAAm membranes demonstrated a flux recovery ratio of 78–99.9% compared to 47% of PES membranes.	[76]

Recently, Vatanpour et al. used polypyrrole, a natural polymer, to form MWCNTs-nanocomposite membranes, which demonstrated high water flux [75]. Finally, the combination of a thermo-responsive polymer, N-isopropyle acryleamide polymer, with MWCNTs also resulted in high water flux and high fouling resistance membranes [76].

It is important to highlight that there are MWCNT-based surfaces that combine both antimicrobial and antifouling properties. Table 5 summarizes the studies addressing these attractive MWCNT-based surfaces.

The application of silver and other noble metals on MWCNT-based coatings continues to yield excellent results. Various studies demonstrated that the association

Table 5 Studies reporting the development of MWCNT-based antifouling and antimicrobial surfaces

Coating	Species	Major conclusions	Refs.
Silver and other noble metals Silver nanoparticle/CNTs (Ag/MWNTs) coated on a polyacrylonitrile (PAN) hollow fiber membrane	*E. coli*	The relative flux drops over Ag/MWNTs/PAN was 6%, which was significantly lower than with pristine PAN (55%). The presence of the Ag/MWNTs inhibited bacterial growth and prevented biofilm formation.	[77]
Silver-CNT/poly(vinylidene fluoride-co-hexafluoropropene) membranes	*E. coli*	The 3 weight % Ag-MWCNTs/PVDF-HFP membrane showed a high fouling resistance rate and bactericidal activity (100% bacterial load reduction).	[78]
Silver nanoparticle with CNTs (Ag-CNT) on ceramic membrane under electrochemical assistance	*E. coli*	Viable cells on the CNT/ceramic membrane were reduced to 3.4 log while bacteria were completely inactivated by Ag-CNT/ceramic membrane.	[19]
Copper grafted on CNTs	*Methylobacterium* spp.	Cu/MWCNTs films were removed in more than 75% of the biofilm area.	[79]
Polyethersulfone (PES) membrane incorporated with zinc oxide (ZnO) and CNTs	*Enterobacter* spp.	ZnO/MWCNT/PES membrane demonstrated efficient antifouling properties with high flux ratios of 28–56 $Lm^{-2}h$ versus 7.8 $Lm^{-2}h$ obtained for the PES membrane. It also showed notable antibacterial properties with few bacteria attached to the membrane.	[80]
Enzymes CNTs with lysostaphin	*B. cereus* *E. coli* *S. aureus (MRSA)* *S. epidermidis*	Enzyme-based composites were highly efficient in killing MRSA (>99%) and inhibiting biofilm formation.	[81]

(continued)

of silver nanoparticles with MWCNT-polymer membranes conferred them a high antimicrobial activity and fouling resistance [19, 77, 78]. Simultaneously, copper grafted on MWCNT surfaces caused bacterial wall damage and inhibited biofilm formation [79]. Lastly, the association of zinc oxide and MWCNTs also demonstrated efficient antifouling and antibacterial properties [80].

Kang et al. described the combination of lysostaphin, an antibacterial enzyme with MWCNTs as a potent enzyme-based nanocomposite with high biocidal (<99%) and

Table 5 (continued)

Coating	Species	Major conclusions	Refs.
Antimicrobial peptides Immobilization of nisin on CNTs	*E. coli* *P. aeruginosa* *S. aureus* *B. subtilis*	The MWNT-nisin composite showed up to sevenfold higher antimicrobial property than pristine MWNTs. The MWNT-nisin deposit film exhibited a 100-fold higher anti-biofilm activity than the MWNT deposit film.	[82]
	B. anthracis	Nisin coating on MWCNT decreased surface hydrophobicity, reduced spore attachment, and reduced the germination of attached spores by 3.5-fold.	[83]
Polymers CNTs with epsilon-polylysine (MEPs)	*E. coli* *P. aeruginosa* *S. aureus*	MEPs nanocomposite killed 97.6, 91.5 and 88.5% of *E. coli*, *P. aeruginosa*, *S. aureus*, respectively. Results indicated that MEPs have also a stronger antiadhesive activity and can prevent biofilms formation.	[84]
N-halamine epoxide and siloxane grafted onto the CNTs (N-Si-MWNTs)	*E. coli* *S. aureus*	The films containing N–Si-MWNTs displayed a flux recovery ratio value above 96.5% and had excellent antibacterial efficacy (98.0 and 95.6% against *S. aureus* and *E. coli*, respectively).	[85]
CNT/poly(vinylidene fluoride-co-hexafluoropropene)	*E. coli*	The 1.5 weight % MWCNT/PVDF-HFP composite membrane showed high fouling resistance rate and bactericidal activity (100% bacterial load reduction).	[78]
Nanoporous solid-state membrane (NSSM) made by a two-step anodization method, and modified with CNTs	*E. coli* *S. aureus*	The BSA protein adsorption capacity reduced from 992 to 97 (μg mL^{-1} cm^{-2}). Results also showed that the percentage of inactivated bacteria was higher on the NSSM-MWCNT surface (98 and 99% for *E. coli* and *S. aureus*, respectively) than controls (8% for *E. coli* and 14% for *S. aureus*) as demonstrated by propidium iodide staining.	[86]

antifouling activities against methicillin-resistant *Staphylococcus aureus* (MRSA) [81].

Other studies also described the immobilization of nisin, an antimicrobial peptide, on MWCNTs. The MWCNT-nisin composite decreased surface hydrophobicity and exhibited higher antimicrobial and anti-biofilm activities than pristine MWCNTs [82, 83].

Multi-walled CNT-polymer composites continue to stand out due to their excellent properties and wide range of applications. Zhou J and Qi demonstrated that MWCNT-epsilon-polylysine killed 97.6, 91.5, and 88.5% of *E. coli*, *P. aeruginosa*, and *S. aureus*, respectively. Additionally, this NC exhibited a stronger antiadhesive activity, preventing biofilm formation [84]. In another study, N-halamine epoxide/PDMS-grafted MWCNTs exhibited high antibacterial effect (98 and 95.6% reduction with *S. aureus* and *E. coli*, respectively). These films also displayed a high flux recovery rate [85]. MWCNT/poly(vinylidene fluoride-co-hexafluoropropene) composite exerted the same effect against *E. coli* [78]. Recently, Alizadeh et al. developed a nanoporous solid-state membrane made through a two-step anodization method and modified with MWCNTs. This new nanocomposite showed a promising effect upon *E. coli* and *S. aureus* and decreased protein absorption [86].

Although the reviewed studies demonstrated good results for both SWCNTs and MWCNTs, the latter seems to be more studied and applied for the development of antifouling and antimicrobial carbon-based nanomaterials.

4 Application of MWCNT-Based Surfaces in Urinary Tract Devices

The large number of published studies concerning MWCNTs confirmed their potential either for decreasing bacterial viability or inhibiting biofilm formation. Because of their outstanding properties, MWCNT-based surfaces have been widely applied in the medical field, in particular for the manufacture of medical devices.

Urinary catheters and ureteral stents are devices commonly used in clinical practice. However, their use often causes urinary tract infections (UTI). The UTIs correspond to about 17% of hospital-acquired bacteremias and have a prevalence of 36 and 27% in USA and Europe, respectively [87–89]. Therefore, these data act as a driving force for the development of new surfaces with antimicrobial/antifouling properties.

As noted above, MWCNTs have been successfully used in the production of hydrophilic silicone coatings. However, the employment of these nanomaterials in urinary tract devices remains understudied and further research is needed.

Recently, a study conducted by Vagos et al. under conditions that mimic the flow in the urinary tract devices demonstrated that bacterial adhesion can be modulated by the incorporation of different types of MWCNTs in PDMS composites. Results showed that the incorporation of small amounts (0.1%) of pristine MWCNTs can lead

to a decrease of up to 20% on *E. coli* adhesion, whereas the use of oxidized MWCNTs (obtained by treatment with nitric acid) can increase bacterial adhesion also by 20% [90]. These results are corroborated by a previous study developed by Arias and Yang, where the MWCNTs-OH did not display significant antimicrobial activity against Gram-positive and Gram-negative bacteria [4]. Contrarily, Chen et al. demonstrated that MWCNTs-OH exhibit a significant and dose-dependent antimicrobial activity suggesting that assay conditions can have a great impact on surface performance [24].

Although these results are promising, further studies are needed to produce efficient MWCNTs/PDMS composites in order to prevent and reduce biofilm formation on device surfaces. A promising strategy may be to test different MWCNT loadings and also introduce chemical and textural variations on MWCNT/PDMS NCs.

5 Conclusions

Carbon nanotubes were described as excellent nanomaterials for numerous applications, particularly for the development of antimicrobial and antifouling surfaces.

Although the CNT mechanism of action is still being discussed by several authors, their antimicrobial and antifouling activities seem to depend on a multiplicity of factors, which may be modulated in order to improve their performance. The functionalization of CNTs surfaces is also essential to increase their hydrophilicity and, consequently, biocompatibility.

According to collected data, there are innumerous materials such as polymer, biomolecules, and metals, that may be blended in order to develop effective CNT-based nanocomposites.

The high antimicrobial activity of CNT-nanocomposites against a broad spectrum of microorganisms was reported. In addition, the significant fouling resistance of these nanocomposites was also proven at distinct levels. However, some studies suggested that MWCNTs are more effective than SWCNTs. Moreover, there are more studies using MWCNTs, which suggests that this type of CNTs is more promising for antimicrobial and antifouling activities. Nevertheless, further studies are needed to produce efficient MWCNT/PDMS composites aiming to develop new antimicrobial and antifouling surfaces.

Acknowledgements This work was financially supported by: Base Funding—UIDB/00511/2020 of the Laboratory for Process Engineering, Environment, Biotechnology and Energy—LEPABE—funded by national funds through the FCT/MCTES (PIDDAC) and by: Project PTDC/BII-BIO/29589/2017—POCI-01-0145-FEDER-029589—funded by FEDER funds through COMPETE2020—Programa Operacional Competitividade e Internacionalização (POCI) and by national funds (PIDDAC) through FCT/MCTES. R. Teixeira-Santos acknowledges the receipt of a junior researcher fellowship from this project.

References

1. Iijima S (1991) Helical microtubules of graphitic carbon. Nature 354(6348):56–58
2. Upadhyayula VKK, Gadhamshetty V (2010) Appreciating the role of carbon nanotube composites in preventing biofouling and promoting biofilms on material surfaces in environmental engineering: a review. Biotechnol Adv 28(6):802–816
3. Murugan E, Vimala G (2011) Effective functionalization of multiwalled carbon nanotube with amphiphilic poly(propyleneimine) dendrimer carrying silver nanoparticles for better dispersability and antimicrobial activity. J Colloid Interface Sci 357(2):354–365
4. Arias LR, Yang L (2009) Inactivation of bacterial pathogens by carbon nanotubes in suspensions. Langmuir 25(5):3003–3012
5. Akasaka T, Watari F (2009) Capture of bacteria by flexible carbon nanotubes. Acta Biomater 5(2):607–612
6. Kang S, Herzberg M, Rodrigues DF, Elimelech M (2008) Antibacterial effects of carbon nanotubes: size does matter! Langmuir 24(13):6409–6413
7. Baughman RH, Zakhidov AA, de Heer WA (2002) Carbon Nanotubes–the Route Toward Applications. Science 297(5582):787
8. He H et al (2013) Carbon nanotubes: applications in pharmacy and medicine. Biomed Res Int 2013:578290
9. Aslan S et al (2010) Antimicrobial biomaterials based on carbon nanotubes dispersed in poly(lactic-co-glycolic acid). Nanoscale 2(9):1789–1794
10. Hirschfeld J et al (2017) Long-term release of antibiotics by carbon nanotube-coated titanium alloy surfaces diminish biofilm formation by Staphylococcus epidermidis. Nanomed: Nanotechnol Biol Med 13(4):1587–1593
11. Saliev T (2019) The Advances in biomedical applications of carbon nanotubes. C–J Carbon Res 5(2)
12. Chaudhari AA et al (2019) A three-dimensional human skin model to evaluate the inhibition of *Staphylococcus aureus* by antimicrobial peptide-functionalized silver carbon nanotubes. J Biomater Appl 33(7):924–934
13. Mehra NK, Jain K, Jain NK (2015) Pharmaceutical and biomedical applications of surface engineered carbon nanotubes. Drug Discov Today 20(6):750–759
14. Vashist SK, Zheng D, Al-Rubeaan K, Luong JH, Sheu FS (2011) Advances in carbon nanotube based electrochemical sensors for bioanalytical applications. Biotechnol Adv 29(2):169–188
15. Vassallo J, Besinis A, Boden R, Handy RD (2018) The minimum inhibitory concentration (MIC) assay with Escherichia coli: an early tier in the environmental hazard assessment of nanomaterials? Ecotoxicol Environ Saf 162:633–646
16. Beigbeder A et al (2008) Preparation and characterisation of silicone-based coatings filled with carbon nanotubes and natural sepiolite and their application as marine fouling-release coatings. Biofouling 24(4):291–302
17. Martinelli E et al (2011) Amphiphilic block copolymer/poly(dimethylsiloxane) (PDMS) blends and nanocomposites for improved fouling-release. Biofouling 27(5):529–541
18. Sun Y, Zhang Z (2016) Anti-biofouling property studies on carboxyl-modified multi-walled carbon nanotubes filled PDMS nanocomposites. World J Microbiol Biotechnol 32(9):148
19. Fan X, Liu Y, Wang X, Quan X, Chen S (2019) Improvement of antifouling and antimicrobial abilities on silver–carbon nanotube based membranes under electrochemical assistance. Environ Sci Technol 53(9):5292–5300
20. Cruz-Silva R et al (2019) New insights in the natural organic matter fouling mechanism of polyamide and nanocomposite multiwalled carbon nanotubes-polyamide membranes. Environ Sci Technol 53(11):6255–6263
21. Hassouna MEM, ElBably MA, Mohammed AN, Nasser MAG (2016) Assessment of carbon nanotubes and silver nanoparticles loaded clays as adsorbents for removal of bacterial contaminants from water sources. J Water Health 15(1):133–144

22. Smith SC, Rodrigues DF (2015) Carbon-based nanomaterials for removal of chemical and biological contaminants from water: a review of mechanisms and applications. Carbon 91:122–143
23. Wick P et al (2007) The degree and kind of agglomeration affect carbon nanotube cytotoxicity. Toxicol Lett 168(2):121–131
24. Chen H et al (2013) Broad-spectrum antibacterial activity of carbon nanotubes to human gut bacteria. Small 9(16):2735–2746
25. Kang S, Pinault M, Pfefferle LD, Elimelech M (2007) Single-Walled carbon nanotubes exhibit strong antimicrobial activity. Langmuir 23(17):8670–8673
26. Nagai H, Toyokuni S (2012) Differences and similarities between carbon nanotubes and asbestos fibers during mesothelial carcinogenesis: Shedding light on fiber entry mechanism. Cancer Sci 103(8):1378–1390
27. Al-Jumaili A, Alancherry S, Bazaka K, Jacob MV (2017) Review on the antimicrobial properties of carbon nanostructures. Materials (Basel, Switzerland) 10(9):1066
28. Johnston HJ et al (2010) A critical review of the biological mechanisms underlying the in vivo and in vitro toxicity of carbon nanotubes: The contribution of physico-chemical characteristics. Nanotoxicology 4(2):207–246
29. Liu S et al (2010) Antibacterial action of dispersed single-walled carbon nanotubes on Escherichia coli and Bacillus subtilis investigated by atomic force microscopy. Nanoscale 2(12):2744–2750
30. Olivi M et al (2013) Inhibition of microbial growth by carbon nanotube networks. Nanoscale 5(19):9023–9029
31. Young Y-F et al (2012) Toxicity mechanism of carbon nanotubes on *Escherichia coli*. Mater Chem Phys 134(1):279–286
32. Zhang Q, Nghiem J, Silverberg GJ, Vecitis CD (2015) Semiquantitative performance and mechanism evaluation of carbon nanomaterials as cathode coatings for microbial fouling reduction. Appl Environ Microbiol 81(14):4744–4755
33. Hartono MR, Kushmaro A, Chen X, Marks RS (2018) Probing the toxicity mechanism of multiwalled carbon nanotubes on bacteria. Environ Sci Pollut Res 25(5):5003–5012
34. Malek I et al (2016) Vertically aligned multi walled carbon nanotubes prevent biofilm formation of medically relevant bacteria. J Mater Chem B 4(31):5228–5235
35. Wang H et al (2006) Unique aggregation of anthrax (*Bacillus anthracis*) spores by sugar-coated single-walled carbon nanotubes. J Am Chem Soc 128(41):13364–13365
36. Zhu Z, Wang Z, Li S, Yuan X (2019) Antimicrobial strategies for urinary catheters. J Biomed Mater Res Part A 107(2):445–467
37. Prabhu S, Poulose EK (2012) Silver nanoparticles: mechanism of antimicrobial action, synthesis, medical applications, and toxicity effects. Int Nano Lett 2(1):32
38. Nepal D, Balasubramanian S, Simonian AL, Davis VA (2008) Strong Antimicrobial coatings: single-walled carbon nanotubes armored with biopolymers. Nano Lett 8(7):1896–1901
39. Levashov PA, Sedov SA, Shipovskov S, Belogurova NG, Levashov AV (2010) Quantitative turbidimetric assay of enzymatic gram-negative bacteria lysis. Anal Chem 82(5):2161–2163
40. Wild P et al (1997) Reevaluation of the effect of lysoyzme on Escherichia coli employing ultrarapid freezing followed by cryoelectronmicroscopy or freeze substitution. Microsc Res Tech 39(3):297–304
41. Ahmed F et al (2012) Antimicrobial applications of electroactive PVK-SWNT nanocomposites. Environ Sci Technol 46(3):1804–1810
42. Aslan S et al (2012) Carbon nanotube-based antimicrobial biomaterials formed via layer-by-layer assembly with polypeptides. J Colloid Interface Sci 388(1):268–273
43. Goodwin DG et al (2015) Interactions of microorganisms with polymer nanocomposite surfaces containing oxidized carbon nanotubes. Environ Sci Technol 49(9):5484–5492
44. Sah U, Sharma K, Chaudhri N, Sankar M, Gopinath P (2018) Antimicrobial photodynamic therapy: single-walled carbon nanotube (SWCNT)-Porphyrin conjugate for visible light mediated inactivation of *Staphylococcus aureus*. Colloids Surf B 162:108–117

45. Cajero-Zul LR et al (2019) Evaluation of the resistance to bacterial growth of star-shaped poly(epsilon-caprolactone)-co-poly(ethylene glycol) grafted onto functionalized carbon nanotubes nanocomposites. J Biomater Sci-Polym Edn 30(3):163–189
46. Tiraferri A, Vecitis CD, Elimelech M (2011) Covalent binding of single-walled carbon nanotubes to polyamide membranes for antimicrobial surface properties. ACS Appl Mater Interfaces 3(8):2869–2877
47. Neelgund GM, Oki A (2011) Deposition of silver nanoparticles on dendrimer functionalized multiwalled carbon nanotubes: synthesis, characterization and antimicrobial activity. J Nanosci Nanotechnol 11(4):3621–3629
48. Neelgund GM, Oki A, Luo Z (2012) Antimicrobial activity of CdS and Ag2S quantum dots immobilized on poly(amidoamine) grafted carbon nanotubes. Colloids Surf B Biointerfaces 100:215–221
49. Ding L, Wang H, Liu D, Zeng X-A, Mao Y (2020) Bacteria capture and inactivation with functionalized multi-walled carbon nanotubes (MWCNTs). J Nanosci Nanotechnol 20(4):2055–2062
50. Zardini HZ et al (2014) Microbial toxicity of ethanolamines—multiwalled carbon nanotubes. J Biomed Mater Res Part A 102(6):1774–1781
51. Gilbertson LM, Goodwin DG, Taylor AD, Pfefferle L, Zimmerman JB (2014) Toward tailored functional design of multi-walled carbon nanotubes (MWNTs): electrochemical and antimicrobial activity enhancement via oxidation and selective reduction. Environ Sci Technol 48(10):5938–5945
52. Jung JH, Hwang GB, Lee JE, Bae GN (2011) Preparation of airborne Ag/CNT hybrid nanoparticles using an aerosol process and their application to antimicrobial air filtration. Langmuir 27(16):10256–10264
53. Mohan R, Shanmugharaj AM, Sung Hun R (2011) An efficient growth of silver and copper nanoparticles on multiwalled carbon nanotube with enhanced antimicrobial activity. J Biomed Mater Res B Appl Biomater 96B(1):119–126
54. Seo Y et al (2014) Antibacterial activity and cytotoxicity of multi-walled carbon nanotubes decorated with silver nanoparticles. Int J Nanomedicine 9:4621–4629
55. Rusen E et al (2014) Design of antimicrobial membrane based on polymer colloids/multiwall carbon nanotubes hybrid material with silver nanoparticles. ACS Appl Mater Interfaces 6(20):17384–17393
56. Sui M, Zhang L, Sheng L, Huang S, She L (2013) Synthesis of ZnO coated multi-walled carbon nanotubes and their antibacterial activities. Sci Total Environ 452–453:148–154
57. Karthika V, Arumugam A (2017) Synthesis and characterization of MWCNT/TiO_2/Au nanocomposite for photocatalytic and antimicrobial activity. IET Nanobiotechnol 11(1):113–118
58. Grover N, Borkar IV, Dinu CZ, Kane RS, Dordick JS (2012) Laccase- and chloroperoxidase-nanotube paint composites with bactericidal and sporicidal activity. Enzyme Microbial Technology 50(6):271–279
59. Venkatesan J, Jayakumar R, Mohandas A, Bhatnagar I, Kim SK (2014) Antimicrobial activity of chitosan–carbon nanotube hydrogels. Materials (Basel) 7(5):3946–3955
60. Mohamed NA, Al-Harby NF, Almarshed MS (2019) Synthesis and characterization of novel trimellitic anhydride isothiocyanate-cross linked chitosan hydrogels modified with multi-walled carbon nanotubes for enhancement of antimicrobial activity. Int J Biol Macromol 132:416–428
61. Kong M, Chen XG, Xing K, Park HJ (2010) Antimicrobial properties of chitosan and mode of action: a state of the art review. Int J Food Microbiol 144(1):51–63
62. Rabea EI, Badawy MET, Stevens CV, Smagghe G, Steurbaut W (2003) Chitosan as antimicrobial agent: applications and mode of action. Biomacromol 4(6):1457–1465
63. Ji Y et al (2018) Effect of CNT/PDMS nanocomposites on the dynamics of pioneer bacterial communities in the natural biofilms of seawater. Materials (Basel, Switzerland) 11(6):902
64. Kim K-I et al (2019) Carbon nanotube incorporation in PMMA to prevent microbial adhesion. Sci Rep 9(1):4921

65. Jing H, Sahle-Demessie E, Sorial GA (2018) Inhibition of biofilm growth on polymer-MWCNTs composites and metal surfaces. Sci Total Environ 633:167–178
66. Zhang Q et al (2017) Interlaced CNT electrodes for bacterial fouling reduction of microfiltration membranes. Environ Sci Technol 51(16):9176–9183
67. Lin C-W et al (2019) Direct grafting of tetraaniline via perfluorophenylazide photochemistry to create antifouling, low bio-adhesion surfaces. Chem Sci 10(16):4445–4457
68. Liu Y-L, Chang Y, Chang Y-H, Shih Y-J (2010) Preparation of amphiphilic polymer-functionalized carbon nanotubes for low-protein-adsorption surfaces and protein-resistant membranes. ACS Appl Mater Interfaces 2(12):3642–3647
69. Celik E, Park H, Choi H, Choi H (2011) Carbon nanotube blended polyethersulfone membranes for fouling control in water treatment. Water Res 45(1):274–282
70. Feng Y, Wang K, Davies CHJ, Wang H (2015) Carbon nanotube/alumina/polyethersulfone hybrid hollow fiber membranes with enhanced mechanical and anti-fouling properties. Nanomaterials (Basel, Switzerland) 5(3):1366–1378
71. Abidin MNZ et al (2016) Antifouling polyethersulfone hemodialysis membranes incorporated with poly (citric acid) polymerized multi-walled carbon nanotubes. Mater Sci Eng C Mater Biol Appl 68:540–550
72. Takizawa Y et al (2017) Antiorganic fouling and low-protein adhesion on reverse-osmosis membranes made of carbon nanotubes and polyamide nanocomposite. ACS Appl Mater Interfaces 9(37):32192–32201
73. Liu Y et al (2017) Synergy of the mechanical, antifouling and permeation properties of a carbon nanotube nanohybrid membrane for efficient oil/water separation. Nanoscale 9(22):7508–7518
74. Kitano H et al (2019) Enhanced antifouling feed spacer made from a carbon nanotube-polypropylene nanocomposite. ACS Omega 4(13):15496–15503
75. Vatanpour V, Ghadimi A, Karimi A, Khataee A, Yekavalangi ME (2018) Antifouling polyvinylidene fluoride ultrafiltration membrane fabricated from embedding polypyrrole coated multiwalled carbon nanotubes. Mater Sci Eng, C 89:41–51
76. Yaghoubi Z, Parsa JB (2019) Preparation of thermo-responsive PNIPAAm-MWCNT membranes and evaluation of its antifouling properties in dairy wastewater. Mater Sci Eng C 103:109779
77. Gunawan P et al (2011) Hollow fiber membrane decorated with Ag/MWNTs: toward effective water disinfection and biofouling control. ACS Nano 5(12):10033–10040
78. Macevele LE, Moganedi KLM, Magadzu T (2017) Investigation of antibacterial and fouling resistance of silver and multi-walled carbon nanotubes doped poly(vinylidene fluoride-co-hexafluoropropylene) composite membrane. Membranes (Basel), 7(3)
79. Seo Y et al (2018) Engineering copper nanoparticles synthesized on the surface of carbon nanotubes for anti-microbial and anti-biofilm applications. Nanoscale 10(33):15529–15544
80. Pang WY, Ahmad AL, Zaulkiflee ND (2019) Antifouling and antibacterial evaluation of ZnO/MWCNT dual nanofiller polyethersulfone mixed matrix membrane. J Environ Manage 249:109358
81. Pangule RC et al (2010) Antistaphylococcal nanocomposite films based on enzyme-nanotube conjugates. ACS Nano 4(7):3993–4000
82. Qi X et al (2011) Covalent immobilization of nisin on multi-walled carbon nanotubes: superior antimicrobial and anti-biofilm properties. Nanoscale 3(4):1874–1880
83. Dong X, McCoy E, Zhang M, Yang L (2014) Inhibitory effects of nisin-coated multi-walled carbon nanotube sheet on biofilm formation from *Bacillus anthracis* spores. J Environ Sci 26(12):2526–2534
84. Zhou J, Qi X (2011) Multi-walled carbon nanotubes/epilson-polylysine nanocomposite with enhanced antibacterial activity. Lett Appl Microbiol 52(1):76–83
85. Huang Y-W et al (2017) Versatile polyvinylidene fluoride hybrid ultrafiltration membranes with superior antifouling, antibacterial and self-cleaning properties for water treatment. J Colloid Interface Sci 505:38–48
86. Alizadeh A, Razmjou A, Ghaedi M, Jannesar R (2019) Nanoporous solid-state membranes modified with multi-wall carbon nanotubes with anti-biofouling property. Int J Nanomed 14:1669–1685

87. Weinstein MP et al (1997) The clinical significance of positive blood cultures in the 1990s: a prospective comprehensive evaluation of the microbiology, epidemiology, and outcome of bacteremia and fungemia in adults. Clin Infect Dis 24(4):584–602
88. Control ECfDPa (2008) Report on the state of communicable diseases in the EU and EEA/EFTA countries
89. Klevens RM et al (2007) Estimating health care-associated infections and deaths in U.S. hospitals, 2002. Public Health Rep 122(2): 160–166
90. Vagos MR, Moreira JMR, Soares OSGP, Pereira MFR, Mergulhao FJ (2019) Incorporation of Carbon Nanotubes in Polydimethylsiloxane to Control Escherichia coli Adhesion. Polym Compos 40:E1697–E1704

Chapter 5
Engineered Phyllosilicate Clay-Based Antimicrobial Surfaces

S. Snigdha, Nandakumar Kalarikkal, Sabu Thomas, and E. K. Radhakrishnan

1 Introduction

Nanostructured materials (NSM) are omnipresent and have been with us since the beginning of time. The presence of nanoparticles was undetected until the development of the electron microscope, after which the use and artificial synthesis of nanoparticles and nanostructured materials exploded. Nanomaterials are currently being applied to improve every field known to man [1–3]. Their unique physical, chemical and biological properties make them very lucrative in biomedical, environmental and industrial applications.

2 Types of Nanostructured Materials

Nanostructured materials also known as nanoparticles can be classified based on their origin, chemical nature and dimensions. Nanoparticles (NPs) can be widely classified into natural and synthetic varieties: Naturally occurring NPs are found in volcanic

S. Snigdha · N. Kalarikkal · S. Thomas
International and Inter University Centre for Nanoscience and Nanotechnology, Mahatma Gandhi University, Kottayam, Kerala 686560, India

E. K. Radhakrishnan (✉)
School of Biosciences, Mahatma Gandhi University, PD Hills (PO), Kottayam, Kerala 686560, India
e-mail: radhakrishnanek@mgu.ac.in

S. Thomas
School of Chemical Sciences, Mahatma Gandhi University, Kottayam, Kerala 686560, India

N. Kalarikkal
School of Pure and Applied Physics, Mahatma Gandhi University, Kottayam, Kerala 686560, India

S. Snigdha et al. (eds.), *Engineered Antimicrobial Surfaces*, Materials Horizons: From Nature to Nanomaterials, https://doi.org/10.1007/978-981-15-4630-3_5

ash, ocean spray, fine sand, dust, and biological entities such as viruses [4–7]. Synthetic NPs are as diverse as their naturally occurring counterparts. Synthetic NPs can be broadly classified into "incidental" and "engineered" nanoparticles. Incidental NPs are usually the by-products of human activity and are of low purity and vary in sizes and shapes [8]. These may also be of varied elemental composition. The NPs produced as a result of mining, and burning of fossil fuels constitutes examples of incidental NPs. Engineered NPs are designed and synthesized in a controlled manner to achieve NPs of uniform shape, size and composition [9–11]. Nanoparticles can also be classified as organic and inorganic nanoparticles. The organic nanoparticles include micelles, dendrimers, liposomes, hybrid and compact polymeric NPs. Quantum dots, silicates and metal NPs constitute the inorganic group of nanomaterials. This report focusses on the use of silicates for microbiological applications [12, 13].

In addition, nanoparticles are also classified according to their dimensions. One-dimensional NPs are with a single dimension less than 100 nm. These NPs exist in layer or sheet forms having thickness of a few nanometres, examples include graphene, clays, graphene oxide and layered hydroxides. These NPs are often referred to as nanosheets or nanolayers. Two-dimensional NPs have two dimensions less than 100 nm and the other dimension larger than 100 nm. 2D NPs possess elongated structure such as carbon nanotubes and cellulose whiskers. Three-dimensional NPs possess all dimensions less than 100 nm. Such NPs are also referred to as isodimensional NPs, and examples include spherical silica, fullerene and quantum dots [14–16].

2.1 Nanostructured Clay Minerals

Nanostructured clay minerals (phyllosilicate clays) have been used by humans since prehistoric times. These were basically used for health-related and cosmetic applications [17, 18]. Layered silicates which are widely used for preparing nanocomposites exist as thin sheets that are stacked together. The basic building blocks of the layered silicates are a tetrahedral sheet with silicon bound to four oxygen atoms and an octahedral sheet with a metal such as aluminium bound to eight oxygen atoms. These building blocks exist either as 1:1 silicate or 2:1 silicate. In 1:1 silicate, a tetrahedral sheet is fused to a octahedral sheet [19]. The 2:1 silicates possess a central octahedral sheet flanked on either sides by tetrahedral silica sheets (Fig. 1). Each silicate layer thickness is around 1 nm, and the length may range from 300 Å to several micrometres. These materials possess a very high aspect ratio (length/thickness) [20].

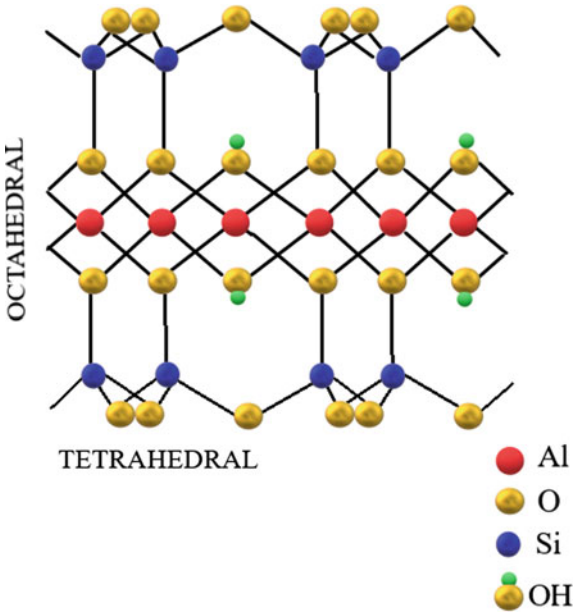


Fig. 1 General structure of phyllosilicates

2.2 *Artificial Nanoclay—Laponite®*

The smectite group of phyllosilicates has been widely used in pharmaceutical and cosmetic applications [21]. Their varied applications made them to be subjected to various physical, chemical and toxicological testing. The natural origin of the clay silicates makes control over their properties and composition challenging. In order to overcome these challenges, the use of synthetic clay minerals was considered. These synthetic clays can be economically produced in bulk with controlled composition, purity and dimensions. Laponite® is a synthetic smectite composed of nanometric crystals produced by BYK Additives and Instruments [21]. Each clay crystal is disc shaped with a diameter of 25 nm and 1 nm height. Laponite has the empirical formula $Na^{+}_{0.7}$ $[(Si_8Mg_{5.5}Li_{0.3})O_{20}(OH)_4]$ [22]. It is a 2:1 crystal with two tetrahedral silica sheets sandwiching an octahedral sheet containing magnesium ions. Lithium ions are also found to randomly replace magnesium ions in the octahedral sheet [23]. This substitution results in rendering a net negative charge on the disc face of the clay crystal. This negative charge is balanced by the positive charge of the sodium ion present between the crystals and in the surrounding aqueous medium. This charge difference produces an electrostatic interaction which promotes stacking in the dry form of the clay. The weak negative charge on the disc face allows more water absorption, resulting in increased clay volume. Under controlled conditions, complete delamination of the crystal stack can occur [24]. Laponite forms clear colloidal dispersions due to the electrostatic repulsions among the crystals, on introducing ions/polar molecules, the attractive forces become dominant compared to the repulsive forces, and this

leads to formation of thixotropic gels. These gels lose their shape upon application of stress, and upon removing the applied stress, the gels can reform [25]. It is also known that the weak positive charges on the edge of the laponite crystals associate with the negatively charges disc face of the other crystals and form 3-D structures by self-assembly (Fig. 2). These three-dimensional structures are often referred to as "house of cards" [26].

Laponite has been known to degrade under acidic conditions into silica ($Si(OH)_4$), sodium, magnesium and lithium ions. It is vulnerable to chemical degradation as well [27, 28]. Laponite has also been observed to be non-toxic to human mesenchymal stem cells at concentrations less than 1 mg/mL [29].

Laponite has been extensively used for biomedical applications due to the ease with which it extends itself for functionalization. It has been used for delivery of drugs and various bioactive molecules, bioimaging and as pH sensitive hydrogel [30–32]. It is also well known for its ability to induce osteogenesis and therefore

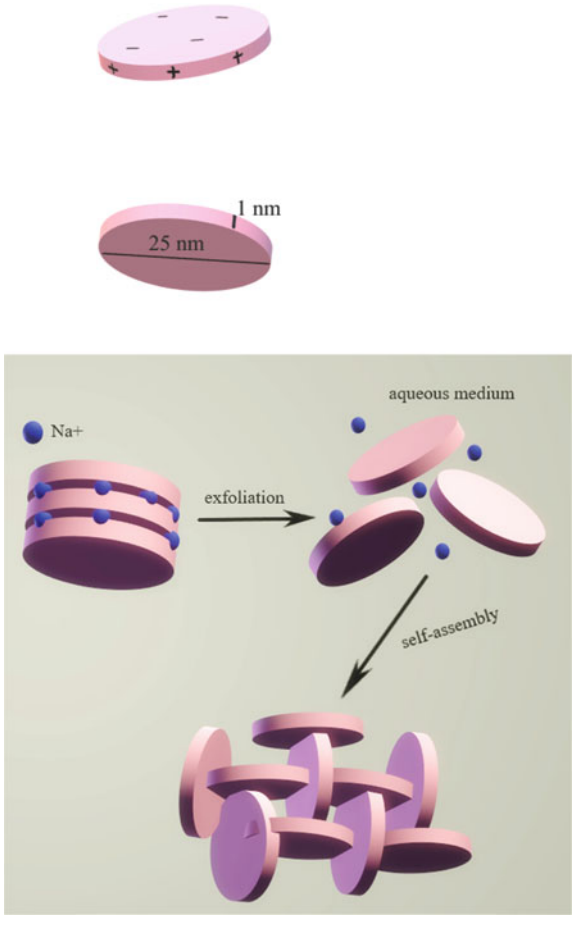


Fig. 2 Laponite clay individual platelets with charge distribution, dimensions and formation of house of cards structure through self-assembly

has its popularity as a bone tissue engineering scaffold. It is also used to modulate gel/scaffold properties [23].

2.3 Organically Modified Nanoclay

Montmorillonite (MMT) is the most researched and applied member of the smectite clay group. It has the general formula $M_x(Al_{4-x}Mg_x)Si_8O_{20}(OH)_4$, where M is a monovalent cation, x is the degree of isomorphism with substitution between 0.5 and 1.3. MMT is a 2:1 phyllosilicate in which the aluminium cations are partially replaced by magnesium ions in the octahedral layer [33]. Overall negative charge is balanced by sodium and calcium ions in the hydrated state of MMT. The layers are therefore held by weak forces, which enable water and other polar molecules to enter the unit structures of MMT causing the lattice to expand [34]. The high aspect ratio and intercalation properties make the MMT clay a very attractive candidate for various applications [35].

Pristine MMT is compatible only with hydrophobic polymers. In order to make MMT compatible with hydrophobic polymers, the alkali counterions such as Na^+ were substituted with a cationic organic moiety [36]. Some of the most commonly used cations are the alkylammonium ions. The sulfonium and phosphonium ions could also be used to substitute the exchangeable cations [33]. The organic cations lower the surface energy and provide better interaction with the polymer matrix. The long chains of the organic moieties also aid in increasing the clay gallery height. The increased gallery height enhances clay–polymer interactions [37]. The alkyl ammonium moieties also could act as initiators and mediate polymerization of monomer units. Therefore, organic modification of layered silicates creates a microenvironment that favours polymer–clay interactions (Fig. 3). The capacity of the clay to exchange ions is expressed as cation exchange capacity (CEC) usually expressed as mequiv/g [33]. It has been estimated that surfactant amounts to about 35–45 wt% in terms of the CEC of the clay [38].

2.4 Hybrid Nanoclay

Silicates are versatile building blocks for preparation of various hybrid materials [39]. One of the oldest reported "clay hybrid" could be the clay/urine organocomplex used for laundry applications in ancient Rome. The Mayan blue pigment can be considered as one of the first clay/nanohydrids. It was based on microfibrous clay (palygorskite) and plant-derived indigo dye. Phyllosilicate clays are being used in combination with various moieties for producing highly effective antimicrobials. When combined with phyllosilicates, the otherwise feebly antimicrobial materials show improved and synergistic microbicidal tendencies. Improved antimicrobial properties in the hybrids are usually attributed to the ability of phyllosilicates to interact and adsorb the bacterial

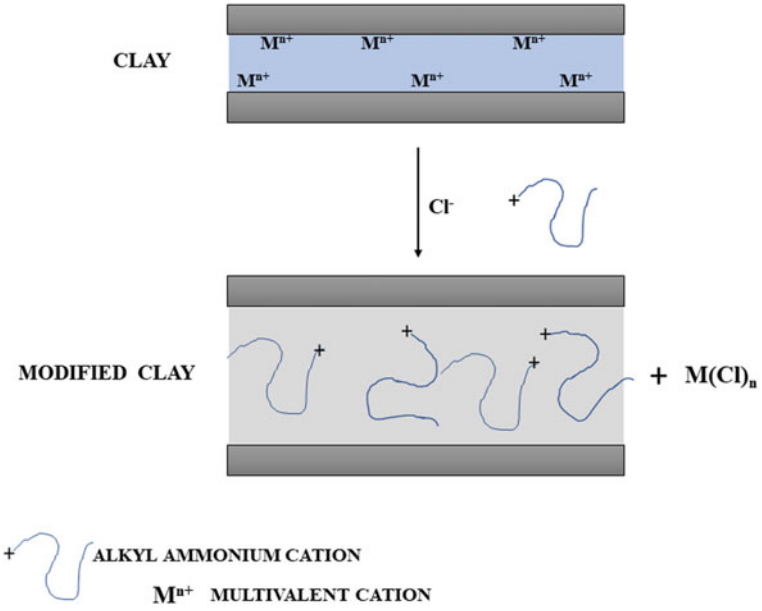


Fig. 3 Representation of cation-exchanged montmorillonite clay

cells onto their surfaces, thereby effectively exposing them to the bactericidal action of incorporated antimicrobial [40, 41]. Table 1 summarizes some of the promising clay-hybrids used for antimicrobial applications. Each passing day witnesses the development of many interesting clay-hybrids and their exceptional antimicrobial activities [42].

3 Polymer Nanocomposites

Polymers are used extensively in every field. In the earlier days, inorganic fillers were used in conjugation with polymers to improve their performance and to make them cost effective. However, these inorganic modifiers added disadvantages such as increase in weight, opacity and brittleness to the polymer matrices [71, 72]. On the other hand, nanocomposites, in which the fillers possess at least one dimension in the nanometric size, showed great performance and exceptional properties [73, 74]. The most widely used nanocomposites are the polymer/clay composites. Polymer/clay nanocomposites have been known for decades, and the earliest study was reported in 1949 by Bower [75]. The Toyota researchers were instrumental in starting detailed research on layered silicate/polymer nanocomposites across the world [76, 77]. Giannelis and co-workers reported the intercalation of polymer chains into clay galleries

Table 1 Promising clay-hybrids for antimicrobial applications

Clay type	Modification	Properties	References
MMT/Laponite	Ag^+	Release of reactive oxygen species (ROS)	[43–47]
MMT/Laponite	Antibiotics	Targeting various proteins and metabolic pathways of bacteria	[43, 48–56]
MMT/Laponite	Cu(II)	Bacterial cell wall injury, enzyme leakage, respiratory cycle inhibition	[57–60]
MMT	Methylene blue	Singlet oxygen generation, greater cell adhesion	[61, 62]
MMT	Various organic modifiers such as alkyl ammonium moieties, 4-aminothiophenol, hexadecyltrimethylammonium (HDTMA)	Bacterial cell adhesion and halide ions release	[40, 41, 43, 63–66]
MMT	TiO_2 nanoparticles		[47, 67, 68]
MMT	ZnO		[67, 69, 70]

to form expanded clay–polymer structures [78, 79]. The renewal of interest in polymer/clay nanocomposites led to great advancements in clay intercalation chemistry and has branched out from thermosetting and thermoplastics to polymer of biological origin as well. The low cost, easy availability and high aspect ratio of nanoclays make polymer clay nanocomposites very attractive for various applications [80, 81]. The three methods used to prepare PNCs are as follows:

3.1 Solution Dispersion or Solvent Casting

The clay is dispersed in a solvent, and the dispersed/swollen clay is mixed with a polymer in solvent and stirred, during the stirring process, the polymer chains move

into the interlayer spaces of the clay. The solvent is then removed by evaporation to yield a PNC membrane [82].

3.2 In Situ Polymerization

Liquid monomer or monomers solution is mixed with clay, and the polymerization of the monomers occurs in the clay galleries. The polymerization occurs in the swollen interlayer of clay in the presence of initiations such as heat, radiation, organic initiator, diffusion by a suitable initiator and catalyst fixed through cationic exchange inside the interlayer before swelling [83, 84].

3.3 Melt Intercalation

The clay can be mixed with the molten polymer under shear or annealing process. Then, polymer chains get inserted into the interlayer of the clay. This is a common method to prepare PCNs. This technique is usually used for industrial applications [84].

3.4 Polymer/Clay Interactions

Polymer/clay interactions in a polymer/clay composite are primarily of three types: Conventional polymer/clay composites or blend, intercalated structures and exfoliated nanocomposites (Fig. 4). Polymer/clay conventional composites consist of phase separated polymer and clay where the clay acts as normal filler and appears as tactoids. The polymer usually does not enter the clay interlayer [85]. Polymer clay nanocomposites comprise either intercalated or exfoliated morphologies [86]. Intercalated structures are formed when one or more polymer chain is intercalated between silicate layers resulting in ordered, multi-layered arrangements of alternating polymeric and inorganic layers [40]. Intercalation causes less than 20–30 Å

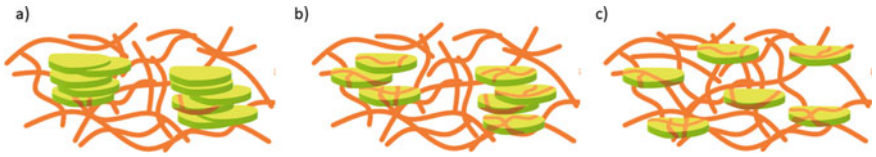


Fig. 4 Clay–polymer interactions: **a** polymer/clay blend, **b** polymer/clay intercalation, **c** exfoliation of clay in polymer matrix

Table 2 Antimicrobial polymer/clay composites

Polymer	Clay	Antimicrobial agent used	References
Chitosan	MMT	Chitosan	[88–90]
Poly(butylene adipate-co-terephthalate) (PBAT)	MMT	PBAT	[91]
Zein	MMT	*H. perforatum* oil	[92]
Poly(caprolactone) (PCL)	Cloisite 30B	Alkyl ammonium moieties	[40]
Polyethylene (PE)	Ag-MMT	Ag^+	[45]

separation between the platelets of the silicates. Exfoliated nanocomposites consist of clay platelets that are completely separated from each other.

Polymer/clay nanocomposites (PNCs) are the dispersion of NPs into a polymer matrix to form hybrid organic–inorganic nanocomposites. The incorporation of nanoclays has showed improvement in several properties of the polymer matrix such as mechanical properties, thermal stability and barrier properties [87]. The PNCs are also extremely versatile and can be engineered to produce effective antimicrobial surfaces. The antimicrobial moieties can be either included in the clay galleries to facilitate slower release, within the polymer matrix or a combination of these two methods to develop effective antimicrobial surfaces that can be engineered according to requirements of the end application. Table 2 shows a few examples of polymer/clay composites that have antimicrobial activity. These nanocomposite materials can be effectively used as coating on various surfaces.

4 Conclusions

Though nanoclays in their native state do not possess antimicrobial properties, they act as versatile platforms that lend themselves to easy modifications. The exchangeable ions in the intergallery spaces of the clay tactoids make the functionalization of clay minerals highly sort after. The high aspect ratio and surface charge of the phyllosilicates tend to be favourable for attracting the bacteria and exposing them effectively to the antimicrobial activities of the exchanged functional groups. One of the interesting uses of phyllosilicates is in the area of antimicrobial studies, wherein various antimicrobial moieties exhibited improved antimicrobial activity upon combining with clay minerals. Various hybrid materials incorporating clay are being engineered, which possess multiple modes of action against the microorganism. Therefore clay minerals hold immense potential for research and development of highly effective antimicrobial materials.

Acknowledgements The authors are grateful to the facilities provided by the International and Inter University Centre for Nanoscience and Nanotechnology, School of Chemical Sciences, School of Pure and Applied Physics and School of Biosciences, Mahatma Gandhi University.

References

1. Chen Y, Fan Z, Zhang Z, Niu W, Li C, Yang N, Chen B, Zhang H (2018) Two-dimensional metal nanomaterials: synthesis, properties, and applications. Chem Rev 118(13):6409–6455
2. Quesada-González D, Merkoçi A (2018) Nanomaterial-based devices for point-of-care diagnostic applications. Chem Soc Rev 47(13):4697–4709
3. Barreca D, Gri F, Gasparotto A, Carraro G, Bigiani L, Altantzis T, Žener B, Štangar UL, Alessi B, Padmanaban DB (2019) Multi-functional MnO_2 nanomaterials for photo-activated applications by a plasma-assisted fabrication route. Nanoscale 11(1):98–108
4. Guo H, Barnard AS (2013) Naturally occurring iron oxide nanoparticles: morphology, surface chemistry and environmental stability. J Mater Chem A 1(1):27–42
5. Hough R, Noble R, Hitchen G, Hart R, Reddy S, Saunders M, Clode P, Vaughan D, Lowe J, Gray D (2008) Naturally occurring gold nanoparticles and nanoplates. Geology 36(7):571–574
6. Theng BK, Yuan G (2008) Nanoparticles in the soil environment. Elements 4(6):395–399
7. Ermolin MS, Fedotov PS, Malik NA, Karandashev VK (2018) Nanoparticles of volcanic ash as a carrier for toxic elements on the global scale. Chemosphere 200:16–22
8. Hochella MF, Mogk DW, Ranville J, Allen IC, Luther GW, Marr LC, McGrail BP, Murayama M, Qafoku NP, Rosso KM (2019) Natural, incidental, and engineered nanomaterials and their impacts on the earth system. Science 363(6434):eaau8299
9. Kumar P, Kumar A, Lead JR (2012) Nanoparticles in the Indian environment: known, unknowns and awareness. ACS Publications
10. Dwivedi AD, Dubey SP, Sillanpää M, Kwon Y-N, Lee C, Varma RS (2015) Fate of engineered nanoparticles: implications in the environment. Coord Chem Rev 287:64–78
11. Ealias AM, Saravanakumar M (2017) A review on the classification, characterisation, synthesis of nanoparticles and their application. IOP Conf Ser Mater Sci Eng 032019
12. Pugazhendhi A, Edison TNJI, Karuppusamy I, Kathirvel B (2018) Inorganic nanoparticles: a potential cancer therapy for human welfare. Int J Pharm 539(1–2):104–111
13. Pan K, Zhong Q (2016) Organic nanoparticles in foods: fabrication, characterization, and utilization. Annu Rev Food Sci Technol 7:245–266
14. Pang X, He Y, Jung J, Lin Z (2016) 1D nanocrystals with precisely controlled dimensions, compositions, and architectures. Science 353(6305):1268–1272
15. Álvarez-Docio C, Reinosa J, Del Campo A, Fernández J (2019) Investigation of thermal stability of 2D and 3D $CoAl_2O_4$ particles in core-shell nanostructures by Raman spectroscopy. J Alloy Compd 779:244–254
16. Zhang N, Huang Y, Wang M (2018) 3D ferromagnetic graphene nanocomposites with ZnO nanorods and Fe_3O_4 nanoparticles co-decorated for efficient electromagnetic wave absorption. Compos B Eng 136:135–142
17. Li Z, Li M, Ashok J, Kawi S (2019) NiCo@ NiCo phyllosilicate@ CeO_2 hollow core shell catalysts for steam reforming of toluene as biomass tar model compound. Energy Convers Manag 180:822–830
18. Sunil B, Pushpalatha M, Basavaprasad VMHT (2018) Modified nano-clay formulation and their application. IJCS 6(4):705–710
19. Beyer G (2002) Nanocomposites: a new class of flame retardants for polymers. Plast Addit Compd 4(10):22–28
20. Komadel P (2003) Chemically modified smectites. De Gruyter
21. Tomás H, Alves CS, Rodrigues J (2018) Laponite®: a key nanoplatform for biomedical applications? Nanomed Nanotechnol Biol Med 14(7):2407–2420
22. Additives B (2014) Instruments. Laponite performance additives, technical information B-RI 21. BYK Additives, Ltd., Cheshire
23. Dawson JI, Kanczler JM, Yang XB, Attard GS, Oreffo RO (2011) Clay gels for the delivery of regenerative microenvironments. Adv Mater 23(29):3304–3308
24. Tawari SL, Koch DL, Cohen C (2001) Electrical double-layer effects on the Brownian diffusivity and aggregation rate of laponite clay particles. J Colloid Interface Sci 240(1):54–66

25. Cummins HZ (2007) Liquid, glass, gel: the phases of colloidal laponite. J Non-Cryst Solids 353(41–43):3891–3905
26. Thompson DW, Butterworth JT (1992) The nature of laponite and its aqueous dispersions. J Colloid Interface Sci 151(1):236–243
27. Mohanty RP, Joshi YM (2016) Chemical stability phase diagram of aqueous laponite dispersions. Appl Clay Sci 119:243–248
28. Jatav S, Joshi YM (2014) Chemical stability of laponite in aqueous media. Appl Clay Sci 97:72–77
29. Gaharwar AK, Mihaila SM, Swami A, Patel A, Sant S, Reis RL, Marques AP, Gomes ME, Khademhosseini A (2013) Bioactive silicate nanoplatelets for osteogenic differentiation of human mesenchymal stem cells. Adv Mater 25(24):3329–3336
30. Xiao S, Castro R, Maciel D, Gonçalves M, Shi X, Rodrigues J, Tomás H (2016) Fine tuning of the pH-sensitivity of laponite–doxorubicin nanohybrids by polyelectrolyte multilayer coating. Mater Sci Eng C 60:348–356
31. Wu Y, Guo R, Wen S, Shen M, Zhu M, Wang J, Shi X (2014) Folic acid-modified laponite nanodisks for targeted anticancer drug delivery. J Mater Chem B 2(42):7410–7418
32. Felbeck T, Behnke T, Hoffmann K, Grabolle M, Lezhnina MM, Kynast UH, Resch-Genger U (2013) Nile-Red–nanoclay hybrids: red emissive optical probes for use in aqueous dispersion. Langmuir 29(36):11489–11497
33. Manias E, Touny A, Wu L, Strawhecker K, Lu B, Chung T (2001) Polypropylene/montmorillonite nanocomposites. Review of the synthetic routes and materials properties. Chem Mater 13(10):3516–3523
34. Ma J, Yu Z-Z, Zhang Q-X, Xie X-L, Mai Y-W, Luck I (2004) A novel method for preparation of disorderly exfoliated epoxy/clay nanocomposite. Chem Mater 16(5):757–759
35. Wang L, Liu W, Li Y, Wu P, Shen S (2019) Mechanical behaviors of methane hydrate-bearing sediments using montmorillonite clay. Energy Procedia 158:5281–5286
36. Zanetti M, Lomakin S, Camino G (2000) Polymer layered silicate nanocomposites. Macromol Mater Eng 279(1):1–9
37. Kornmann X, Lindberg H, Berglund LA (2001) Synthesis of epoxy–clay nanocomposites: influence of the nature of the clay on structure. Polymer 42(4):1303–1310
38. Yu ZZ, Yang M, Zhang Q, Zhao C, Mai YW (2003) Dispersion and distribution of organically modified montmorillonite in nylon-66 matrix. J Polym Sci Part B Polym Phys 41(11):1234–1243
39. Kawasumi M (2004) The discovery of polymer-clay hybrids. J Polym Sci Part A Polym Chem 42(4):819–824
40. Babu SS, Kalarikkal N, Thomas S, Radhakrishnan E (2018) Enhanced antimicrobial performance of cloisite 30B/poly (ε-caprolactone) over cloisite 30B/poly (l-lactic acid) as evidenced by structural features. Appl Clay Sci 153:198–204
41. Babu SS, Mathew S, Kalarikkal N, Thomas S (2016) Antimicrobial, antibiofilm, and microbial barrier properties of poly (ε-caprolactone)/cloisite 30B thin films. 3 Biotech 6(2):249
42. Malachová K, Praus P, Pavlíčková Z, Turicová M (2009) Activity of antibacterial compounds immobilised on montmorillonite. Appl Clay Sci 43(3–4):364–368
43. Parolo M, Fernández L, Zajonkovsky I, Sánchez M, Bastion M (2011) Antibacterial activity of materials synthesized from clay minerals. In: Science against microbial pathogens: communicating current research and technological advances. Formatex, microbiology series, vol 3, pp 144–151
44. Costa C, Conte A, Buonocore GG, Del Nobile MA (2011) Antimicrobial silver-montmorillonite nanoparticles to prolong the shelf life of fresh fruit salad. Int J Food Microbiol 148(3):164–167
45. Roy A, Joshi M, Butola B, Ghosh S (2020) Evaluation of biological and cytocompatible properties in nano silver-clay based polyethylene nanocomposites. J Hazard Mater 384:121309
46. Kheiralla ZMH, Rushdy AA, Betiha MA, Yakob NAN (2014) High-performance antibacterial of montmorillonite decorated with silver nanoparticles using microwave-assisted method. J Nanopart Res 16(8):2560

47. Zhang GK, Ding XM, He FS, Yu XY, Zhou J, Hu YJ, Xie JW (2008) Low-temperature synthesis and photocatalytic activity of TiO_2 pillared montmorillonite. Langmuir 24(3):1026–1030
48. Jayrajsinh S, Shankar G, Agrawal YK, Bakre L (2017) Montmorillonite nanoclay as a multifaceted drug-delivery carrier: a review. J Drug Deliv Sci Technol 39:200–209
49. Herrera P, Burghardt R, Phillips T (2000) Adsorption of *s* by cetylpyridinium-exchanged montmorillonite clays. Vet Microbiol 74(3):259–272
50. Kevadiya BD, Rajkumar S, Bajaj HC, Chettiar SS, Gosai K, Brahmbhatt H, Bhatt AS, Barvaliya YK, Dave GS, Kothari RK (2014) Biodegradable gelatin–ciprofloxacin–montmorillonite composite hydrogels for controlled drug release and wound dressing application. Colloids Surf B 122:175–183
51. Gulen B, Demircivi P (2020) Adsorption properties of flouroquinolone type antibiotic ciprofloxacin into 2: 1 dioctahedral clay structure: Box-Behnken experimental design. J Mol Struct 127659
52. Kaur N (2020) Nanoantimicrobials: an emerging technological approach in food preservation. In: Technological developments in food preservation, processing, and storage. IGI Global, pp 146–165
53. Hamilton A, Hutcheon G, Roberts M, Gaskell E (2014) Formulation and antibacterial profiles of clay–ciprofloxacin composites. Appl Clay Sci 87:129–135
54. Kalwar K, Zhang X, Bhutto MA, Dali L, Shan D (2017) Incorporation of ciprofloxacin/laponite in polycaprolactone electrospun nanofibers: drug release and antibacterial studies. Mater Res Express 4(12):125401
55. Ghadiri M, Chrzanowski W, Rohanizadeh R (2014) Antibiotic eluting clay mineral (Laponite®) for wound healing application: an in vitro study. J Mater Sci Mater Med 25(11):2513–2526
56. Wang S, Wu Y, Guo R, Huang Y, Wen S, Shen M, Wang J, Shi X (2013) Laponite nanodisks as an efficient platform for doxorubicin delivery to cancer cells. Langmuir 29(16):5030–5036
57. Tong G, Yulong M, Peng G, Zirong X (2005) Antibacterial effects of the Cu(II)-exchanged montmorillonite on *Escherichia coli* K88 and *Salmonella choleraesuis*. Vet Microbiol 105(2):113–122
58. Hu C, Xu Y, Xia M, Xiong L, Xu Z (2007) Effects of Cu^{2+}-exchanged montmorillonite on growth performance, microbial ecology and intestinal morphology of Nile tilapia (*Oreochromis niloticus*). Aquaculture 270(1–4):200–206
59. Bagchi B, Kar S, Dey SK, Bhandary S, Roy D, Mukhopadhyay TK, Das S, Nandy P (2013) In situ synthesis and antibacterial activity of copper nanoparticle loaded natural montmorillonite clay based on contact inhibition and ion release. Colloids Surf B 108:358–365
60. Tang X, Dai J, Sun H, Nabanita S, Petr S, Tang L, Cheng Q, Wang D, Wei J (2018) Copper-doped nano laponite coating on poly (butylene succinate) scaffold with antibacterial properties and cytocompatibility for biomedical application. J Nanomater 2018
61. Bujdák J, Jurečeková J, Bujdáková H, Lang K, Šeršeň F (2009) Clay mineral particles as efficient carriers of methylene blue used for antimicrobial treatment. Environ Sci Technol 43(16):6202–6207
62. Chakraborty U, Singha T, Chianelli RR, Hansda C, Paul PK (2017) Organic-inorganic hybrid layer-by-layer electrostatic self-assembled film of cationic dye Methylene Blue and a clay mineral: Spectroscopic and Atomic Force microscopic investigations. J Lumin 187:322–332
63. Yilmaz YY, Yalcinkaya EE, Demirkol DO, Timur S (2020) 4-aminothiophenol-intercalated montmorillonite: organic-inorganic hybrid material as an immobilization support for biosensors. Sens Actuators B Chem 127665
64. Ganguly S, Dana K, Ghatak S (2009) Thermogravimetric study of n-alkylammonium-intercalated montmorillonites of different cation exchange capacity. J Therm Anal Calorim 100(1):71–78
65. Xi Y, Frost RL, He H (2007) Modification of the surfaces of Wyoming montmorillonite by the cationic surfactants alkyl trimethyl, dialkyl dimethyl, and trialkyl methyl ammonium bromides. J Colloid Interface Sci 305(1):150–158
66. He A, Wang L, Li J, Dong J, Han CC (2006) Preparation of exfoliated isotactic polypropylene/alkyl-triphenylphosphonium-modified montmorillonite nanocomposites via in situ intercalative polymerization. Polymer 47(6):1767–1771

67. Mustapha S, Ndamitso M, Abdulkareem A, Tijani J, Shuaib D, Ajala A, Mohammed A (2020) Application of TiO_2 and ZnO nanoparticles immobilized on clay in wastewater treatment: a review. Appl Water Sci 10(1):1–36
68. Mulewa W, Tahir M, Amin NAS (2017) MMT-supported Ni/TiO_2 nanocomposite for low temperature ethanol steam reforming toward hydrogen production. Chem Eng J 326:956–969
69. Ye J, Li X, Hong J, Chen J, Fan Q (2015) Photocatalytic degradation of phenol over ZnO nanosheets immobilized on montmorillonite. Mater Sci Semicond Process 39:17–22
70. Hu C, Gu L, Luan Z, Song J, Zhu K (2012) Effects of montmorillonite–zinc oxide hybrid on performance, diarrhea, intestinal permeability and morphology of weanling pigs. Anim Feed Sci Technol 177(1–2):108–115
71. Alexandre M, Dubois P (2000) Polymer-layered silicate nanocomposites: preparation, properties and uses of a new class of materials. Mater Sci Eng R Rep 28(1):1–63
72. Fischer H (2003) Polymer nanocomposites: from fundamental research to specific applications. Mater Sci Eng C 23(6–8):763–772
73. Giannelis EP (1996) Polymer layered silicate nanocomposites. Adv Mater 8(1):29–35
74. Krishnamoorti R, Vaia RA, Giannelis EP (1996) Structure and dynamics of polymer-layered silicate nanocomposites. Chem Mater 8(8):1728–1734
75. Bower C (1949) Studies on the form and availability of organic soil phosphorous. IOWA Agric Exp Stat Res Bull 362–339
76. Usuki A, Kojima Y, Kawasumi M, Okada A, Fukushima Y, Kurauchi T, Kamigaito O (1993) Synthesis of nylon 6-clay hybrid. J Mater Res 8(5):1179–1184
77. Usuki A, Koiwai A, Kojima Y, Kawasumi M, Okada A, Kurauchi T, Kamigaito O (1995) Interaction of nylon 6-clay surface and mechanical properties of nylon 6-clay hybrid. J Appl Polym Sci 55(1):119–123
78. Vaia RA, Ishii H, Giannelis EP (1993) Synthesis and properties of two-dimensional nanostructures by direct intercalation of polymer melts in layered silicates. Chem Mater 5(12):1694–1696
79. Mehrotra V, Giannelis E (1989) Conducting molecular multilayers: intercalation of conjugated polymers in layered media. MRS Online Proc Libr Arch 171
80. Wypych F, Bergaya F, Schoonheydt RA (2018) From polymers to clay polymer nanocomposites. In: Developments in clay science, vol 9. Elsevier, pp 331–359
81. Bee S-L, Abdullah M, Bee S-T, Sin LT, Rahmat A (2018) Polymer nanocomposites based on silylated-montmorillonite: a review. Prog Polym Sci 85:57–82
82. Rodríguez FJ, Abarca RL, Bruna JE, Moya PE, Galotto MJ, Guarda A, Padula M (2019) Effect of organoclay and preparation method on properties of antimicrobial cellulose acetate films. Polym Compos 40(6):2311–2319
83. Gohari DP, Kalaee MR, Sharif A (2019) Interfacial in situ polymerization of layered-silicate/poly (hexamethylene isophthalamide) nanocomposites. J Inorg Organomet Polym Mater 1–9
84. Reddy KR, Reddy CV, Babu B, Ravindranadh K, Naveen S, Raghu AV (2019) Recent advances in layered clays–intercalated polymer nanohybrids: synthesis strategies, properties, and their applications. In: Modified clay and zeolite nanocomposite materials. Elsevier, pp 197–218
85. Chen H-B, Schiraldi DA (2019) Flammability of polymer/clay aerogel composites: an overview. Polym Rev 59(1):1–24
86. Fu X, Qutubuddin S (2001) Polymer–clay nanocomposites: exfoliation of organophilic montmorillonite nanolayers in polystyrene. Polymer 42(2):807–813
87. Zhu TT, Zhou CH, Kabwe FB, Wu QQ, Li CS, Zhang JR (2019) Exfoliation of montmorillonite and related properties of clay/polymer nanocomposites. Appl Clay Sci 169:48–66
88. Ferfera-Harrar H, Aiouaz N, Dairi N, Hadj-Hamou AS (2014) Preparation of chitosan-g-poly (acrylamide)/montmorillonite superabsorbent polymer composites: studies on swelling, thermal, and antibacterial properties. J Appl Polym Sci 131(1)
89. Reddy AB, Manjula B, Jayaramudu T, Sadiku E, Babu PA, Selvam SP (2016) 5-Fluorouracil loaded chitosan–PVA/Na^+ MMT nanocomposite films for drug release and antimicrobial activity. Nano-Micro Lett 8(3):260–269

90. Wang X, Du Y, Yang J, Tang Y, Luo J (2008) Preparation, characterization, and antimicrobial activity of quaternized chitosan/organic montmorillonite nanocomposites. J Biomed Mater Res Part A 84(2):384–390
91. Mondal D, Bhowmick B, Mollick MMR, Maity D, Ranjan Saha N, Rangarajan V, Rana D, Sen R, Chattopadhyay D (2014) Antimicrobial activity and biodegradation behavior of poly (butylene adipate-co-terephthalate)/clay nanocomposites. J Appl Polym Sci 131(7)
92. Gunes S, Tamburaci S, Tihminlioglu F (2020) A novel bilayer zein/MMT nanocomposite incorporated with *H. perforatum* oil for wound healing. J Mater Sci Mater Med 31(1):7

Chapter 6
Modulating Surface Energy and Surface Roughness for Inhibiting Microbial Growth

Sasmita Majhi and Abhijit Mishra

1 Introduction

Bacterial adhesion and subsequent biofilm formation on surfaces poses significant health risk and also limits material application [1, 2]. Material design has emerged as an effective alternative in reducing bacteria adhesion onto surfaces, in lieu of using biocides, to overcome the increased risk of multi-drug-resistant strains [3, 4]. Various material properties such as surface topography, wettability, charge, and surface energy need to be considered while designing bacteria-resistant surfaces [5–7]. Surfaces with moderate wettability have enhanced bacterial attachment compared to more hydrophobic/philic surfaces. A hydrophobic surface with lower surface energy reduces bacteria adhesion compared to materials having higher surface energy. Superhydrophic surfaces (contact angle > 150°) with extremely low wettability implemented by rough surfaces with low surface energy resist bacterial adhesion very effectively. The reason for this is that air gets entrapped between the roughening structures on the surface, thereby reducing the interface contact area and adhesion force with the bacteria, hence reducing bacterial attachment on surface. Surface topography described by spatial distribution of roughening structures and macro or microscopic patterns on surfaces with relative to bacteria shape and size defines bacterial interaction with surfaces [8–11]. To address these surface features, nanoscience-based approaches including active (bactericidal) and/or passive (anti-adhesive) strategies have been explored to develop antimicrobial surfaces. The approaches based on nanoscience strategies for designing antibacterial surfaces combine the interdisciplinary knowledge from physics, chemistry, and materials science research [12]. Various improvements have been carried out by researchers to modify molecular properties of surfaces at micro- and nanoscale by engineering surface physical properties such as size, shape, spacing distance, and their organization on

S. Majhi · A. Mishra (✉)
Materials Science and Engineering, Indian Institute of Technology Gandhinagar, Palaj, Gandhinagar, Gujarat 382355, India
e-mail: amishra@iitgn.ac.in

S. Snigdha et al. (eds.), *Engineered Antimicrobial Surfaces*, Materials Horizons: From Nature to Nanomaterials, https://doi.org/10.1007/978-981-15-4630-3_6

surfaces to form a specific micro- and nano-patterned structures [13, 14]. Other ways include furnishing the surfaces with nanoparticles, nanocarriers, polymer brushes having inherent antimicrobial properties [15–18].

In this review, we focus on modulation of surface roughness and topography based on nanoscience-based strategies and surface energy based on fluorinated and non-fluorinated surfaces that influences bacterial adhesion.

2 Nature Inspired Micro- and Nanostructured Surfaces

Micro- and nanostructured surfaces available in nature, among insects, animals, and plants, have inspired many researchers to mimic their antibacterial properties. Such structured surfaces represent passive antimicrobial mechanism as they do not release any antimicrobial agents to the environment. Surface textures of animals and plants such as shark skin, lotus leaves, and taro leaves repel microbial adhesion due to the presence of micro- and nano superhydrophobic surfaces and thus form anti-adhesive surfaces. Similarly, surface patterns on gecko skin, cicada wings, dragon fly, kill bacteria forming bactericidal surfaces. These architectures on natural surfaces are mimicked onto desired surfaces by engineering the micro- and nano-patterns and the hydrophobicities [19–21]. Various patterns such as pillars, pits, ribs, channels, tubes, and ridges are fabricated on surfaces using various lithography, etching, and deposition techniques such as electron beam lithography (EBL), nanoimprint lithography (NIL), photolithography, soft lithography, chemical and vapour deposition, sol–gel, hydrothermal synthesis, and others [13]. Few of these techniques have been discussed below.

3 Lithography

Lithography is a micro- and nano-fabrication technique that enables precise small-scale designing of 2D or 3D structures.

Nanoimprint lithography (NIL) is a contact form of lithography (also called hot embossing) that uses a liquid polymer, called resist, to be micro/nano-patterned onto substrate surface through direct mechanical deformation of the resist using an imprint mould. NIL does not utilize photons or electrons to modify the resist, and therefore, the resolution of NIL is determined only by minimum features of the imprint mould. The advantage of this technique is that it can fabricate patterns with high resolution (sub 2 nm is also achievable) with high fidelity and high throughput rate [22]. Few disadvantages of NIL include damage to structures during removal of mould, chances of mould damage, and mould fabrication cost.

Soft lithography is a technique used to replicate micro- and nanostructures onto soft materials, such as polymers, gels, and organic monolayers. The patterns are

fabricated onto surfaces from a master surface using mould usually made up of poly(dimethylsiloxane) (PDMS) (key component). Thus, this technique combines printing, moulding, and embossing with stamps to fabricate patterned surfaces at the micron and submicron levels [23]. Advantage of this technique involves reusability of mould, thus making the technique cost-effective. Despite of being an effective technique, it can only be applied onto organic and polymeric materials.

Reactive ion etching (RIE) is a plasma etching technique to create nanostructured patterns [24]. High-energy ions, generated by plasma under vacuum conditions, or gas required for etching is injected into process chamber and subjected to radio frequency (RF) plasma source that bombards high-energy ions to substrate causing localized surface etching, thereby forming nano-patterned surfaces. This process usually etches surface textures with depth $< 1\ \mu m$.

Hydrothermal synthesis is a wet-chemical synthesis process that produces nanomaterials with uniform structure in large scale. It can produce multiple nanostructures, such as nanoparticles, nanotubes, nanowires, and nanorods. The nanostructure morphologies (shape, size, and roughness) can be tuned by altering experimental parameters such as precursor concentrations, solvent composition and pH, operation temperature and pressure, reaction duration [25, 26]. This method is widely used for the fabrication of nanostructural materials because of its reliability, high efficiency, environment friendliness, and low cost.

Chemical vapour deposition (CVD) and physical vapour deposition (PVD) are also used for nano-fabrication process while being used for preparing numerous materials and coatings. Both processes involve evaporation of target material followed by its deposition and condensation on substrate surface placed in a vacuum chamber. CVD technique uses a chemical reaction in chamber unlike PVD technique. Magnetron sputtering is a commonly used process of PVD technique that bombards high-energy ions to target material causing local removal of ions from target surface, and thus, various process parameters (target power, substrate temperature, deposition time, etc.) can be varied to obtain different nano-patterns [27].

4 Micro- and Nano-patterned Antibacterial Surfaces

Researchers have used different techniques to develop micro- and nano-patterns on surfaces to inhibit the microbial growth, thereby making the surfaces antibacterial and antifouling. Ye et al. prepared a cicada and catkin-inspired dual biomimetic structure on poly (ether-ether-ketone) (PEEK, model implant) surface to have reduced bacterial adhesion with wide antimicrobial range for longer durability [28]. Cicada wings-like biomimetic pattern having nanopillar-shape was prepared on PEEK surface by "template printing" method followed by deposition of catkins-like ZnO nanoslices on the ordered nanopillars by "hydrothermal method". Cicada wings-shaped nanopillar

structures could kill bacteria through direct contact by damaging their cell membrane followed by cell lysis; however, it usually kills less than 60% (especially gram-positive) bacteria. ZnO being an FDA-approved nanomaterial having good antibacterial effects can be combined with cicada biomimetic antibacterial structures to improve their antibacterial performance, particularly for gram-positive bacteria. Initially, burst release of ZnO nanoslices from implant surfaces could effectively kill the pathogenic bacteria around the implant, following which the exposed insect-like nanopillar structures would exert its long-term antibacterial activities against pathogens. ZnO showed better antibacterial activity against *S. aureus* with its rapid release at the initial stage of implantation, and the nanopillars were more effective in killing *E. coli*. The decreasing ratio of adhered *S. aureus* bacteria was over 50% after 5 h of attachment on nanopillar surface, which is also attributed towards increased hydrophobic feature of nanopillar structures. The developed dual biomimetic PEEK biomaterial also maintained an ideal biocompatibility.

Laser-treated surfaces affect the surface morphology and wettability, thereby influencing the bacteria adhesion. Laser exposure can create spikes (size: 20–40 μm), laser-induced periodic surface structures (LIPSS, size: 0.5–0.9 μm), and nanopillars (size: 0.8–1.3 μm). Lutey et al. examined the effect of laser-textured specimens on bacterial retention by analysing surface morphology, wettability, and topography [29]. *E. coli* is found to be retained on the specimens having spikes, while it gets reduced by 99.8% and 99.2% for LIPSS and nanopillars structures, respectively, whereas *S. aureus* retention is found to be inhibited on spike surface features, and their viability is also reduced by 84.7% and 79.9% for LIPSS and nanopillar specimens, respectively. Both LIPSS and nanopillars have low surface roughness (~60–90 nm) and moderate hydrophobicity (water contact angle ~ 119–140°) and exhibited fine features similar to size of bacterial cells, and hence, surface attachment points are limited. *E. coli* retention on surfaces depends on the relationship between cell dimensions and surface morphology, while *S. aureus* retention depends on surface wettability and average surface roughness. This shows both surface roughness and wettability together decide bacteria retention.

Researchers have also combined both passive and active strategies for designing antibacterial surfaces. Shark skin-inspired surface topographies have shown reduced microbial adhesion through a biocide-free structure–property relationship [30, 31]. Diamond-like riblets on shark skin facilitate self-cleaning by reducing drag. Sharklet AF is one such coating designed to mimic shark's skin, to reduce bacterial adhesion because of their unique hierarchical design and engineered roughness. However, similar to other microtopographic-patterned surfaces, bacteria can also accumulate onto Sharklet AF-patterned surfaces upon providing sufficient time of adhesion. Hence, along with microtopography, effective antimicrobial agents can be incorporated to develop multifunctional surfaces that decrease microbial adhesion and inactivate attached microbes. To this end, Arisoy et al. combined antifouling shark-skin patterns with antibacterial titanium dioxide (TiO_2) nanoparticles (NPs), to produce antifouling and antibacterial surfaces [32]. Shark-skin microstructure is imprinted using a UV-crosslinkable adhesive material (Norland Optical Adhesive), loaded with

varying TiO_2 NPs (0, 10, or 50 wt%) via solvent-assisted soft nanoimprint lithography (NIL) on poly (ethylene terephthalate) (PET) substrate followed by 10 s curing of near-infrared (NIR) irradiation. TiO_2 NPs introduced in the shark-skin surfaces increased the contact angle hysteresis from 30 to 100° and reduced the *E. coli* attachment by 70–85% and was able to kill 85–95% of *E. coli* and *S. aureus* after 1 h of UV light exposure because of the photocatalytic properties of TiO_2.

Insect wing-inspired nano-architectures on surfaces can also make them superhydrophobic, self-cleaning, and antibacterial. Hasan et al. prepared such nanostructured "super surface" using deep reactive ion etching (DRIE) of silicon wafer inspired by surface topographical features of dragonfly wings [33]. The prepared super surfaces have nanopillars of 4 μm height and 220 nm diameter with random inter-pillar spacing. The surfaces also exhibited superhydrophobicity with a static water contact angle (WCA) of 154.0° and contact angle hysteresis of 8.3°. The superhydrophobic surface having a low adhesion makes it self-cleaning. Nanostructured surfaces also showed low surface energy compared to control surfaces making it low adhesive in nature. The sharp nanopillars structures of the modified surfaces showed six-fold higher bactericidal activity against both gram-negative (*E. coli*) and gram-positive (*S. aureus*) strains through mechanical rupture of the cells. However, these nanostructured surfaces also killed mammalian cells (mouse osteoblasts) by mechanical rupture of the cell membrane, and this can be attributed to aspect ratio and packing of nanostructures, which may have different impact on different kinds of mammalian cells as observed in other studies [34, 35].

Minoura et al. explained the antibacterial effect of nanostructured moth-eye films against both *E. coli* and *S. aureus* as the synergistic role of both physical and chemical properties of the films [36]. They fabricated nanopillar structured moth-eye film composed of hydrophilic ultraviolet curable resins, urethane trifunctional acrylate having polyethylene glycol (PEG) derivatives as spacers on polyethylene terephthalate (PET) film using nanoimprint method, then compared them with flat structured films formed of the hydrophilic resin on PET and bare PET film. Antibacterial activity of prepared films, tested using bacterial droplet method which allows drying of bacterial suspension without any film cover, showed moth-eye film was more potent compared to bare film. The super-hydrophilic moth-eye film spreads the bacterial suspension readily on it, thus leading to enhancement in contact of bacteria with the surface. This leads to antibacterial activity of the surface because the bacterial suspension thickness decreases with the spreading causing shorter drying time which subsequently affects the survival of bacteria as the dry conditions hamper bacterial growth. This also explains antibacterial condition in case of hydrophilic flat-surfaced film as well, having the hydrophilic resin on PET surface. In addition, bacteria in contact with the hydrophilic moth-eye films get trapped with the adhesive property of PEG derivatives and get killed. The antibacterial property of moth-eye film is also aided by their nanostructures (nanopillars on surfaces) that enhances their physical adherence along with the chemical properties of the resin.

5 Synthetic Micro- and Nanostructured Surfaces

Biomaterial-related infections, especially postoperative infection associated with implants induced by bacterial invasion and biofilm formation on surfaces, are one of the most serious complications resulting in patient suffering, at times demanding implant removal and/or repeated surgeries leading to extended hospitalization, sometimes fatalities and increased financial burden. One of the effective ways to combat these infections includes bestowing biomaterials with antimicrobial ability without compromising their cytocompatibility. Nanoscale topographical modification on biomaterial surfaces can effectively improve their biological performance [31]. Recently, TiO_2 nanotube (NT) arrays have gained much interest in biomedical coatings because of their self-organizing nature with highly ordered and controlled dimensions that can be easily fabricated by electrochemical anodization of titanium (Ti) and its alloys. TiO_2 NTs have excellent biological performance but have inadequate antibacterial ability. Gao et al. prepared TiO_2 NT arrays on Ti with embedded Ag_2O nanoparticle (diameter, 5–20 nm) in the nanotube wall using TiAg magnetron sputtering and anodization [37]. These NT-Ag_2O arrays can effectively kill 97% of *E. coli* and *S. aureus* even after 28 days of immersion due to controlled release of Ag^+ from TiO_2 nanotube wall, suggesting long-lasting antibacterial ability without having any effect on osteoblast viability and differentiation. Release of Ag^+ from Ag induces inactivation bacterial proteins, DNA condensation, and bacterial cell membrane degradation. Additionally, direct contact with Ag NPs damages the bacterial plasma membrane causing bacteria lysis with release of cytosol. Herein, antibacterial activity of NT-Ag_2O arrays is synergistic effect of both Ag NPs and released Ag^+.

Wang et al. prepared antibacterial nano-silver(Ag)-functionalized Ti surfaces against two representative epidemic *Staphylococcus* strains. They incorporated Ag-nanoparticles on PEO-modified Ti surfaces following a hydrothermal chemical treatment [38]. Ag-nanoparticle-modified Ti surface was found to inhibit bacterial adhesion and biofilm formation of *Staphylococcus epidermidis* (*S. epidermidis*, RP62A) and *Staphylococcus aureus* (*S. aureus*, USA 300) by regulating their biofilm-related genes (icaA and icaR for *S. epidermidis*; fnbA and fnbB for *S. aureus*). TiO_2-Ag-0.1 samples presented better antibacterial activity (damaging effects on the planktonic bacteria) than TiO_2-Ag-0.01 samples. Anti-biofilm activity of the TiO_2-Ag samples can be explained by direct contact of Ag NPs with bacterial cell membranes which can damage the membrane integrity causing the leakage of inner cellular components and thus induces cell death. Further, Ag^+ ions released from Ag NPs can have a secondary bactericidal effect on cells starting with ion attachment to bacterial cell membrane followed by its entry into cytoplasm which eventually can destroy the intracellular structures leading to cell death. Additionally, modified surfaces also supported survival, adherence, and spreading of mammalian cells more effectively than *Staphylococcus* strains, thereby resulting in a mammalian cell-assisted, antibacterial functional Ag-modified Ti surfaces.

Similarly, Kim et al. studied the effect of morphology on antibacterial activity in case of titania nanoarrays (nanowire vs. nanoparticle) [39]. Anatase TiO_2 nanowire

(TNW) and TiO_2 nanoparticle (TNP) films were prepared by hydrothermal method and doctor blade method, respectively. The interaction of 1D nanoarrays with cell membranes depends on nanostructure properties (shape, aspect ratio and density) and cell type. Thus, nanostructures having higher aspect ratio topography and density shows better bactericidal activity compared to nanostructures having lower aspect ratio and density. This property is also reflected in case of TNW and TNP films; the vertical TNW films exhibited better antibacterial activity compared to the flat TNP films. This enhanced antibacterial property is attributed to the topography of nanowires that causes physical damage to bacterial outer membrane even though surface area is higher in case of TNP films.

Fisher et al. demonstrated tunable topographical features of nano-patterned surfaces affect their interaction with bacteria that includes shape, size, distribution, and spacing of nano-patterns in relation to shape and size of bacteria [40]. They fabricated two different diamond nanocone surfaces on silicon wafer using microwave plasma chemical vapour deposition (MPCVD), followed by electron cyclotron resonance (ECR)-assisted plasma reactive ion etching (RIE) having different substrate bias. Surface having higher bias (−200 V, surface A) produced more homogenous nanocones with higher density and sharp tips compared to the surface having lower bias (−150 V, surface B) that produced inhomogeneous nanocone arrays with decreased cone density. The diamond nanocone-shaped arrays showed bactericidal capabilities against *P. aeruginosa* by stretching and puncturing the bacterial cell wall resulting in bacterial cell lysis. Surface B showed better bactericidal activity (cell damage) compared to surface A because of its greater nonuniformity that allows the cells to lie across the tops of cones or falls between the cones that leads to nonrecoverable cell death.

Dickson et al. prepared bactericidal nanopillared polymer surfaces using nanoimprint lithography (NIL) [41]. They imprinted nanopillars onto the surfaces of poly (methyl methacrylate) (PMMA) films using commercial silicon and nickel moulds. Based on their periodicity, samples are named as "P600" and "P300". Another sample having cicada wings replicate fabricated using silicon negative mould of cicada wings in PMMA is named as "P200". Pillared films showed better antibacterial activity against *E. coli* compared to flat films, particularly films having smaller pillar geometry and closely spaced pillars as observed in case of P200. In comparison with flat surface, dead cells percentage increased by 16%, 97%, and 114% as measured on P600, P300, and P200 surfaces, respectively. Small and closely spaced pillars would exert more local stress on the bacterial cell membranes because as cells adsorb to the pillars, wetted surface area increases causing local cell stretching and eventually cell lysis.

Lee et al. investigated effect of non-thermal atmospheric pressure plasma jet (NTAPPJ) treatment on surface properties of titanium, namely surface roughness, surface free energy, and chemical composition that ultimately affects bacterial adhesion and viability [42]. NTAPPJ treatment was found to affect the surface chemistry and energy of titanium (Ti) surfaces without having an effect on the surface roughness. NTAPPJ treatment changed the surface chemistry by increasing levels of hydroxyl-related ions, such as OH– and COOH– while decreasing the hydrocarbon content. These changes increased the hydrophilicity as well as the surface

energy, and thus, indicated NTAPPJ treatment can effectively control the surface chemistry of Ti, including the surface energy without affecting its physical properties. Bacterial adhesion and biofilm formation rate were significantly reduced on the NTAPPJ-treated Ti surfaces compared to untreated surfaces. The relative adhesion rate of *S. mutans*, *S. aureus*, *K. oxytoca*, and *K. pneumoniae* on 10 min NTAPPJ-treated surfaces was found to be 6.9, 14.2, 0.66, and 0.42%, respectively, compared to the control surfaces. Similarly, biofilm formation rate was observed to be 67.866 ± 2.605, 65.853 ± 1.781, 46.887 ± 2.673, and 45.411 ± 1.658% for *S. mutans*, *S. aureus*, *K. oxytoca*, and *K. pneumoniae*, respectively, compared to control. The results demonstrated both adhesion and the bioflm formation rate were significantly lower for gram-negative bacteria (*K. oxytoca* and *K. pneumoniae*) than gram-positive bacteria (*S. mutans* and *S. aureus*) on NTAPPJ-treated Ti surfaces. Thus, NTAPPJ treatment could be an effective way for Ti-based implants in reducing implant-based diseases and implantation failure.

Pietrzyk et al. studied the change in surface topography, surface wettability, and antibacterial properties of SiO_2-based hydrophobic coatings prepared by sol–gel method modified with hydrophobizing compounds and zinc compounds [43]. The sols were prepared by modification of tetraethoxysilane (TEOS), with methyltrimethoxysilane (MTMS), hexamethyldisilazane (HMDS) and addition of zinc nitrate or zinc acetate. Coatings showed changes in surface topography and surface wettability upon modification with hydrophobizers (MTMS and HMDS). MTMS/HMDS-modified coatings showed increase in surface hydrophobicity due to introduction of $–CH_3$ (methyl) groups into the coating structure; however, these coatings did not have significant impact on *E. coli* adhesion, whereas introduction of Zn content to the coatings reduced the surface susceptibility to *E. coli* colonization. This antibacterial property of the coatings is aided by incorporation Zn atoms to coating structure by chemical bonds with oxygen (Zn–O) and alkyl groups. Reduction in bacterial colonization on surfaces along with bactericidal activity of Zn-based coatings provides a key feature for antibacterial touch surfaces.

6 Antimircobial Peptide Immobilization on Surfaces

Alternative synthetic strategies to obtain antibacterial surfaces are based on coating of organic compounds having antibacterial properties which are aided by change in surface topography and wettability. Majhi et al. have prepared such an antibacterial polystyrene (PS) surface by immobilizing a short, in-house designed antimicrobial peptide (AMP), KLR (KLLLRLRKLLRR) [44]. KLR is covalently immobilized on to PS surfaces both via its C-terminal using EDC/NHS chemical coupling and via its N-terminal using maleimide–thiol coupling with a Cysteine added to KLR (CKLLLRLRKLLRR) first. These AMP-modified polystyrene surfaces showed excellent antibacterial activity against both *E. coli* and *S. aureus*, killing 100% of both species and without having any cytotoxic effect towards fibroblasts. Chemical modification of PS surfaces for AMP immobilization has also affected the surface roughness and

wettability. This indicates it is the combined effect of surface physical properties and bacterial interaction with surfaces that have imparted antibacterial ability to the AMP-modified PS surfaces.

Chen et al. prepared an antibacterial surface by immobilizing a novel AMP, GL13K on titanium surface [45]. Peptide-coated surfaces were prepared by covalent conjugation of GL13K solutions with silanes ((3-chloropropyl) triethoxysilane (CPTES)) modified on hydroxyl (–OH) activated Ti surfaces. GL13K-coated Ti surfaces showed homogeneous distribution of GL13K peptides on surface being highly hydrophobic with WCA higher than 120 °C compared to control Ti surface (WCA ~ 5 °C). GL13K-coated surfaces substantially reduced adhered *S. gordonii* viability, metabolic activity and also prevented biofilm formation compared to control surfaces demonstrating direct bactericidal effect of coated GL13K peptide. Peptide-coated surfaces also showed disrupted bacterial cell wall forming holes or cracks on cell wall or along the circumferential lines of walls. This suggests peptide's ability towards bacterial interaction while causing membrane disruption and cell lysis.

7 Modulating Surface Energy

Surface energy also plays an important role in determining bacterial adhesion to surfaces. Micro/nano-patterned surfaces combined with low surface energy increase surface hydrophobicity or anti-wetting properties, thereby reduces bacterial adhesion. Kayes et al. modified polypropylene (PP) substrates through various oxygen and fluorine reactive ion etching (RIE) treatments to impart anti-biofouling properties by influencing surface chemistry, morphology, energy, and wettability [46]. The employed fluorine etch chemistry generated hierarchical microstructure/nanofibrils with low surface energy in PP substrates. Low power (LP) oxygen etch process creates hydrophilic surface and reduces *E. coli* adhesion by 68.7% compared to untreated PP, while high power (HP) oxygen etch process increases bacterial adhesion with same surface energy. This difference in antibacterial activity of HP-treated PP surfaces is attributed to microscale roughness of nanofibril structures that reduces the effectiveness of the liquid barrier due to the presence of air pockets. Oxygen-treated surfaces have high surface energy, with low static contact angles and high hysteresis. In contrast, fluorine plasma etch process creates hierarchical microstructure/nanofibrils exhibiting lotus-leaf wetting with high static water contact angle (~155°), low hysteresis (<10°), and low surface energy. These fluorinated surfaces reduce *E. coli* by 99.6% compared to control surfaces. These nanofibrils are smaller in size than bacteria and being aided by low surface energy, thus reduce *E. coli* adhesion significantly.

Incorporation of antibacterial cationic polymers enhances antibacterial durability of fabrics, but bacterial antiadhesion is usually difficult to achieve. Bacterial antiadhesion can be achieved by repelling or killing approaching bacteria by introducing highly negatively charged polymers or polymers with low surface energy that acts by electrostatic repulsion or by ultrahydrophobic repulsion, respectively. Lin et al.

prepared antibacterial and bacterially anti-adhesive cotton fabrics by spray-coating polymers having antibacterial quaternary ammonium monomers with different alkyl chain lengths and fluorine-containing monomers [47]. The coexistence of quaternary ammonium and fluorine components imparts antibacterial activity and bacterial antiadhesion properties to the prepared cotton fabrics. Incorporation of increased fluorine component to the fabrics decreases the surface energy, thus enhances the bacterial anti-adhesive capability. These fluorine-treated fabrics make the surface hydrophobic, thereby reduces the chances of bacterial suspension to wet the surface thus prevents bacterial colonization and enhances bacterial antiadhesion.

8 Concluding Remarks

Microbial contamination and biofilm formation on biomaterials are associated with major medical complications and increased healthcare cost. Advancements based on nanoscience-based technologies are needed to develop novel strategies that can reduce bacterial interaction with surfaces. With the use of numerous fabrication techniques, the surface structures can be engineered with aspect to its shape, size, spacing, and height to width ratio to alter the antibacterial and antifouling efficiency of surfaces. Use of few antimicrobial agents such as AMPs or antimicrobial polymers was recently used as effective approaches that avoid both cytotoxicity and bacterial resistance. The balance between advantages versus disadvantages and antibacterial potency versus cytotoxicity of the antibacterial strategies can be considered to decide the appropriate strategies for eliminating bacterial adhesion onto surfaces. Still, there is a long way to go in designing of effective, ecological, and economic antibacterial and/or antifouling surface strategies while improving the understanding of surface–microbe interactions. Integration of current knowledge and new technologies can be a key factor in developing such multifunctional surfaces.

References

1. Garrett TR, Bhakoo M, Zhang Z (2008) Bacterial adhesion and biofilms on surfaces. Prog Nat Sci 18:1049–1056. https://doi.org/10.1016/j.pnsc.2008.04.001
2. Khatoon Z, McTiernan CD, Suuronen EJ, Mah TF, Alarcon EI (2018) Bacterial biofilm formation on implantable devices and approaches to its treatment and prevention. Heliyon 4:e01067. https://doi.org/10.1016/j.heliyon.2018.e01067
3. Desrousseaux C, Sautou V, Descamps S, Traoré O (2013) Modification of the surfaces of medical devices to prevent microbial adhesion and biofilm formation. J Hosp Infect 85:87–93. https://doi.org/10.1016/j.jhin.2013.06.015
4. Costello CM, Yeung CL, Rawson FJ, Mendes PM (2012) Application of nanotechnology to control bacterial adhesion and patterning on material surfaces. J Exp Nanosci 7:634–651. https://doi.org/10.1080/17458080.2012.740640
5. Wu S, Zhang B, Liu Y, Suo X, Li H (2018) Influence of surface topography on bacterial adhesion: a review (review). Biointerphases 13:060801. https://doi.org/10.1116/1.5054057

6. Tuson HH, Weibel DB (2013) Bacteria-surface interactions. Soft Matter 9:4368–4380. https://doi.org/10.1039/c3sm27705d
7. Cheng Y, Feng G, Moraru CI (2019) Micro- and nanotopography sensitive bacterial attachment mechanisms: a review. Front Microbiol 10. https://doi.org/10.3389/fmicb.2019.00191
8. Yuan Y, Hays MP, Hardwidge PR, Kim J (2017) Surface characteristics influencing bacterial adhesion to polymeric substrates. RSC Adv 7:14254–14261. https://doi.org/10.1039/c7ra01571b
9. Kelleher SM, Habimana O, Lawler J, O'Reilly B, Daniels S, Casey E, Cowley A (2015) Cicada wing surface topography: an investigation into the bactericidal properties of nanostructural features. ACS Appl Mater Interfaces 8:14966–14974. https://doi.org/10.1021/acsami.5b08309
10. Preedy E, Perni S, Nipiĉ D, Bohinc K, Prokopovich P (2014) Surface roughness mediated adhesion forces between borosilicate glass and gram-positive bacteria. Langmuir 30:9466–9476. https://doi.org/10.1021/la501711t
11. Perera-Costa D, Bruque JM, González-Martín ML, Gómez-García AC, Vadillo-Rodríguez V (2014) Studying the influence of surface topography on bacterial adhesion using spatially organized microtopographic surface patterns. Langmuir 30:4633–4641. https://doi.org/10.1021/la5001057
12. Hasan J, Crawford RJ, Ivanova EP (2013) Antibacterial surfaces: the quest for a new generation of biomaterials. Trends Biotechnol 31:295–304. https://doi.org/10.1016/j.tibtech.2013.01.017
13. Jaggessar A, Shahali H, Mathew A, Yarlagadda PKDV (2017) Bio-mimicking nano and microstructured surface fabrication for antibacterial properties in medical implants. J Nanobiotechnol 15:64. https://doi.org/10.1186/s12951-017-0306-1
14. Rigo S, Cai C, Gunkel-Grabole G, Maurizi L, Zhang X, Xu J, Palivan CG (2018) Nanoscience-based strategies to engineer antimicrobial surfaces. Adv Sci 5:1700892. https://doi.org/10.1002/advs.201700892
15. Hu R, Li G, Jiang Y, Zhang Y, Zou JJ, Wang L, Zhang X (2013) Silver-zwitterion organic-inorganic nanocomposite with antimicrobial and antiadhesive capabilities. Langmuir 29:3773–3779. https://doi.org/10.1021/la304708b
16. De Giglio E, Cafagna D, Cometa S, Allegretta A, Pedico A, Giannossa LC, Sabbatini L, Mattioli-Belmonte M, Iatta R (2013) An innovative, easily fabricated, silver nanoparticle-based titanium implant coating: development and analytical characterization. Anal Bioanal Chem 405:805–816. https://doi.org/10.1007/s00216-012-6293-z
17. Wang B, Liu H, Wang Z, Shi S, Nan K, Xu Q, Ye Z, Chen H (2017) A self-defensive antibacterial coating acting through the bacteria-triggered release of a hydrophobic antibiotic from layer-by-layer films. J Mater Chem B 5:1498–1506. https://doi.org/10.1039/c6tb02614a
18. Huh AJ, Kwon YJ (2011) "Nanoantibiotics": a new paradigm for treating infectious diseases using nanomaterials in the antibiotics resistant era. J Control Release 156:128–145. https://doi.org/10.1016/j.jconrel.2011.07.002
19. Tripathy A, Sen P, Su B, Briscoe WH (2017) Natural and bioinspired nanostructured bactericidal surfaces. Adv Colloid Interface Sci 248:85–104. https://doi.org/10.1016/j.cis.2017.07.030
20. Han S, Ji S, Abdullah A, Kim D, Lim H, Lee D (2018) Superhydrophilic nanopillar-structured quartz surfaces for the prevention of biofilm formation in optical devices. Appl Surf Sci 429:244–252. https://doi.org/10.1016/j.apsusc.2017.07.164
21. Vasudevan R, Kennedy AJ, Merritt M, Crocker FH, Baney RH (2014) Microscale patterned surfaces reduce bacterial fouling-microscopic and theoretical analysis. Colloids Surf B Biointerfaces 117:225–232. https://doi.org/10.1016/j.colsurfb.2014.02.037
22. Barcelo S, Li Z (2016) Nanoimprint lithography for nanodevice fabrication. Nano Converg 3:21. https://doi.org/10.1186/s40580-016-0081-y
23. Zhao XM, Xia Y, Whitesides GM (1997) Soft lithographic methods for nano-fabrication. J Mater Chem 7:1069–1074. https://doi.org/10.1039/a700145b
24. Franssila S (2010) Introduction to microfabrication. https://doi.org/10.1002/9781119990413
25. Oh JK, Lee JK, Kim SJ, Park KW (2009) Synthesis of phase- and shape-controlled TiO_2 nanoparticles via hydrothermal process. J Ind Eng Chem 15:270–274. https://doi.org/10.1016/j.jiec.2008.10.001

26. Zhu K, Hu G (2014) Supercritical hydrothermal synthesis of titanium dioxide nanostructures with controlled phase and morphology. J Supercrit Fluids 94:165–173. https://doi.org/10.1016/j.supflu.2014.07.011
27. Surmenev R, Vladescu A, Surmeneva M, Ivanova A, Braic M, Grubova I, Cotrut CM (2017) Radio frequency magnetron sputter deposition as a tool for surface modification of medical implants. In: Modern technologies for creating the thin-film systems and coatings, pp 213–248
28. Ye J, Deng J, Chen Y, Yang T, Zhu Y, Wu C, Wu T, Jia J, Cheng X, Wang X (2019) Cicada and catkin inspired dual biomimetic antibacterial structure for the surface modification of implant material. Biomater Sci 7:2826–2832. https://doi.org/10.1039/c9bm00082h
29. Lutey AHA, Gemini L, Romoli L, Lazzini G, Fuso F, Faucon M, Kling R (2018) Towards laser-textured antibacterial surfaces. Sci Rep 8:10112. https://doi.org/10.1038/s41598-018-28454-2
30. Graham MV, Cady NC (2014) Nano and microscale topographies for the prevention of bacterial surface fouling. Coatings 4:37–59. https://doi.org/10.3390/coatings4010037
31. Hasan J, Chatterjee K (2015) Recent advances in engineering topography mediated antibacterial surfaces. Nanoscale 7:15568–15575. https://doi.org/10.1039/c5nr04156b
32. Dundar Arisoy F, Kolewe KW, Homyak B, Kurtz IS, Schiffman JD, Watkins JJ (2018) Bioinspired photocatalytic shark-skin surfaces with antibacterial and antifouling activity via nanoimprint lithography. ACS Appl Mater Interfaces 10:20055–20063. https://doi.org/10.1021/acsami.8b05066
33. Hasan J, Raj S, Yadav L, Chatterjee K (2015) Engineering a nanostructured "super surface" with superhydrophobic and superkilling properties. RSC Adv 5:44953–44959. https://doi.org/10.1039/c5ra05206h
34. Bucaro MA, Vasquez Y, Hatton BD, Aizenberg J (2012) Fine-tuning the degree of stem cell polarization and alignment on ordered arrays of high-aspect-ratio nanopillars. ACS Nano 6:6222–6230. https://doi.org/10.1021/nn301654e
35. Qi S, Yi C, Ji S, Fong CC, Yang M (2009) Cell adhesion and spreading behavior on vertically aligned silicon nanowire arrays. ACS Appl Mater Interfaces 1:30–34. https://doi.org/10.1021/am800027d
36. Minoura K, Yamada M, Mizoguchi T, Kaneko T, Nishiyama K, Ozminskyj M, Koshizuka T, Wada I, Suzutani T (2017) Antibacterial effects of the artificial surface of nanoimprinted moth-eye film. PLoS ONE 12:e0185366. https://doi.org/10.1371/journal.pone.0185366
37. Gao A, Hang R, Huang X, Zhao L, Zhang X, Wang L, Tang B, Ma S, Chu PK (2014) The effects of titania nanotubes with embedded silver oxide nanoparticles on bacteria and osteoblasts. Biomaterials 35:4223–4235. https://doi.org/10.1016/j.biomaterials.2014.01.058
38. Wang J, Li J, Guo G, Wang Q, Tang J, Zhao Y, Qin H, Wahafu T, Shen H, Liu X, Zhang X (2016) Silver-nanoparticles-modified biomaterial surface resistant to staphylococcus: new insight into the antimicrobial action of silver. Sci Rep 6:32699. https://doi.org/10.1038/srep32699
39. Kim EJ, Choi M, Park HY, Hwang JY, Kim HE, Hong SW, Lee J, Yong K, Kim W (2019) Thorn-like TiO_2 nanoarrays with broad spectrum antimicrobial activity through physical puncture and photocatalytic action. Sci Rep 9:1–12. https://doi.org/10.1038/s41598-019-50116-0
40. Fisher LE, Yang Y, Yuen M-F, Zhang W, Nobbs AH, Su B (2016) Bactericidal activity of biomimetic diamond nanocone surfaces. Biointerphases 11:011014. https://doi.org/10.1116/1.4944062
41. Dickson MN, Liang EI, Rodriguez LA, Vollereaux N, Yee AF (2015) Nanopatterned polymer surfaces with bactericidal properties. Biointerphases 10:021010. https://doi.org/10.1116/1.4922157
42. Lee MJ, Kwon JS, Jiang HB, Choi EH, Park G, Kim KM (2019) The antibacterial effect of non-thermal atmospheric pressure plasma treatment of titanium surfaces according to the bacterial wall structure. Sci Rep 9:1938. https://doi.org/10.1038/s41598-019-39414-9
43. Pietrzyk B, Porębska K, Jakubowski W, Miszczak S (2019) Antibacterial properties of Zn doped hydrophobic SiO_2 coatings produced by sol-gel method. Coatings 9:362. https://doi.org/10.3390/coatings9060352
44. Majhi S, Arora A, Mishra A (2019) Surface immobilization of a short antimicrobial peptide (AMP) as an antibacterial coating. Materialia 6:100350. https://doi.org/10.1016/j.mtla.2019.100350

45. Chen X, Hirt H, Li Y, Gorr S-U, Aparicio C (2014) Antimicrobial GL13K peptide coatings killed and ruptured the wall of *Streptococcus gordonii* and prevented formation and growth of biofilms. PLoS ONE 9:e111579. https://doi.org/10.1371/journal.pone.0111579
46. Kayes MI, Galante AJ, Stella NA, Haghanifar S, Shanks RMQ, Leu PW (2018) Stable lotus leaf-inspired hierarchical, fluorinated polypropylene surfaces for reduced bacterial adhesion. React Funct Polym 128:40–46. https://doi.org/10.1016/j.reactfunctpolym.2018.04.013
47. Lin J, Chen X, Chen C, Hu J, Zhou C, Cai X, Wang W, Zheng C, Zhang P, Cheng J, Guo Z, Liu H (2018) Durably antibacterial and bacterially antiadhesive cotton fabrics coated by cationic fluorinated polymers. ACS Appl Mater Interfaces 10:6124–6136. https://doi.org/10.1021/acsami.7b16235

Chapter 7
Antimicrobial Metal-Based Nanomaterials and Their Industrial and Biomedical Applications

Ehsan Nazarzadeh Zare and Pooyan Makvandi

1 Introduction

Nowadays, nanotechnology has presented great potentials in many arenas of science and technology. Indeed, nanotechnology is the study of extremely small structures [1]. It is the treatment of individual compounds into dimensional structures to yield materials with superior properties. Nanotechnology is widely used in target drug delivery [2], tissue engineering [3], sensors [4], water treatment [5] and other fields.

Owing to the extensive existence of drug-resistant pathogens as a serious health issue, there is growing interest in the employ of novel nanostructured materials, specifically metal-based nanomaterials (MNMs) as antimicrobials [6]. Thus, MNMs could aid as a substitute to antibiotics to control bacterial infections. In this regard, fabrication of metal compounds in nanoscale is very important.

The MNMs are classified to metals (e.g., Au, Ag, Ti, Zn, Cu), metal oxides (e.g., Fe_3O_4, TiO_2, ZnO, CuO, Ag_2O) and metal alloys (e.g., $ZnFe_2O_3$, $CuFe_2O_3$). They are synthesized by various methods such as chemical, physical and plant or microorganism-mediated biosynthesis. For example, Fe_3O_4 can be produced by co-precipitation method (chemical method) from iron (II and III) salts [7]; Ag can be synthesized by green synthesis of plants extract (gums, green tea, etc.) [8]. It is well known that the procedures and conditions for synthesis of MNMs can effect their physicochemical and biological properties. One of the important factors in MNMs

E. N. Zare
School of Chemistry, Damghan University, 36716-41167 Damghan, Iran
e-mail: e.nazarzadeh@du.ac.ir

P. Makvandi (✉)
Institute for Polymers, Composites, and Biomaterials (IPCB), National Research Council (CNR), Naples 80125, Italy
e-mail: pooyan.makvandi@ipcb.cnr.it

Department of Chemistry, College of Science, Shahid Chamran University of Ahvaz, Ahvaz 61357-43337, Iran

S. Snigdha et al. (eds.), *Engineered Antimicrobial Surfaces*, Materials Horizons: From Nature to Nanomaterials, https://doi.org/10.1007/978-981-15-4630-3_7

is their particle size which influences the biological properties such as antimicrobial activities and toxicity.

The nanomaterials, specifically MNMs, have higher antibacterial activity than that those in microscale, as surface area in nanomaterials is very larger than their volume [9]. Actually, in the nanometer dimensions, some properties of the particles, e.g., thermal stability, reactivity, dissolution, are noticeably increased. In this chapter, the antimicrobial activity of MNMs is presented along with their applications in various arena from industrial to biomedical fields.

2 Antimicrobial Activity

Today, infectious illnesses are one of the chiefs led to disease and fatality in the world, and thus, there is the requisite for study on antimicrobial agents [10]. With an increase in resistance of antibiotics, a rising interest in developing new and effective antimicrobial agents has paramount importance [11]. Antimicrobial agents mention the materials that led to killing or inhibiting the microbes causing disease. Numerous antimicrobial agents are used for this purpose. In recent years, nanotechnology has been employed to prepare new antimicrobial systems and devices, capable of fighting opportunistic infections. Metal-based nanomaterials (MNMs) are able to identify bacterial cells from mammalian cells and can supply long-term antibacterial and biofilm inhibition [12].

Metal-based nanomaterials, e.g., Au, Ag, Si, CuO, ZnO, TiO_2, MgO, Fe_3O_4, Fe_2O_3, MgO, CaO, etc., have been applied in different fields to impart antimicrobial activity [6]. Because of dimensions, such as smaller size and the larger surface area to volume ratio of MNMs than bacteria, they provide strong and extended antimicrobial interaction with bacteria and biofilms at smaller dosages [13]. There are many physical and chemical factors such as size, zeta potential, shape and roughness that affect the antimicrobial activity of MNTs [14]. Changing these factors has a reflective effect on the antimicrobial activity of MNTs as presented in Table 1. Typically, smaller MNTs have higher antimicrobial activity. Nevertheless, some studies have demonstrated that size alone is not the most significant factor of the antimicrobial activity of MNTs [14]. Other parameters such as the media, the preparation process, the defense mechanism of the microbe and the physical features of the MNMs can also affect the microbicidal activity (Fig. 1) [15]. Actually, the logical reason for more toxic of the small MNMs than the large MNMs can be explained by increase the reactive oxygen species (ROS) production, which therefore can destruct and deactivate vital biomolecules [16, 17]. The precise mechanisms for antimicrobial activity of MNMs are still being studied. In general, three main possible mechanisms in this regard are [18]:

- Metal ions release that from dissolution of the metals from MNMs surface, react with the cell membrane and other cellular components.
- ROS generation on the MNMs surface that led to oxidative stress.
- Physical destruction of the cell membrane through the MNMs.

Table 1 Size, shape and antibacterial activities of the some of the metal-based nanomaterials

Metal-based nanomaterials	Size (nm)	Shape	Strain	Activity	Ref.
Au	8.4	Spherical	*A. baumannii, E. coli, P. aeruginosa, S. aureus*	MIC = 8 μg/mL	[20]
	50–100	Spherical	*E. faecium, S. oneidensis*	MIC = 32 μg/mL	
CeO_2	6	Square	*E. coli*	Z = ~0.2 mm	[21]
	7	NR	*E. coli*	MIC = 500 μg/mL	[22]
	15	Circular, ovoid	*E. coli*	Z = ~3.3 mm	[21]
TiO_2	12	Spherical	*E. coli*	MIC = 100 μg/mL	[23]
	17	Spherical	*E. coli*	MIC = 100 μg/mL	
	21	Spherical	*E. coli*	MIC = 100 μg/mL	
ZnO	12	Spherical	*E. coli*	Z = 31 mm	[24]
	19	Spherical-like	*E. coli*	MIC = 50 μg/mL	[25]
Ag	9	Spherical	*E. coli*	IC50 = 6.4 μg Ag^+/mL	[26]
	19	Spherical	*E. coli*	IC50 = 15.7 μg Ag^+/mL	
	43	Spherical	*E. coli*	IC50 = 40.9 μg Ag^+/mL	

MIC minimal inhibitory concentration; *Z* zone of inhibition; *LC50* lethal concentration; *NR* not reported

3 Applications

3.1 Industrial Applications

As discussed in the previous section, the MNMs show efficient antimicrobial effects against pathogenic microorganisms. Consequently, some of the MNMs, e.g., Ag, Au, ZnO, TiO_2, MgO, Fe_3O_4, etc., have got significant attention as antimicrobials fillers in industrial products such as antimicrobial coatings, food packaging and water treatment.

Antimicrobial food packaging: It is an imperative to evaluate the amount of the released antimicrobial materials from the package to foods/bioproducts during lengthy storage [27, 28]. There are many literatures published on the use of the

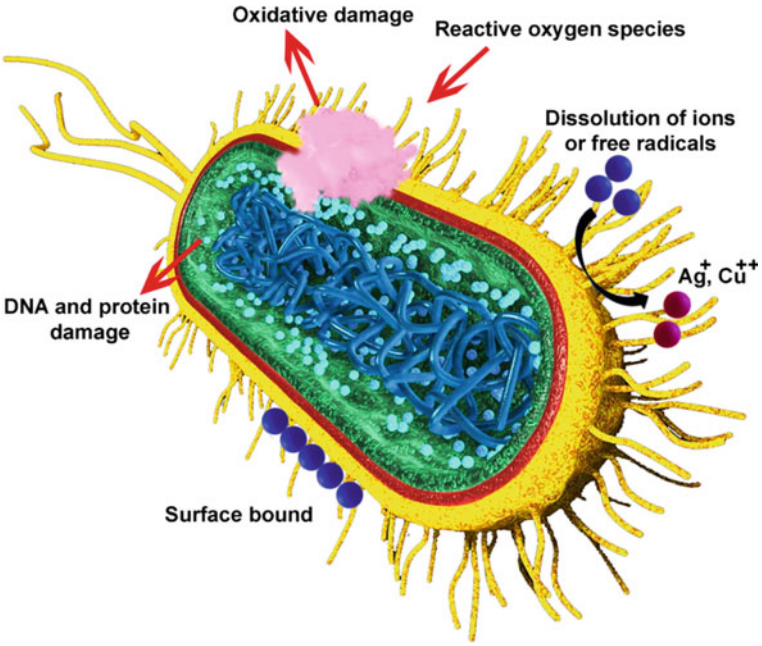


Fig. 1 Schematic of possible interactions and modes of toxicity when engineered MNMs interact with bacterial cells. Different nanoparticle forms are bactericidal through one or several of these mechanisms. DNA: deoxyribonucleic acid. Reprinted from [19] with permission from the publisher

MNMs as an antimicrobial filler for manufacturing antimicrobial packaging [27, 29]. In most of them, a polymer metric such as poly(vinyl alcohol), poly(ethylene glycol), poly(ε-caprolactone), natural polymers containing and MNMs is used [29, 30]. In general, the antimicrobial packaging can be fabricated by two main methods include: (i) Incorporation of antimicrobial agent into a polymer backbone by means of covalent or ionic bonds. (ii) Adsorption of antimicrobial agent on the polymer surface [29]. Numerous researchers have used Ag, Ag-Cu, CuO, ZnO and TiO_2 due to their great antimicrobial properties and high stability.

For example, low-density polyethylene/Cu film with antimicrobial activity was prepared by using solvent evaporation. The presence of Cu nanoparticles into low-density polyethylene improved the antimicrobial and mechanical properties [31]. Starch/poly(vinyl alcohol)/ZnO as an antimicrobial nanofilm was fabricated by the solution casting method. The antimicrobial, UV barrier and mechanical properties were enhanced by addition of ZnO nanoparticles [32]. In another work, montmorillonite/CuO nanocomposite was used for enhancing antimicrobial, optical and mechanical properties of chitosan film [33]. Recently, MNMs in combination of gums such as carboxymethyl guar gum and chewing gum used for the preparation of antimicrobial food packaging [34, 35].

Water treatment: Water contamination via toxic metal ions, dyes, pathogenic microorganisms, etc., is a serious issue for humans life. Consequently, the development of technologies for removing them from polluted water is an environmental challenge [5]. The use of antimicrobial MNs alone and/or in combination with synthetic/natural polymers is a facile method for the removal of contaminations from water [36]. Metal-based nanomaterials, i.e., Ag, TiO_2 and ZnO have been investigated for application in the disinfection of different waterborne illness-causing by microorganisms. One main problem in the use of antimicrobial MNMs alone is their low dispersion and instability in water. This problem can be solved by embedding and/or surface coating by polymers which give them great applications in water purification.

For example, Ag nanoparticles synthesized by *Penciillium Citreonigum Dierck* and *Scopulaniopsos brumptii Salvanet-Duval* fungi employed for pathogenic bacteria removal from wastewater [37]. Antimicrobial TiO_2/tragacanth gum nanocomposite was used for photocatalytic elimination of methylene blue dye from wastewater [38].

Animicrobial coating: Generally, antimicrobial activity of coatings can be categorized as biocidal or biostatic [39]. The agents that kill microorganisms are biocide, whereas the agents that inhibit growth of the microorganisms are biostatic [39]. Most of the antimicrobial coatings usually use biocides such as Ag, TiO_2, ZnO and Au as active agents. For example, in textile industry, these antimicrobial agents are incorporated into the polymer fibers during extrusion or attached to the surface of polymer fibers during finishing. Metal-based nanocomposites also are proposed to be used in different places (for instance, hospitals wall and ground) and devices (e.g., medical devices, doorknob, keyboard) that are prone to the microbial growth.

The antimicrobial textiles modified with MNMs such as Ag, Au, Cu, Ag–Cu, ZnO are reported by many researchers [40–44]. For example, chitosan/Au biomedical textiles fabricated via exhaustion method. Compared with Au nanoparticles or chitosan alone, the chitosan/Au coating demonstrated a better antimicrobial effect against bacteria [41].

Cosmestic: One of the industrial applications of MNMs is in cosmetic materials. Cosmetic products include sunscreens, soaps, shampoo, toothpastes and face creams. Sunscreens are the most common cosmetic products that protect against ultraviolet radiation [45]. They are usually divided into organic and inorganic agents. The MNMs such as ZnO, SiO_2 and TiO_2 are used as photo-stable and physical blocker agents in sun protection cream. Although, the MNMs are commonly found in cosmetics products, unfortunately, they are can be caused serious problems to the lungs. In recent years, several scientists studied the use of MNMs in cosmetic products [46]. For instance, Leong et al. synthesized the antimicrobial TiO_2 via modified sol–gel reaction for cosmetics applications. The azelaic acid was used for enhancing of antimicrobial activity of TiO_2. In another work, Spoiala et al. fabricated SiO_2/ZnO nanocomposite to be applied in cosmetic creams [47]. Antimicrobial and antioxidant ZnO nanoparticles synthesized by Adhatoda vasica leaf extract for use in cold cream formulation [48]. It was reported that cold cream containing ZnO displayed significant resistance against clinical skin pathogens [49]. Ag nanoparticles are also

applied into toothpaste, shampoo and soap as preservatives and also in deodorants, lip products, wet wipes, face and body foams. Ag and Au nanoparticles have been implemented in day and night creams to provide the skin a fresher appearance [50].

3.2 Biomedical Applications

Bioactive MNMs represent an interesting alternative for the development of advanced biomaterials with antimicrobial properties, due to their good physicochemical and mechanical properties. The antimicrobial metal-based nanomaterials are widely used in biosensors, drug delivery, dentistry, tissue engineering, etc. This section represents a summary of recent development in the antimicrobial metal-based nanomaterials for biomedical applications.

Drug delivery: In general, drugs or biomolecules delivery to a target site (e.g., tumors and organs) is known as targeted delivery [51, 52]. Many efforts have been devoted to enhance the efficiency of MNM carriers for drug delivery. Numerous literatures studied the use of MNMs in target drug delivery, for example, Au/poly(lactic acid) nanocomposite for photothermally controlled drug delivery [53], graphene oxide/Ag for chemo-photothermal therapy [54], Fe_3O_4/glucose/Ag for light-responsive drug delivery [55], Ag/SiO_2/TiO_2 for doxorubicin drug delivery [56], poly(*N*-isopropylacrylamide-*co*-acrylamide)/SiO_2/Au for insulin delivery [57], Au/Au_2S for cis-platin delivery [58]. Table 2 summarizes the MNMs that have been studied for potential biomedical applications.

Biosensor: Biosensors are devices that associate the biological detecting to a detector or transducer. In general, three chief constituents such as a biorecognition part, a hold surface, e.g., MNMs and polymers, and a detector/transducer part exist in biosensors systems [59, 60]. Biosensors have been used in several clinical applications, e.g., glucose and cholesterol detection in patients. Biosensors based on antimicrobial MNMs and conducting polymers (polyaniline, polyfuran and polypyrrole) have been extensively used for detecting hydrogen peroxide, tyrosinase, glucose, cholesterol (Table 2).

Wound healing: Wound healing is a complex process involving a cascade of biological reactions in response to injury. The healing rate of acute wounds differs from chronic wounds, and it depends on the immunological status of patients [8]. It has to be highlighted that infection is a crucial and generally unsolved issue in wound healing. Therefore, the MNMs are good candidates for wound healing applications as they can inhibit or decline infections. For instance, at present, silver as a useful antibacterial agent is reemerging as a viable treatment option for burn wound treatment [61]. The combination natural polymers with antimicrobial MNMs to enhance the physicochemical and biocompatibility can offer a faster healing process [62]. In recent years, antimicrobial MNs/natural polymer nanocomposites have been investigated for wound healing, for instance, tragacanth gum/Ag,

Table 2 Antimicrobial metal-based nanomaterials that have been studied in biomedical applications

Metal-based nanomaterials	Size (nm)	Applications	Remarks	References
Au/Au_2S	20–80	Drug delivery	Drug release sensitive to near-infrared irradiation	[58]
Au/poly(lactic acid)	32.4	Drug delivery	The drug release rate could be tuned by controlling the intensity of near-infrared exposition	[53]
Chitosan-encapsulated ZnO quantum dots	Zn quantum dots 2–4	Drug delivery	Chitosan enhanced the stability of the ZnO quantum dots because of the hydrophilicity and cationic charge	[72]
WS_2/Fe_3O_4/mesoporous silica/poly(ethylene glycol)	WS_2/Fe_3O_4/mesoporous silica: 2.48	Drug delivery	In vivo synergistic therapeutic effect, effective inhibition of tumor growth is realized after the combined photothermal and chemotherapy delivered by WS2/Fe_3O_4/mesoporous silica/poly(ethylene glycol)/doxorubicin	[73]
Polyaniline/TiO_2/graphene oxide	17	Biosensor	Good selectivity and stability at 82% of the initial activity for 30 days maintained	[74]
Polypyrrole/Au	N.R	Biosensor	The biosensor showed only 40% loss of its initial activity after its 200 uses over 100 days, when stored at 4 °C	[75]

(continued)

Table 2 (continued)

Metal-based nanomaterials	Size (nm)	Applications	Remarks	References
Tragacanth/Ag	77.55	Wound dressing	The treated cotton fabrics showed good water absorption properties along with reasonable antibacterial effectiveness	[76]
Chitosan/poly(vinyl alcohol)/ZnO	ZnO: 50–100	Wound healing	Chitosan/poly(vinyl alcohol)/ZnO showed strong antimicrobial, wound healing effect, hemocompatibility and biocompatibility	[77]
Polyurethane/CuO	Thickness < 10 μm	Dentistry	Significant reduction of bacterial populations was demonstrated with 10% w/w CuO over a 4-h period	[78]
Poly(methyl methacrylate)/TiO_2	56–170	Dentistry	Poly(methyl methacrylate)/TiO_2 nanocomposite was successfully used for complete denture manufacturing	[79]
poly(ethylene glycol)/poly(lactic acid-co-glycolic acid)/Au	20	Photothermal therapy	Upon laser irradiation, the system releases the encapsulated drug with higher efficiency	[80]
Hydroxyapatite/poly(vinyl alcohol)/TiO_2	15	Bone tissue engineering	Hydroxyapatite/poly(vinyl alcohol)/TiO_2 nanocomposite leads to improved mechanical properties by achieving the initial mechanical strength up to 0.99 ± 0.19 MPa and enhanced in vitro bioactivity	[81]
Hydroxyapatite/TiO_2	TiO_2: 20, Hydroxyapatite: 100	Bone tissue engineering	SEM showed grains sizes of less than 1 μm and high granular interface quality, which are factors that favor cell attachment to granules and porous surfaces, ensuring good hydrophilic capacity and capillarity	[82]

N.R. not reported; *MNSs* metal nanostructures

gellan gum/TiO_2, sodium alginate/acacia gum/ZnO, chitosan/TiO_2, chitosan/ZnO, chitosan/Ag, chitosan/poly(vinyl alcohol)/ZnO, CuO/TiO_2/poly(ethylene glycol) (Table 2).

Bone tissue: However, infections during or post scaffold transplantation are still challenging as they reduce the efficacy of bone healing. After the transplantation, infections may also be distributed to the scaffold from other sources of inflammation through bloodstream [63]. Implantation of a typically metallic orthopedic prosthetic is a common treatment for bone fractures [64, 65]. Many efforts have been made to change orthopedic implant materials. A recent investigation showed that nanotechnology might generally improve all materials employed for bone regeneration. Metal-based nanomaterials have displayed better properties related to their micron structure owing to their physicochemical, mechanical and biological properties. On the other hand, nanocomposites fabricated by MNMs and polymers or ceramic may become good alternative materials in bone tissue, in view of their better mechanical and biological properties [66]. Table 2 showed the nanometal-based composites in bone tissue engineering.

Dentistry: In spite of the notable advances obtained, biomaterials in dentistry accumulate microbial biofilms. Photogenic microorganisms are the main parameter of dental treatment defeat, creating secondary caries and infections which necessitate retreatment [18, 67]. Dental materials employed for the microorganism-induced illness treatment in the oral cavity are unable to hinder microorganism colonization and biofilm formation, while the combination of polymeric matrices and MNMs is capable of inhibiting microorganism proliferation [67–69]. Antimicrobial dental restorative nanocomposites improve the restorative treatment outcome and present an opportunity to extend their useful lifetime by reducing secondary caries caused by bacterial recolonization.

There are many literatures in which Ag, ZnO, TiO_2, CuO and other metal-based nanomaterials are utilized in dentistry (Fig. 2). For example, poly(lactic-*co*-glycolic acid)/Ag–Fe_3O_4 nanocomposite has been prepared via solvent casting and employed as a coating on implant surfaces [70]. In another work, poly(lactic-*co*-glycolic acid)/TiO_2 nanocomposites have also been used as growth factor sustained release systems for dental implants [71]. In addition, the structure of composite may also be employed as a reservoir for antibacterial and anti-inflammatory agents.

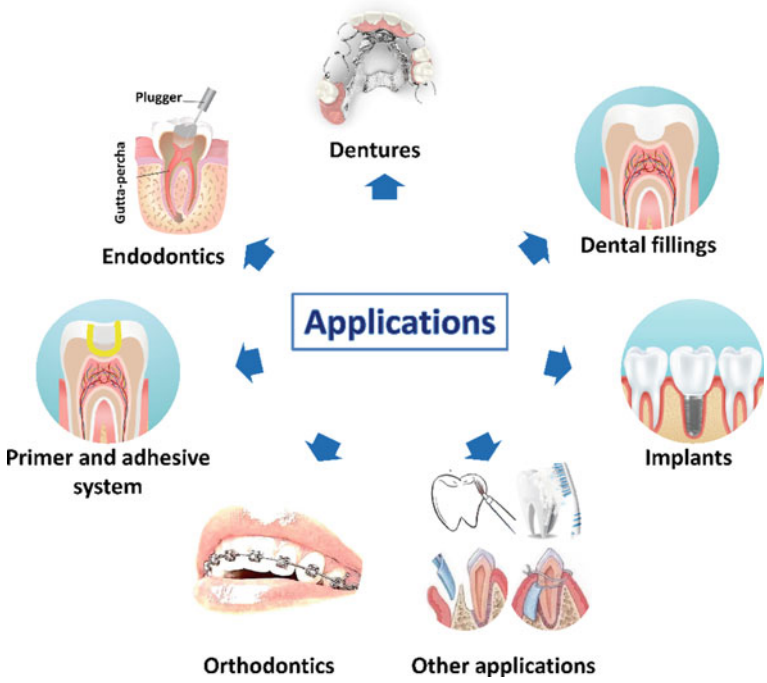


Fig. 2 Polymeric and inorganic antimicrobial nanosized fillers can be applied in various areas in dentistry, including endodontics, dental fillings, dentures, orthodontics, implants, periodontics and preventive dentistry as well as primer and adhesive systems [18]

References

1. Nas FS, Ali M (2018) Arch Nanomed Open Access J 1:59–64
2. Choi KY, Han HS, Lee ES, Shin JM, Almquist BD, Lee DS, Park JH (2019) Adv Mater 1803549
3. Ashtari K, Nazari H, Ko H, Tebon P, Akhshik M, Akbari M, Alhosseini SN, Mozafari M, Mehravi B, Soleimani M (2019) Adv Drug Deliv Rev. https://doi.org/10.1016/j.addr.2019.06.001
4. Mirzaei A, Yousefi HR, Falsafi F, Bonyani M, Lee J-H, Kim J-H, Kim HW, Kim SS (2019) Int J Hydrogen Energy
5. Zare EN, Motahari A, Sillanpää M (2018) Environ Res 162:173–195
6. Dizaj SM, Lotfipour F, Barzegar-Jalali M, Zarrintan MH, Adibkia K (2014) Mater Sci Eng, C 44:278–284
7. Nazarzadeh Zare E, Lakouraj MM, Baghayeri M (2015) Int J Polym Mater Polym Biomater. https://doi.org/10.1080/00914037.2014.936588
8. Zare EN, Makvandi P, Tay FR (2019) Carbohydr Polym 212:450–467
9. Graves J, Thomas M, Ewunkem J (2017) Nanomaterials 7:283
10. Vega-Jiménez AL, Vázquez-Olmos AR, Acosta-Gío E, Álvarez-Pérez MA (2019) In: Koh KS, Wong VL (eds) Nanoemulsions-properties, fabrications and applications, 1st edn. IntechOpen, pp 1–18
11. Gold K, Slay B, Knackstedt M, Gaharwar AK (2018) Adv Ther 1:1700033–1700048
12. Khairnar SD, Shinde SG, Shrivastava VS (2019) J Nanomed Biother Discov 9:163–170

13. Vimbela GV, Ngo SM, Fraze C, Yang L, Stout DA (2017) Int J Nanomed 12:3941–3965
14. Slavin YN, Asnis J, Häfeli UO, Bach H (2017) J Nanobiotechnol 15:65–85
15. Singh P, Garg A, Pandit S, Mokkapati V, Mijakovic I (2018) Nanomaterials 8:1009–1028
16. Wang L, Hu C, Shao L (2017) Int J Nanomed 12:1227–1249
17. Niño-Martínez N, Salas Orozco MF, Martínez-Castañón GA, Torres Méndez F, Ruiz F (2019) Int J Mol Sci 20:2808–2823
18. Makvandi P, Gu JT, Zare EN, Ashtari B, Moeini A, Tay FR, Niu L (2020) Acta Biomater 101:69–101
19. Zare EN, Makvandi P, Ashtari B, Rossi F, Motahari A, Perale G (2020) J Med Chem 63:122
20. Lai H-Z, Chen W-Y, Wu C-Y, Chen Y-C, Appl ACS (2015) Mater Interfaces 7:2046–2054
21. Pelletier DA, Suresh AK, Holton GA, McKeown CK, Wang W, Gu B, Mortensen NP, Allison DP, Joy DC, Allison MR (2010) Appl Environ Microbiol 76:7981–7989
22. Thill A, Zeyons O, Spalla O, Chauvat F, Rose J, Auffan M, Flank AM (2006) Environ Sci Technol 40:6151–6156
23. Simon-Deckers A, Loo S, Mayne-L'hermite M, Herlin-Boime N, Menguy N, Reynaud C, Gouget B, Carriere M (2009) Environ Sci Technol 43:8423–8429
24. Padmavathy N, Vijayaraghavan R (2008) Sci Technol Adv Mater 9:35004
25. Li M, Zhu L, Lin D (2011) Environ Sci Technol 45:1977–1983
26. Ivask A, ElBadawy A, Kaweeteerawat C, Boren D, Fischer H, Ji Z, Chang CH, Liu R, Tolaymat T, Telesca D (2013) ACS Nano 8:374–386
27. Hoseinnejad M, Jafari SM, Katouzian I (2018) Crit Rev Microbiol 44:161–181
28. Sekhavat Pour Z, Makvandi P, Ghaemy M (2015) Int J Biol Macromol 80:596–604
29. Dobrucka R, Ankiel M (2019) J Food Saf 39:e12617
30. Valencia GA, Zare EN, Makvandi P, Gutiérrez TJ (2019) Compr Rev Food Sci Food Saf 18:2009–2024
31. Lomate GB, Dandi B, Mishra S (2018) Food Packag Shelf Life 16:211–219
32. Nair IC, Radhakrishnan EK (2019) Int J Biol Macromol 136:395–403
33. Nouri A, Yaraki MT, Ghorbanpour M, Agarwal S, Gupta VK (2018) Int J Biol Macromol 109:1219–1231
34. Gour A, Jain NK (2019) Artif Cells Nanomed Biotechnol 47:844–851
35. Durán N, Durán M, de Jesus MB, Seabra AB, Fávaro WJ, Nakazato G (2016) Nanomed Nanotechnol Biol Med 12:789–799
36. Pradeep T (2009) Thin Solid Films 517:6441–6478
37. Moustafa MT (2017) Water Sci 31:164–176
38. Rahimdokht M, Pajootan E, Ranjbar-Mohammadi M (2019) Polym Int 68:134–140
39. Zille A, Almeida L, Amorim T, Carneiro N, Esteves MF, Silva CJ, Souto AP (2014) Mater Res Express 1:32003
40. Durán N, Marcato PD, De Souza GIH, Alves OL, Esposito E (2007) J Biomed Nanotechnol 3:203–208
41. Silva IO, Ladchumananandasivam R, Nascimento JHO, Silva KKOS, Oliveira FR, Souto AP, Felgueiras HP, Zille A (2019) Nanomaterials 9:1064
42. Chattopadhyay DP, Patel BH (2010) J Eng Fiber Fabr 5:155892501000500300
43. Paszkiewicz M, Gołąbiewska A, Rajski Ł, Kowal E, Sajdak A, Zaleska-Medynska A (2016) J Nanomater
44. Verbič A, Gorjanc M, Simončič B (2019) Coatings 9:550
45. Patel A, Prajapati P, Boghra R (2011) Asian J Pharm Clin Res 1:40–55
46. Spoiala A, Nedelcu I, Ficai D, Ficai A, Andronescu E (2013) Dig J Nanomater Bios 8:1235
47. Spoiala A, Albu MG, Ficai A, Andronescu E, Voicu G, Ungureanu C (2014) Dig J Nanomater Biostruct 9:1729–1737
48. Leong HJ, Oh S-G (2018) J Ind Eng Chem 66:242–247
49. Sonia S, Ruckmani K, Sivakumar M (2017) Mater Sci Eng C 79:581–589
50. Gajbhiye S, Sakharwade S (2016) J Cosmet Dermatol Sci Appl 6:48
51. Jamaledin R, Di Natale C, Onesto V, Taraghdari ZB, Zare EN, Makvandi P, Vecchione R, Netti PA (2020) J Clin Med 9:542

52. Battisti M, Vecchione R, Casale C, Pennacchio FA, Lettera V, Jamaledin R, Profeta M, Di Natale C, Imparato G, Urciuolo F (2019) Front Bioeng Biotechnol 7:296
53. Campardelli R, Della Porta G, Gomez L, Irusta S, Reverchon E, Santamaria J (2014) J Mater Chem B 2:409–417
54. Shi J, Wang L, Zhang J, Ma R, Gao J, Liu Y, Zhang C, Zhang Z (2014) Biomaterials 35:5847–5861
55. Chen J, Guo Z, Wang H-B, Gong M, Kong X-K, Xia P, Chen Q-W (2013) Biomaterials 34:571–581
56. Wang Y, Chen L, Liu P (2012) Chem Eur J 18:5935–5943
57. Bikram M, Gobin AM, Whitmire RE, West JL (2007) J Control Release 123:219–227
58. Ren L, Chow GM (2003) Mater Sci Eng C 23:113–116
59. Makvandi P, Wang C, Zare EN, Borzacchiello A, Niu L, Tay FR (2020) Adv Funct Mater. https://doi.org/10.1002/adfm.201910021
60. Zare EN, Jamaledin R, Naserzadeh P, Afjeh-Dana E, Ashtari B, Hosseinzadeh M, Vecchione R, Wu A, Tay FR, Borzacchiello A, Makvandi P (2020) ACS Appl Mater Interfaces 12:3279–3300
61. Makvandi P, Ali GW, Della Sala F, Abdel-Fattah WI, Borzacchiello A (2019) Carbohydr Polym 223:115023
62. Zare EN, Makvandi P, Borzacchiello A, Tay FR, Ashtari B, Padil VTV (2019) Chem Commun 55:14871–14885
63. Nair MB, Kretlow JD, Mikos AG, Kasper FK (2011) Curr Opin Biotechnol 22:721–725
64. Balasundaram G, Webster TJ (2006) Nanomedicine (Lond) 1(2):169–176
65. Makvandi P, Ali GW, Della Sala F, Abdel-Fattah WI, Borzacchiello A (2019) Mater Sci Eng C 107:10195
66. Kotela I, Podporska J, Soltysiak E, Konsztowicz KJ, Blazewicz M (2009) Ceram Int 35:2475–2480
67. Makvandi P, Ghaemy M, Mohseni M (2016) Eur Polym J 74:81–90
68. Makvandi P, Pollini M, Gallo AL, Maffezzoli A, Esposito Corcione C, Montagna F, Paladini F, Jamaledin R (2017) Polym Adv Technol 29:364–371
69. Makvandi P, Jamaledin R, Jabbari M, Nikfarjam N, Borzacchiello A (2018) Dent Mater 34:851–867
70. Yang Y, Ren S, Zhang X, Yu Y, Liu C, Yang J, Miao L (2018) Int J Nanomed 13:3751
71. Sun S, Zhang Y, Zeng D, Zhang S, Zhang F, Yu W (2018) J Mater Sci Mater Med 29:141
72. Yuan Q, Hein S, Misra RDK (2010) Acta Biomater 6:2732–2739
73. Yang G, Gong H, Liu T, Sun X, Cheng L, Liu Z (2015) Biomaterials 60:62–71
74. Tang W, Li L, Zeng X (2015) Talanta 131:417–423
75. Devi R, Yadav S, Pundir CS (2012) Colloids Surf A Physicochem Eng Asp 394:38–45
76. Montazer M, Keshvari A, Kahali P (2016) Carbohydr Polym 154:257–266
77. Gutha Y, Pathak JL, Zhang W, Zhang Y, Jiao X (2017) Int J Biol Macromol 103:234–241
78. Ahmad Z, Vargas-Reus MA, Bakhshi R, Ryan F, Ren GG, Oktar F, Allaker RP (2012) Methods in enzymology, vol 509. Elsevier, Amsterdam, pp 87–99
79. Totu EE, Nechifor AC, Nechifor G, Aboul-Enein HY, Cristache CM (2017) J Dent 59:68–77
80. Fazio E, Scala A, Grimato S, Ridolfo A, Grassi G, Neri F (2015) J Mater Chem B 3:9023–9032
81. Stipniece L, Narkevica I, Sokolova M, Locs J, Ozolins J (2016) Ceram Int 42:1530–1537
82. Camargo NHA, de Lima SA, Gemelli E (2012) Am J Biomed Eng 2:41–47

Chapter 8
Potential Environmental Effects of Engineered Antimicrobial Surfaces

K. Sapna, J. Sonia, B. N. Kumara, A. Nikhitha, Manjunath M. Shenoy, A. B. Arun, and K. Sudhakara Prasad

1 Introduction

Microbes are the living organisms found everywhere, which constantly affect the environment, and these microbes cause some of the serious infections around the world. The impact of these organisms on the environment may be harmful to the adjacent regions where they live. The bacteria, fungi and parasites are some of the organisms which are considered as the source of the infection and which have the potential to transmit the disease or infection in rapid rate [1]. Antimicrobial agent contains a large variety of chemical compounds and physical agents that are used to destroy microorganisms or to prevent their development. Antimicrobial agents can be used to eradicate microbes or reduce their growth as there are lots of infections which are difficult to treat and need rapid control [2]. There are several methods used for making use of these antimicrobial agents for controlling the growth of the microbes. In general, different combinations of antimicrobial agents have been widely used to make antimicrobial surfaces, where the antimicrobial agent is either coated or combined and is used in different applications.

K. Sapna · J. Sonia · B. N. Kumara · K. S. Prasad (✉)
Nanomaterial Research Laboratory (NMRL), Nano Division, Yenepoya Research Centre, Yenepoya (Deemed to Be University), Deralakatte, Mangalore 575018, India
e-mail: ksprasadnair@yenepoya.edu.in

K. Sapna · A. Nikhitha · A. B. Arun · K. S. Prasad
Yenepoya Research Centre, Yenepoya (Deemed to Be University), Deralakatte, Mangalore 575018, India

A. Nikhitha · M. M. Shenoy
Department of Dermatology, Yenepoya (Deemed to Be University), Mangalore, Karnataka 575018, India

K. S. Prasad
Centre for Nutrition Studies, Yenepoya (Deemed to Be University), Deralakatte, Mangalore 575018, India

S. Snigdha et al. (eds.), *Engineered Antimicrobial Surfaces*, Materials Horizons: From Nature to Nanomaterials, https://doi.org/10.1007/978-981-15-4630-3_8

Among the widely known antimicrobial agents, nanoparticles are receiving the most attention due to their extraordinary physical and chemical properties and are used commonly for making EAS. They are fabricated with high specificity considering shape, size, surface properties and chemistry. Most of these ENPs are produced in different forms such as aerosols, colloids or powders through physical, chemical and biological synthesis. Relatively, the biologically synthesized nanoparticles are eco-friendly and less disruptive to the environment [3].

A record of ENPs-enabled product applications indicates that over 1814 products are being manufactured, and it is projected that the number of products will triple by 2020 (Fig. 1) [4]. Among the commercial ENPs, titanium dioxide (TiO_2) and silver oxide (Ag_2O) nanoparticles are the NPs with high production applications. Such an increase in production is mainly due to their usage in industrial, agricultural applications, consumer products and variety of medical applications. Nevertheless, ENPs contribute to their unintended release into the environment through industries and acting in unknown manner on soils, waters and biota [5]. Other than accidental release during production, it is plausible that ENPs will remain bound to the products at the end of the product life cycle [6]. For instance, there are empirical evidence that ENPs are present in sewage sludge [7], wastewater effluents [8] and landfill leachates [9]. Indeed, sewage sludge is used for various purposes such as agriculture and soil amendment (55%), thermal energy generation (25%) and solid waste landfills (20%). Thus, wastewater is a primary point source of aged-ENPs input into the environment, most likely either through wastewater-sludge-digestate-soil pathway or wastewater-effluent-surface water.

Antibiotics were being used for preventing the growth and killing of bacteria for several decades. The antimicrobial agent-coated implants, impregnated bone cements and surfaces gained more attention for the control of the diseases and also due to

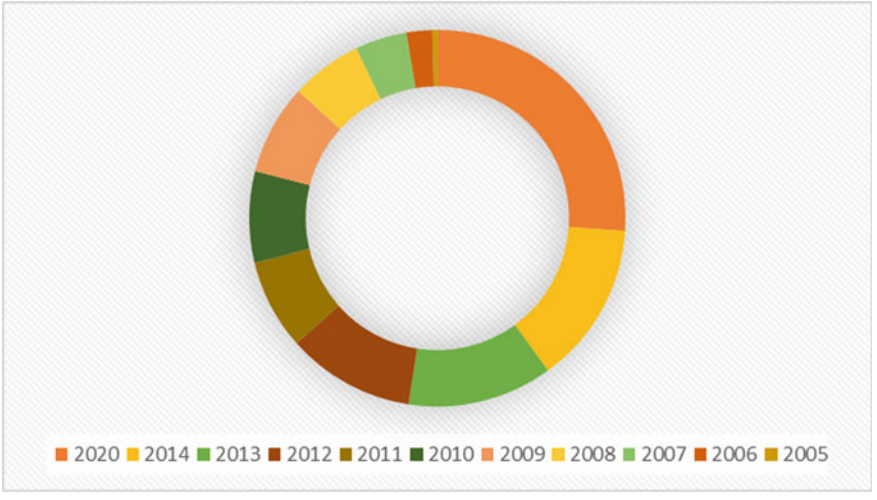


Fig. 1 Trends and estimated projection in the number of ENPs-enabled product applications [4]

their specificity and sensitivity. On the other hand, antimicrobial resistance (AMR) is becoming more and more serious due to the increase in global antibiotics use, inappropriate use of antibiotics in medical practice and the widespread and uncontrolled use in animals to increase meat production [10, 11]; consequently, drug-resistant pathogens such as multidrug resistant (MDR) bacteria have been increasing globally to alarming levels [12–14]. Hence, it is the need of the hour to have EAS to modify the current medical instruments to avoid the unwanted infections and over use of antibiotics. Here, in this book chapter, we overview the current ENPs available to make antimicrobial surfaces and their potential hazards.

2 ENPs

ENPs are materials with size ranging between 1 and 100 nm in diameter, synthesized and incorporated into a variety of consumer products because of their novel physical and chemical properties. ENPs are purposely fashioned and premeditated with very precise properties related to shape, size, surface properties and chemistry. These characteristics are exhibited in various forms such as colloids, aerosols or powders. As colloids, most ENPs are insoluble in aqueous medium and not retained on saturated porous media against the prediction of the Derjaguin–Landau–Verwey–Overbeek (DLVO) theory in which sorbed particles are expected to remain attached to media surfaces [15]. In relation to their optical property, conductivity and reactivity, ENPs obey the laws of quantum physics instead of colloidal chemistry which enhances their functional characteristics (Fig. 2).

The metal nanoparticles (NPs) such as Ag, Au, Zn, Cu, Ti, Mg, Ni, Se, Al, Cd and Pd show tremendous activity towards antimicrobial therapy. The surface is modified or coated with inorganic NPs such as Au, TiO_2, AgNPs and ZnO NPs which have

Fig. 2 Potential toxicological effects of surface-modified ENPs

been shown good antimicrobial activity. Interestingly, Ag, Zn and Cu can boost the antimicrobial activity in its bulk form, whereas iron oxide will exhibit the same property in its nano-forms [16, 17]. The numerous mechanisms of action against NPs would require various coincident gene transformations in the bacterial cell; hence, it is very difficult for bacterial cells to become resistance to NPs [18]. These NPs can enhance antimicrobial activity due to their large surface-to-volume ratio and also can destroy the microorganism family. The ENPs extensively required for fabrication of antimicrobial surfaces and have been excessively used in agricultural, industrial purposes, consumer products and storage wares, as nutritional additives, hospital consumables, kitchen appliances, cosmetics and also in medical applications [19, 20]. In order to make EAS, among the ENPs, polymer-based ENPs, carbogenic nanomaterials, chitosan, metal and metal oxide-based NPs have been widely used. Herein we have briefly focused their toxicological impact on our environment, soil, water bodies and microorganisms.

2.1 Polymer-Based ENPs

Antimicrobial polymers are the polymers which are having the enhanced antibacterial/fungal activity by which it inhibits or suppresses the bacterial or fungal growth, and these polymers are majorly non-volatile and stable compounds. The antimicrobial activity of the polymer is directly proportional to the size of the particle [21]. The polymer ENPs are bounded with poly-cations so that it is having the property of disagreement with the negatively charged cell sheaths. The most available reactive groups present on the polymer ENPs surface are quaternary phosphonium, tertiary sulphonium, quaternary ammonium and guanidinium ions [22, 23]. The interaction mechanism speculated to be, the cations existing in the polymer intermingle with fungal and bacterial cell membrane, leads to the rupture of lipid membrane and distress the transference of vital compounds crossways on the membrane and initiates cell death [24]. Scientists had focused on synthesizing new type of polymers with sulphonamide or sulphapyridine-formaldehyde copolymers, sulphonamide-dimethylolurea copolymers and N-acylsulphanilamide groups for extended antimicrobial activities. The size reliant on the volume of quaternary ammonium groups in the copolymers with N-vinylpyrrolidone with (2-methacryloxyethyl) triethylammonium iodine or bromide towards antimicrobial activities showed further insight on quaternary ammonium group's presence in polymers [1]. Many research outputs concluded that polymers will exhibit enhanced antimicrobial activity, and the antimicrobial polymeric materials' activity is taken into account by considering different parameters such as type and degree of alkylation, distribution of charge, hydrophilic/hydrophobic ratio and their influence on the activity and molecular weight.

Primarily antimicrobial polymers are associated with the antimicrobial activity by covalent bond groups/linkages. Some authors are deep rooted that functional groups encompassing non-degradable polymers, i.e. polymers and copolymers of (4-vinylsalicylic acid) and (5-vinylsalicylic acid) by-products, stood very dynamic against gram-positive and/or gram-negative bacteria [1], and these products are not with respect to their molecular weight [25]. Researchers interleaved polymers into carbon-based material to improve supplementary activity through π-π interface bonding. It is concluded that low concentration of carbon nanomaterials with poly vinyl(N-carbazole) (PVK) shows high activity against gram-positive and gram-negative bacteria, and because the high solubility of carbon-based nanomaterials exhibited good bacterial interaction and antibacterial activity [26, 27]. Moreover, antimicrobial activity can be accomplished by enhancing antimicrobial toxicity using polymers linked with capping agents or carbonaceous nanomaterials and antimicrobial agents [28].

The antimicrobial polymers are used in countless applications such as water filters, surface coating materials and also in fibrous sterilizers because these polymer surfaces will not allow fungal mediators or bacteria to pass through the filter membrane [29]. In the food industry, food grade antimicrobial polymers are used to hinder the adulteration of food to suppress the bacterial infection or fungal infection, and even these polymers can enhance the lifetime of packaged foods [30]. In health care and clinical applications, antimicrobial agents are used as meticulously in dental healing resources because of their high activity, and blended polymers are used as antimicrobial wound recuperations.

2.2 Carbogenic Nanomaterials

Carbon nanomaterials such as carbon nanotubes (CNTs), graphene, quantum dots (QDs), etc., are generally used because of its unique and adaptable physicochemical, mechanical and electrical properties. And also, these carbogenic nanomaterials are suggested for use in various applications such as superconductor materials, construction, optical devices, biomedicine, molecular switches, quantum computers and agricultural smart delivery systems. Additionally, it is possible that CNTs are one of the least degradable man-made materials. Multi-walled carbon nanotubes (MWCNTs) are toxic to soil microbial community structure and functioning, because of its functionalization and composition [31]. The CNTs on the microorganisms negatively affect the bacterial growth and also in microorganisms, so it leads to cell death and cell viability [32]. Fullerenes (C_{60}) and CQD inhibit the growth of the bacteria in the soil [33]. Graphene present in soil influences the antimicrobial intensity on particular organism [34].

2.3 *Chitosan*

Chitosan is a flexible material which is derived from chitin; it is the principal structural polymer in arthropod exoskeletons. Additionally, studies on the antibacterial activity of chitosan oligomers proved that chitosan is supplementary active in preventing the progress of bacteria than chitosan oligomers [35]. The primary amine groups are at the position of C-2, primary and secondary hydroxyl groups are at the position of C-3, and C-6 is very responsive in chitosan, respectively. Out of these groups, C-2 amine functional group is the highly reactive group for all environmental activities [36–38]. Chitosan film is viewed as bio-purposeful material, well endured by the existing materials, predominantly pertinent as eatable coatings to lengthen shelf life and preserve eminence of fresh nutrients. It is a hydrophilic polysaccharide having high antimicrobial activity inclined to broad spectrum of gram-negative, gram-positive bacteria and fungi. Researchers presented many mechanisms for the antimicrobial activity of chitosan; the activity will be depending on the necessity of polymeric molecular weight (MW), degree of deacetylation (DDA), pH and temperature. The effectiveness of the antimicrobial activity will be depending on the species of target microorganisms and the organic properties also [39].

2.3.1 Molecular Weight

Molecular weight (MW) has been revealed to be a significant feature in chitosan properties such as crystallinity, deprivation, ductile strengths and humidity content [40–43], and these properties typically influence the chitosan antimicrobial activity. Researchers had proposed some specific criteria such as absorption range, and grade of deacetylation correlates the MWs with respect to antimicrobial activity [44], However, there are still some inconsistency in reports on the correlation of MW and corresponding antimicrobial activity [45, 46]. MW is also inversely related to deacetylation reaction period and temperature. The MW of chitosan is dependent on the primary source material such as shrimp, crab, fungi, and the larger MW chitosan has higher antibacterial activity in some cases. The pH effects, antibacterial activity, chitosan MW and zeta potential (ZP) vary with water solubility [44, 46].

2.3.2 Degree of Deacetylation (DDA)

Deacetylation is the process of eviction of acetyl group from the molecular chained chitin molecule. The DDA can be used to find out the occurrence of amino moieties in the polysaccharides, and even differences in temperature and pressure can be monitored by DDA of chitosan. Several studies have demonstrated the influence of degree of acetylation on the antimicrobial effectiveness against fungi, gram-negative and gram-positive bacteria [45]. However, still there is a controversy on the effect of DDA on microbial activity.

2.3.3 The pH

pH is the measure of concentration of hydrogen in the solution; majorly, antibacterial activity and chitosan MW are affected by pH, and it is inversely proportional to the antimicrobial activity of chitosan. At acidic pH (pH 5.0 and 6.0), chitosan activity increased as the MW increased, by the same way, at neutral pH the chitosan's with MWs > 29.2 kDa exhibits loss of activity. With high temperature and low pH, the amino moieties of chitosan become ionized and also will enhance the positive charge due to the higher fraction of amino moieties, insolubility and de-protonation. The unmodified chitosan will not be showing any activity towards the pH-7 [44].

2.3.4 Temperature

Temperature plays significant role on the antibacterial action of chitosan nanoparticles. The antibacterial activity is increased with respect to temperature, and even in microorganisms also, the same effect is observed. Higher temperature and acidic pH amplified the bactericidal properties of chitosan, and the reaction or kinetic rate also changes as per temperature.

2.4 Gold Nanoparticles (Au NPs)

In the field of research, NPs play a vital role because of the exhibiting eventual properties towards science and engineering. The ultimate property of NPs is due to high surface area to volume ratio [47, 48]. The antimicrobial activity of the Au NPs is becoming a scorching subject of researchers because of the widespread chem-physio properties for the ultimate use of antimicrobials [49]. Au NPs which are non-toxic, inert, more stable, size controllable, particle size and surface charge are directly proportional to the antibacterial activity [50, 51]. Due to high-class optical-electronic properties Au NPs are appropriate in drug delivery, sensory investigations and antimicrobial mediators application in medical fields. Functionalization is the main criteria to achieve the antimicrobial activity against the gram-positive, gram-negative and multi-drug resistant pathogens, and it can be done by Au NPs [52].

2.5 Silver Nanoparticles (AgNPs)

Silver (Ag) plays a vital role in the antimicrobial progression of the nanoparticles. Numerous studies were confirmed the antimicrobial activity of AgNPs against the activity of fungi, viruses, parasites and bacteria [53–55]. The antimicrobial activity of AgNPs chiefly depends on the Ag^+ release; Ag^+ is having the capability of accumulation of more numbers on cell walls and also having the competence to

penetrate the cell walls. The activity of Ag^+ with the microbial cell wall leads to the annihilation of microbial cells [7, 56]. The toxicity of AgNPs is purely dependent on the surface characteristics such as shape and size. These are directly proportional to the generation of Ag^+, and these can able to attach on the cell wall or cell membrane [8] and increase the antimicrobial activity [57]. Triangular-shaped NPs are more toxic compared to spherical and rod-shaped NPs because of the higher density of atoms per area on the edges [58]. The unmodified nanofibers are used to control the bacterial growth, so there is no outcome on the evolution, but the renewed cellulose nanofibers were improved with AgNPs which are used as an antibacterial agent to inhibit the growth of bacterial species. The amount and concentration of Ag^+ in AgNPs are important, since Ag^+ has a sturdy affinity for the microbial cell wall, so it has a high effect on inhibition activity of bacteria. Hence, AgNPs are the more advanced antimicrobial agents and also cause the formation of reactive oxygen species (ROS) with hydrogen peroxide (H_2O_2) in microbial cells. Many researches proved that AgNPs will affect the environment by suppressing the nitrification rate and nitrogen-producing bacteria in plants such as *Nitrobacter*, *Nitrosomonas* and *Bacillus subtilis* [59].

2.6 Zinc Oxide Nanoparticles (ZnO NPs)

Zinc (Zn) is a vital element, and for the dynamic and universal property, it is used in all fields of medicine and also named as vivacious component to enhance the activity of enzymes in some viruses and humans [9, 60–62]. The probable intake of Zn in adult is 8–15 mg/day, and half of the intake will be lost through urine and sweat. The oxide of Zn, that is, zinc oxide (ZnO) is listed as "generally recognized as safe" (GRAS) by the U.S. Food and Drug Administration (21CFR182.8991) [63]. Other than the aforementioned NPs, nanomaterials inducing the intercellular reactive oxygen species such as OH, H_2O_2 and O_2^{2-} (strong oxidizing agent) can be harmful to cells of bacteria [64]; these strong oxidizing agents can be generated from ZnO NPs through ultraviolet (UV) and visible light. The ZnO NPs are extensively used as antibacterial agents and are more dynamic towards gram-positive bacteria relative to other NPs of the same group of elements. The surface-to-volume ratio is directly proportional to antibacterial activity against both gram-negative and gram-positive bacteria and inversely proportional to the size of the metal oxide NPs, i.e. thinner NPs boost greater antibacterial activity than minute particles (microscale particles) [65]. The ZnO NPs antibacterial properties vary with particle size, shape, concentration and bacterial exposure time. Since NPs and metal ions are very minute in thickens compared to bacterial cells, it can distort the cell membrane and can inhibit the growth of microorganisms by penetrating the cell walls. The injected concentrations of NPs are proportional to growth inhibition property of microbes [66–68] and are better functional against various microorganisms such as *Staphylococcus aureus*, *Klebsiella pneumonia*, *Pseudomonas vulgaris*, *Pseudomonas aeruginosa*.

Recent studies had proved that these nanoparticles have discerning toxicity to bacteria but display minimal effects on human cells [69]. Several ENPs are derivatives of metal oxides which are fabricated by chemical, biological and physical synthesis procedures. However, eco-friendly ENPs are derived through biological processes, and these ENPs will not affect the environment. The ZnO NPs are used for packaged foods, preservatives and as antimicrobial agents due to their antimicrobial activity. These ZnO NPs are non-toxic towards human consumption and only exhibit toxicity against the microorganisms [70]. Because of inhibiting the growth of microbes, it is widely used in packaged food industries. Additionally, these NPs do not have any pungent smell, taste and also non-reactive with food or food containers [71, 72].

Including above-mentioned metal and metal oxide nanoparticles, some other NPs also directly or indirectly affect the environment such as lanthanide oxide nanoparticles (LnO NPs), dysprosium oxide NPs (nDy_2O_3), also contributing negative effects on humans and environment excessively, and still, these NPs are used in medical applications [73–77]. The TiO_2 NPs will effect on the intestinal bacteria of Drosophila which depends on the size and even in in vivo studies [78, 79]. TiO_2 alone will act as an obstacle for bacterial growth through photochemical activation, which leads to cell death by the formation of H_2O_2 and reactive oxygen species (ROS) in the presence of UV radiation. Studies found that lithium nickel manganese cobalt oxide (NMC) nanoparticles ($L_xNi_yMn_zCo_{1-y-z}O_2$, $0 < x, y, z < 1$) have been used excessively in battery industries. When the NMC inbuilt batteries are exposed to the environment, toxic elements such as Li^+, Ni^{+2} and Co^{+2} are released. The dissolved Ni and Co ions as well as Mn and Li act as potential bacterial toxicants, also affecting the beneficial soil-based microspecies [80] (Table 1).

3 Toxicity and ENPs Concentrations

The ENPs are commonly present as metals, dust or other various forms. These nanoparticles may contaminate the surroundings of the environment because of the requirement of chemical and physical synthesis methods which are not eco-friendly. The potential effect of the ENPs in the environment is termed as nanotoxicity. Ag nanoparticles is one of the ENPs widely used; generally, the toxic effect of Ag is at high concentrations, for example, the lethal dose (LD50) for rats was higher than 1600 mg kg^{-1} $days^{-1}$ for oral administration [89]. The toxicological effect of silver nanoparticles on bacteria such as nitrifying bacteria (*Nitrosomonas* and *Nitrobacter*), *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis* has been reported [93] and is due to release of Ag^+. This harms the algal community at different concentrations [59]. ZnO is another ENPs with its antimicrobial effects which creates a certain level of uncertainty in nano-ecotoxicity. ZnO NPs are found to inhibit different microorganisms with varying concentrations. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of 0.1 and 0.8 μg mL^{-1} on *E. coli* K88 [94], 3 and 12 mmol L^{-1} inhibited the growth of *E. coli* O157:H7 [95], 179 and 1790 μg mL^{-1} antibacterial effect on *S.*

Table 1 Types of commonly used ENPs for EAS and their effect on different organisms

ENP's	Particle size (nm)	Test organism	Effect on organism	References
Polyvinyl alcohol (PVA) and adenosine triphosphate disodium (Na_2ATP)-doped Ag	7 ± 3 40 ± 14	*Nitrosomonas europaea* ATCC19718	Size- and capping material-dependent inhibition of ammonia oxidation, disintegration of nucleoid, damage of cell wall	[81]
nZVFe (zero-valent iron)	20–90	Paracoccus sp.	Dose-dependent inactivation with low cell density, 50 mg L^{-1} promoted both cell growth and biodegradation of nitrate	[82]
ZnO	2–28	Pseudomonas sp., Fusarium sp.	Growth inhibition and disruption of bacterial and fungal cell wall/membrane	[83]

(continued)

Table 1 (continued)

ENP's	Particle size (nm)	Test organism	Effect on organism	References
ZnO	70 ± 15	*Botrytic cinerea*, *Penicillium expansum*	3 mmol L^{-1} significantly inhibited fungal growth with *P. expansum* as the most sensitive to the treatment. Deformation of fungal hyphae and inhibition of cellular activities in *B. cinerea*. Prevented conidiophore development in *P. expansum* resulting in the death of fungal hyphae	[84, 85]
ZnO	$>1.3 \times 10^{-3}$	*S. aureus* and *E. coli*	More 1.3×10^{-3}M and with some small molecules and macromolecules in DEG medium were damaged, and the cell contents may have leaked out	[69]
TiO_2 (coated)		*Daphnia magna*	Not specified for TiO_2 with Si/Al coatings (90/7/1 wt% TiO_2/Al/Si):EC50, 48 h [100 mg L^{-1}]. The TiO_2 had a crystalline phase determination of 79% rutile and 21% anatase, a median particle size of 140 nm in water, and BET SSA was 38.5 m^2/g and 140 nm in water	[86]

(continued)

Table 1 (continued)

ENP's	Particle size (nm)	Test organism	Effect on organism	References
TiO_2 C_{60} $C_{60}HxC_{70}Hx$	C_{60} and $C_{60}HxC_{70}Hx$: 10–20 nm in suspension TiO_2: 30 nm	*Daphnia magna*	Behavioural and physiological changes solvent (THF) exposure to 260 μg L^{-1} C_{60} increased heart rate of *D. magna*. Exposure to both 260 μg L^{-1} $C_{60}HxC_{70}Hx$ as well as to 260 μg L^{-1} C_{60} resulted in increased hopping frequency and increased appendage movement. Recovery after exposure to $C_{60}HxC_{70}Hx$, but not for C_{60}. No significant effects on any of the three parameters were found for TiO_2	[87]
C_{60}	Aggregate size: 2 nm to several microns	*Daphnia magna*	Addition of 5–8 mg L^{-1} C_{60} increased the toxicity of phenanthrene more than 10 times when results were expressed as water phase concentrations. Uptake of phenanthrene was faster with C_{60}; 1.7 times higher steady-state concentrations were found, but due to very fast clearance after transfer to clean water, accumulation of phenanthrene was not affected by the presence of C_{60}	[88]

(continued)

Table 1 (continued)

ENP's	Particle size (nm)	Test organism	Effect on organism	References
SWCNT (Single-walled carbon nanotube coated)	1.2 nm	*Daphnia magna*	Daphnids were able to modify the SWCNT upon ingestion (removing the lipid lysophophatidyl choline coating), hereby reducing solubility of the SWCNT. 100% mortality was observed after 48 h at 20 mg L^{-1} exposure	[89]
CdTe—quantum dots (QDs)	80% of the aggregates (450 nm)	*Elliptio complanata*	CdTe was found to aggregate. 15% of the Cd was found in the molecular fraction below 1 kDa. Exposure concentrations of 1.6, 4 and 8 mg L^{-1} CdTe did not cause mortality or weakly closed shells. CdTe QDs were found to induce oxidative stress in gills and digestive gland tissues. Oxidative stress in the gills was also detected when mussels were exposed to 0.5 mg L^{-1} of molecular cadmium sulphate ($CdSO_4$). Results indicated that CdTe acts not only through release of free Cd^{2+} ions but also effects were caused by colloidal CdTe	[90]

(continued)

Table 1 (continued)

ENP's	Particle size (nm)	Test organism	Effect on organism	References
SWCNT/MWCNT (Multi-walled carbon nanotube) (^{14}C labelled)	MWNTs: 30–70 nm SWCNTs: 1–2 nm	*Lumbriculus variegatus*	CNTs were found to not readily absorb into organism tissues. CNTs detected in the organisms were found to be associated with sediments remaining in the organism guts	[91]
Au bioconjugate	Gold nanoparticle at 50 mg/L inhibited the bacterial growth by cup–plate test	*E. coli*; *P. aeruginosa*; *B. subtilis*; *S. aureus*; *S. cerevisiae*; *C. albicans*	1. Damaged the cellular membrane integrity 2. A significant decrease in cell viability, with 90% increase in red stained (dead) cells gold nanoparticle	[92]

aureus [96] MIC of 500 ± 306.18 μg mL^{-1} and MBC of 500 μg mL^{-1} on *Streptococcus mutants* [97], respectively. In complex environmental matrix such as the soil, ZnO concentrations that ranged from 0 to 200 mM g^{-1} [98], 140 to 1400 mg kg^{-1} [99] and 238 to 2500 mg kg^{-1} [100] at different exposures exerted inhibitory effect on microbial community activities (Fig. 3).

However, interpretation of toxic effect based on the concentration can potentially mislead because a particular ENPs dose in the soil matrix can stimulate microbial community activity, whereas individual organisms or groups are inhibited [96]. In addition, different outcomes are exhibited by diverse microbes that interact with varying types of ENPs and their concentrations. Typically, low concentrations of ENPs can exhibit different outcomes in environment. For example, 0–2.0 mg L^{-1} TiO_2 stimulated and also inhibited microbes in loamy soil [97], whereas 0.072–0.708 mg L^{-1} of AgO nanoparticles was toxic to microbes in deciduous soil [98]. And also, it reduces the plant growth and reduction in root elongation and weight [101]. Thus, the interpretation of the bioavailable dose of different ENPs in the environment and the associated biotic responses in simple and complex media vary due to factors such as the presence of natural organic matter (NOM), colloids, physicochemical and biological transformations, complexation reaction with ligands, physicochemical properties of the ENPs and contact time [102].

ENPs are repeatedly released into the soil and aquatic environments because of the increasing industrial application of metal and metal oxides. However, there are some intrinsic challenges posed by repeated exposure compared to single exposures. The studies show that ENPs of Zn and nano-form of Cu oxides in soil were highly toxic to bacteria. While in the case of their bulk forms, CuO was non-toxic, whereas that of Zn in all forms was more toxic than its nano-form [98]. Similarly, the repeated exposure to Ag_2O NPs was more toxic to ammonia-oxidizing bacterial (AOB) biomass than a single acute exposure [102]. ENPs harmful effect on the aquatic organisms and

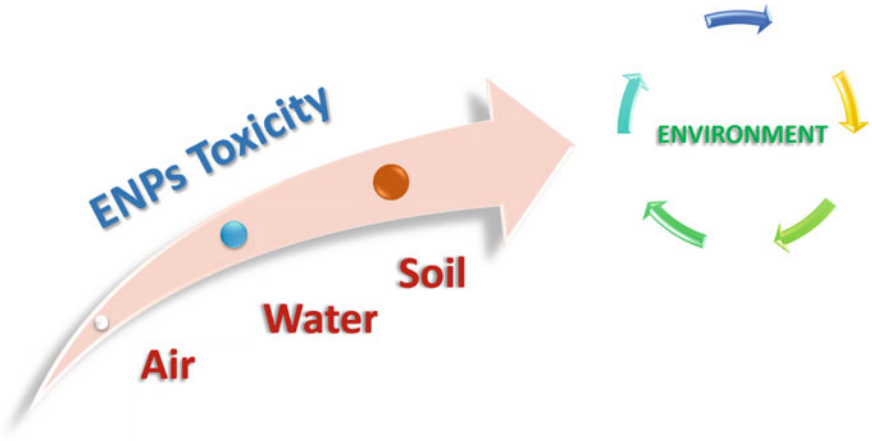


Fig. 3 Toxicity of ENPs towards the environment

food web is challenging; the dissolution of nanoparticles in the aqueous matrix will affect the reactive nature of the nanoparticles by changing the size, surface charges and release of ions, thus influencing the toxic effects [103]. Particularly, bacterio-plankton and phytoplankton populations had their photosynthetic efficiency significantly reduced when exposed to 500 $\mu g\ L^{-1}$ of Ag_2O NPs. This is consistent with the assertion that ENPs in complex medium such as soil [104, 105] and activated sludge exert selective toxic effect on the different microbial groups and species. However, the uncontrollable use of ENPs has introduced numerous toxic groups of compounds into the ecosystem, leaving a toxicological challenge to deal with. [106].

The paths of exposure of nanomaterials to living organisms such as plants, animals, fishes depend on the habitat of the organism. The entry of nano particles is present in the different environment to living organisms by gills, mouth, to the gut, etc. For example, in plants, the entry of nanoparticles through root, for fish in water and worms in soil, etc. As per the reports, the studies on ecotoxicology report different effects of antimicrobial agents like bacterial inhibition, stimulation, survival and death, which largely depend on dose, species and test procedure [15]. It is essential to determine their impact on surrounding non-target organisms and ecological processes not only during leaching, but also during their production, especially since that production is estimated to grow to 58,000 tons per year by 2020 [107] (Fig. 4).

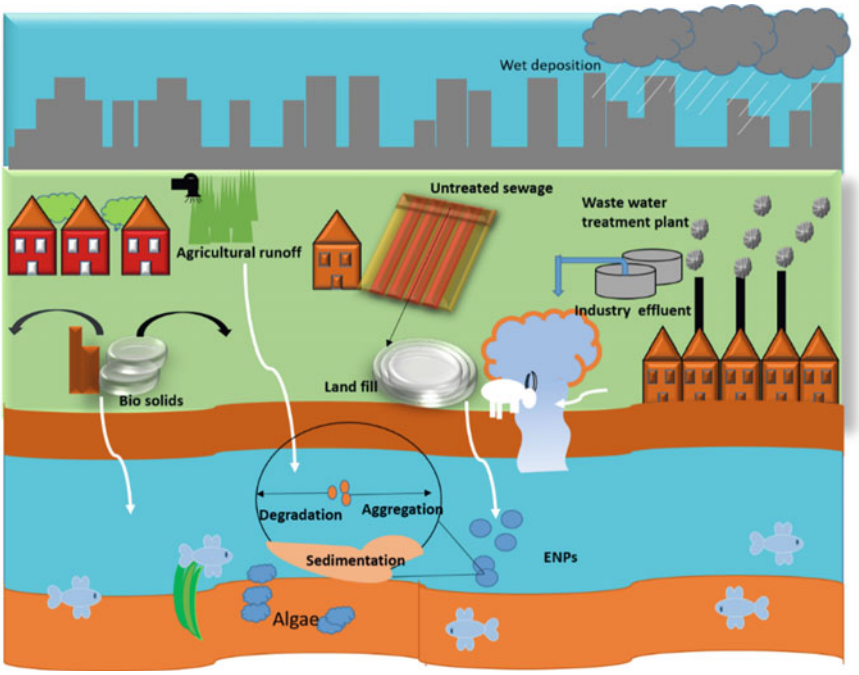


Fig. 4 Toxicity of ENPs to organisms from wide-ranging parts of the ecosystem food webs

Another one important factor is air, which determines the fate of nanoparticles in the environment. This can be analysed by different factors such as the duration of time in which the particles remain in the air, their interaction with other particles or molecules in the atmosphere and the distance that they can travel in the air. And also, some important factors which determine the ENPs in the atmosphere are diffusion, agglomeration, wet and dry deposition and gravitational sedimentation. Usually, particles on the nano-metre scale are considered to have a shorter residence time in air, compared to medium-sized particles, because they quickly agglomerate into much larger particles and settle to the ground [108].

4 Ecological Effects of ENPs in Soil

Soil plays a major role in ecological sustenance and is also important in maintaining the quality of the water [109]. Hence, the protection of quality of the soil is of vital need. Among different factors, biotic factors are the major factors that indicate the soil health. Biotic factors include the soil organisms (earthworm and other microorganisms) and biotic parameters. The inevitable release of ENPs to the environment can be direct and indirect. Direct release of ENP is by leaching from various consumer products and building surfaces, and indirect release via wastewater sludges used as fertilizers [110, 111]. The partition of ENPs into transformation products and their disposal into soil can also be one of the leading reasons for soil contamination [112]. The release of metal oxides such as TiO_2 NPs, cupric oxide nanoparticles (CuO NPs) into the ecosystem such as agricultural land has raised major concerns in pollution of environment. TiO_2 NPs are widely used in consumer product as well as in agriculture field as nano-pesticides [113]. High exposure doses of TiO_2 NPs and CuO NPs have found to inhibit the activity soil enzymes (urease and phosphatase) drastically. The presence of these enzymes is an indirect indicator for metabolic activity of microbes and their inherent ability to purify pollutants in soils [114]. The activity of the enzyme is hindered due to reaction of these metal oxides with the enzymes binding sites and also by reacting protein groups of enzymes [115]. However, in a study TiO_2 NPs, even at a low concentration have found to distort the nitrogen cycle and a modification of the bacterial community structure in an agricultural soil even at low realistic concentration. Ammonia oxidation represents the first step in *nitrification*, and *Nitrososphaera* is the archaea which is required for ammonia oxidation. During the exposure of TiO_2 NPs, Nitrososphaera (ammonia-oxidizing archaeans) was inhibited. During the exposure of TiO_2 NPs, *Nitrososphaera* (ammonia-oxidizing archaeans) was inhibited [116]. Studies have also found that TiO_2 NPs may affect foetus health indirectly during pregnancy by its accumulation in placental tissue [117]. Earthworms are the key players in soil health. When ENPs containing metal ions in the soil were exposed to earthworm, reduction in reproduction of 90% was observed [118]. Metal oxides such as CuO-based ENPs are toxic to the reproduction of earthworm, altering the soil health [119]. The porosity of soils as well as its pH plays a key role, allowing ENPs to pass through the pore system, leaching into

aquifer systems and eventually marine environments [120]. ENPs mobility is more efficient across mineral soils than in organic soils [121]. Thus, by reaching the soil subsurface, it interacts with plants and invertebrates such as annelids, nematodes, insects and microorganisms and causes physical, biochemical and cellular damage. They also affect organisms at tissue, organismal and community levels, with various outcomes. In plants, TiO_2 NPs and AgNPs induce cytotoxic and genotoxic damage by generating reactive oxygen species (ROS), thereby affecting the germination, growth and photosynthetic activity [122, 123]. In a study, when the root tips were exposed to AgNPs, less growth of root hair was observed on the surface of *Arabidopsis thaliana*. This negative effect on the root hair will reduce the water intake of the plant, leading to improper growth and development [124]. Later, the same group also found increased ROS generation in *Arabidopsis thaliana* leading to cellular damage during exposure to AgNPs [125]. Unfortunately, these ENPs are also active against natural enemies of the mosquito, suggesting a potential public health problem through disruption of biological control of mosquito populations [126].

5 Ecological Effects of ENPs in the Aqueous Environment

Since there is an increase in ENPs commercial usage, the leaching and release of ENPs occur through wastewater which ultimately leads to marine ecosystems despite of several safety measures taken. Also, with the large-scale production of ENPs, the chance of these NPs entering the aquatic ecosystem is also high [127, 128]. This makes the aquatic ecosystems; a terminal sink for ENPs introduced to natural systems and thereby increased risk of exposure of ENPs to organisms living in it. Because of the release of zinc ions with their ability to aggregate and dissolute, ZnO NPs exhibit comparatively high toxicity to marine organisms [129]. Taking this into account, different ecological parameters such as temperature, pH, ionic strength, electrolyte type and organic matter and their effect on ZnO behaviour and its dissolution and toxicity to marine organisms were studied. The study concluded that among the various factors, organic matter was the primary agent leading to aggregation and toxicity of ZnO [130]. In the study, animal models such as zebrafish, daphnids and an algal species were used to determine the toxic effects of Ag, Cu, Al, Ni, Co and TiO_2 NPs both as NPs and as their soluble salts. Among these NPs, Ag and Cu in nano-form were found to be toxic in all the organisms tested, while TiO_2 NPs were not toxic. Filter-feeding invertebrates were more susceptible to nanometals, and soluble forms of nanometals were more toxic. Additionally, the health of zebrafish was also impaired by both ionic Ag and AgNPs [131]. On the contrary, in another study, the increased cytotoxicity by TiO_2 NP towards *Escherichia coli* in aquatic system was observed in with increased salinity which was observed by a group, and the same was validated in natural estuarine water [132]. The bioavailability of metal ions from ENPs compared to their bulk counterparts, released into the aquatic environment, is also one of the reasons of them being toxic to marine organisms. The toxic effects of CuO to algae were due to bioavailable copper ions from Cu_2O NPs. Further, they showed

that ZnO NPs were more toxic to the microalga than Cu_2O NPs [133]. Another study lead by a group [134] stressed on the importance of testing NPs toxicity in natural waters, rather than artificial ones and also confirmed that ZnO and AgNPs ecotoxicity was mostly due to release of the toxic ions. Several toxic effects of NPs in their dissolved forms are also shown in other studies [135, 136]. Free ENPs are likely to aggregate in the aquatic environment, which ultimately settle down during sedimentation process. These aggregated ENPs are usually less mobile and tend to come in contact with the sediment-dwelling animals. Factors affecting aggregation of NPs include pH, ionic strength, electrolytes, natural organic matter, diffusion coefficients, weight average diameters of NPs and hydrodynamic conditions. Thus, the transport of NPs in the aquatic environment can be affected by aggregation, dissolution and/or transformation [137]. The extracellular polymeric substances (EPS) are synthesized by microorganisms, which are abundant in natural aquatic systems. The EPS could also act as a protective material against NPs. However, a study showed that the Ag ions released from AgNPs above a certain threshold caused detrimental effect on the EPS layer [138]. The presence of EPS can affect ENP stability and dissolution; the dissolution of CuO NPs was increased by EPS [139], leading to their ultimate stability in the environment, thereby affecting the aquatic organisms subsiding nearby. Therefore, it is a challenge to predict the environmental fate and distribution of NPs. Toxic effects of ZnO NPs were studied in freshwater snails, and was found that the ZnO NPs lead to increase in ROS causing DNA damage after 24 and 96 h of exposure in the digestive gland of the snails [140]. Mahaye et al. [141] stressed the need for further ENPs genotoxicity research using a wider range of test organisms, particularly those that play important trophic roles in complex aquatic communities. While ENPs released into soil and water are obvious concerns, John et al. [142] pointed out that air contamination can also occur accidentally. This is less common than soil and water pollution but should be further studied.

6 ENPs Interactions with Microorganisms

Microorganisms play vital roles in ecological process regulating the biogeochemical systems [143, 144]. Hence, the interactions of ENPs and microorganisms depend on variety of characteristics such as the size, shape, chemical composition, capping agent and environmental factors including natural organic matter, ligands, surfactants, pH and colloids [145, 146]. Besides having antibacterial activity against infectious pathogens, the ENPs are found to be toxic even to the soil beneficial non-target microorganisms. For example, ZnO NPs are widely used as antimicrobial agent and in environmental remediation. However, in a study, indole acetic acid production on plant growth promoting rhizobacteria was inhibited by ZnO NPs [147]. The soil type also determines the variability of toxicity of ENPs in soil microorganisms. In a study, ZnO NPs were found to be more toxic in acidic soil than in neutral soil [148].

Compared to fullerenes and carbon nanotubes, metal and metal oxides NPs are reported to have more toxic effect on soil microorganisms [113]. In order to understand the ENPs microorganism interaction, factors such as solubility, bioavailability and bioreactivity are crucial. During the aggregation of the ENPs, the bioavailability to microorganisms also decreases drastically. There is a competition of the ENPs like AgO with divalent cations when binding to the teichoic acid present in the gram-positive bacterial cell. This results in less toxicity of AgO in gram-positive organisms. Whereas in the case of gram-negative cell walls, the presence of lipopolysaccharides (LPS) hinders the passage of toxic substances [149]. Although ENPs pose a risk to ecologically sensitive microbial species and processes, the growth of methanogens and heterotrophs in the presence and chronic exposure to toxic ENPs concentration in activated sludge provides strong evidence that *Methanosarcina*, *Acidovorax*, *Rhodoferax* and *Commamonas* are nano-tolerant microorganisms [150]. Direct supply of ENPs such as Fe and TiO_2 NPs in water treatment and environmental remediation inhibits and stimulates target organisms, respectively and at the same time exerts adverse effect on non-target microorganisms and other biological systems [151, 152]. The ENPs undergo variety of transformations like photochemical transformations, dissolutions, precipitation, oxidation, reduction, adsorption and desorption, combustion, abrasion and bio-transformations in the environmental matrix causing toxicity to non-target organisms [153].

Gut microbiota are community of microorganisms living in the gastrointestinal tract. Alteration of the physiological functions of this microbiota can lead to various diseases. The ENPs such as carbon nanotubes (SWCNT and MWCNT), Ti_2O, cerium dioxide (CeO_2), ZnO, nano-silica and nano-silver may affect the microbiota in a dose-dependent manner and further cause physiological alterations which leads to diseases such as colitis, obesity and immunological dysfunctions. Also, in another study, when the model colon with microbial community was exposed to titanium dioxide (TiO_2), ZnO and CeO_2 at varying doses, colonic bacteria was phenotypically altered leading to obesity [154].

Other factors such as the size of ENPs [18, 155], the presence of divalent cations/anions and surface charges [156, 157], the bacterial cell wall composition and their charges [158, 159] and the use of capping agents which repel ENPs by electrostatic, steric or electro-steric forces to avoid forming aggregate [160, 161] can either enhance or attenuate ENP micro-biocidal effect. The surface capping agent is associated with positive or negative influence on the toxicity of the ENPs on pure and mixed microbial cultures, bio-solid amended and unamended soils. Also, the addition of a functional group enhances the toxic potential of ENPs to cell cultures and whole organisms. CNT functionalized with hydroxyl (–OH), carboxyl (C=O) and amine ($–NH_2$) was more toxic than pristine CNTs on aquatic microbial community composition [162], whereas sodium citrate-coated silver oxide nanoparticle exerted low inhibitory effect on heterotrophic, mesophilic bacteria [163].

With wastewater bio-solids serving as a sink and source of ENPs-enabled waste into the environment, soil microorganisms thus constitute the bulk of unintentional target of the toxic effects. The adverse effect of ENPs on soil microorganisms is gradually emerging, and the mechanism of action needs to be studied. This inference

is as a result of the established antimicrobial properties of several ENPs on most pure cultures of different microorganisms and emerging evidence of ENPs toxic effect on soil microbial community [4].

7 Conclusion

The EASs offer exciting application opportunities in diverse fields ranging from biomedical, agriculture, environmental, cosmetics to household commodities. Increasing use of EAS with ENPs in day-to-day applications is also boosting their exposure to environment and ecosystems significantly, which has raised the concern for environmental safety due to their potential adverse and toxicological effects on microbial community. Although, several risk and safety assessment studies to evaluate the fate of NPs in the environment and their effect on living organisms are being carried out in the recent years, still the current knowledge on impact of these NPs on the environment is limited because of their size-dependent activities and species-specific behaviours. The present chapter comprehensively summarizes the different types of ENPs and interprets the impact of ENPs on the environmental system. The environmental science community needs to provide appropriate testing protocols and predictive tools for addressing the crucial issue of risk of harmful impacts and corrective and preventive measures of these ENPs. The development of an effective working relationship between industry, government subsidiaries, socially responsible directories and an independent environmental science community will facilitate the development of a coherent approach to the identification and preventive approaches of environmental hazards and the design of nano-risk protocols.

Acknowledgements KS is thankful for Senior Research Fellowship and funds from ICMR. JS, BNK and KSP are greatful to Department of Biotechnology (DBTBT/PR21309/MED/32/557/2016) and Department of Science and Technology (DST-SERB- CRG/2018/00033), Govt. of India for funding, ABA is indebted to ICMR extramural grant (Leptos/15/2013-ECD-I).

References

1. Muñoz-Bonilla A, Cerrada ML, Fernández-García M (2013) Introduction to antimicrobial polymeric materials. In: Polymeric materials with antimicrobial activity: from synthesis to application, vol 10, p 1
2. Pranantyo D, Xu LQ, Kang ET, Chan-Park MB (2018) Chitosan-based peptidopolysaccharides as cationic antimicrobial agents and antibacterial coatings. Bio Macromol 19(6):2156–2165
3. Duran N, Marcato PD, Conti RD, Alves OL, Costa F, Brocchi M (2010) Potential use of silver nanoparticles on pathogenic bacteria, their toxicity and possible mechanisms of action. J Braz Chem Soc 21:949–959

4. Eduok S, Coulon F (2017) Engineered nanoparticles in the environments: interactions with microbial systems and microbial activity. In: Microbial ecotoxicology. Springer, Cham, pp 63–107
5. Eduok S (2013) Evaluation of the impact of engineered nanoparticles on the operation of wastewater treatment plant. Ecotox Environ Safe 95:1–9
6. Schumann GE, Philippe A, Bundschuh M, Metreveli G, Klitzke S, Rakcheev D, Lang F (2015) Understanding the fate and biological effects of Ag- and TiO_2-nanoparticles in the environment: the quest for advanced analytics and interdisciplinary concepts. Sci Total Environ 535:3–19
7. Daughton CG, Ternes TA (1999) Pharmaceuticals and personal care products in the environment: agents of subtle change? Environ Health Perspect 107:907–938
8. Wigger H, Hackmann S, Zimmermann T, Köser J, Thöming J, von Gleich A (2015) Influences of use activities and waste management on environmental releases of engineered nanomaterials. Sci Total Environ 535:160–171
9. Hennebert P, Avellan A, Yan J, Aguerre-Chariol O (2013) Experimental evidence of colloids and nanoparticles presence from 25 waste leachates. Waste Manage 33:1870–1881
10. Siedenbiedel F, Tiller JC (2012) Antimicrobial polymers in solution and on surfaces: overview and functional principles. Polymers 4(1):46–71
11. Ali I (2012) New generation adsorbents for water treatment. Chem Rev 112(10):5073–5091
12. Xu P, Zeng GM, Huang DL, Feng CL, Hu S, Zhao MH, Lai C, Wei Z, Huang C, Xie GX, Liu ZF (2012) Use of iron oxide nanomaterials in wastewater treatment: a review. Sci Total Environ 424:1
13. Ayati A, Ahmadpour A, Bamoharram FF, Tanhaei B, Mänttäri M, Sillanpää M (2014) A review on catalytic applications of Au/TiO_2 nanoparticles in the removal of water pollutant. Chemosphere 107:163–174
14. Gómez-Pastora J, Bringas E, Ortiz I (2014) Recent progress and future challenges on the use of high performance magnetic nano-adsorbents in environmental applications. Chem Eng J 256:187–204
15. Eduok S, Martin B, Nocker A, Villa R, Jefferson B, Coulon F (2013) Evaluation of engineered nanoparticle toxic effect on wastewater microorganisms: current status and challenges. Ecotox Environ Safe 95:1–9
16. Hemeg HA (2017) Nanomaterials for alternative antibacterial therapy. Int J Nanomedicine 12:8211
17. Li Q, Mahendra S, Lyon DY, Brunet L, Liga MV, Li D, Alvarez PJ (2008) Antimicrobial nanomaterials for water disinfection and microbial control: potential applications and implications. Water Res 42(18):4591–4602
18. Wang L, Hu C, Shao L (2017) The antimicrobial activity of nanoparticles: present situation and prospects for the future. Int J Nanomedicine 12:1227
19. Trudinger PA, Bubela B (1967) Microorganisms and the natural environment. Miner Depos 2(3):147–157
20. Kenawy ER, Worley SD, Broughton R (2007) The chemistry and applications of antimicrobial polymers: a state-of-the-art review. Biomacromolecules 8(5):1359–1384
21. Kamal GD, Pfaller MA, Rempe LE, Jebson PJ (1991) Reduced intravascular catheter infection by antibiotic bonding: a prospective, randomized, controlled trial. JAMA 265(18):2364–2368
22. Santos M, Fonseca A, Mendonça P, Branco R, Serra A, Morais P, Coelho J (2016) Recent developments in antimicrobial polymers: a review. Materials 9(7):599
23. Hoque J, Akkapeddi P, Yadav V, Manjunath GB, Uppu DS, Konai MM, Yarlagadda V, Sanyal K, Haldar J (2015) Broad spectrum antibacterial and antifungal polymeric paint materials: synthesis, structure–activity relationship, and membrane-active mode of action. ACS Appl Mater Interfaces 7(3):1804–1815
24. Cornell RJ, Donaruma LG (1965) 2-Methacryloxytropones. Intermediates for the synthesis of biologically active polymers. J Med Chem 8(3):388–390
25. Santos CM, Tria MC, Foster E, Advincula RC, Rodrigues DF (2013) Carbon-based polymer nanocomposites: from material preparation to antimicrobial applications. In: Polymeric materials with antimicrobial activity: from synthesis to applications, vol 13, no 10, p 327

26. Ahmed F, Santos CM, Vergara RAMV, Tria MCR, Advincula R, Rodrigues DF (2012) Antimicrobial applications of electroactive PVK-SWNT nanocomposites. Environ Sci Technol 46(3):1804–1810
27. Zhou J, Qi X (2011) Multi-walled carbon nanotubes/epilson-polylysine nanocomposite with enhanced antibacterial activity. Lett Appl Microbiol 52(1):76–83
28. Aslan S, Loebick CZ, Kang S, Elimelech M, Pfefferle LD, Van Tassel PR (2010) Antimicrobial biomaterials based on carbon nanotubes dispersed in poly (lactic-co-glycolic acid). Nanoscale 2(9):1789–1794
29. Kenawy ER, Abdel-Hay FI, El-Magd AA, Mahmoud Y (2006) Biologically active polymers: VII. Synthesis and antimicrobial activity of some crosslinked copolymers with quaternary ammonium and phosphonium groups. React Funct Polym 66(4):419–429
30. Plascencia-Jatomea M, Viniegra G, Olayo R, Castillo-Ortega M, Shirai K (2003) Effect of chitosan and temperature on spore germination of *Aspergillus niger*. Macromol Biosci 3(10):582–586
31. Mukherjee A, Majumdar S, Servin AD, Pagano L, Dhankher OP, White JC (2016) Carbon nanomaterials in agriculture: a critical review. Front Plant Sci 7:172
32. Goodwin DG Jr, Marsh KM, Sosa IB, Payne JB, Gorham JM, Bouwer EJ, Fairbrother DH (2015) Interactions of microorganisms with polymer nanocomposite surfaces containing oxidized carbon nanotubes. Environ Sci Technol 49(9):5484–5492
33. Gao J, Wang Y, Hovsepyan A, Bonzongo JCJ (2011) Effects of engineered nanomaterials on microbial catalyzed biogeochemical processes in sediments. J Hazard Mater 186(1):940–945
34. Zou X, Zhang L, Wang Z, Luo Y (2016) Mechanisms of the antimicrobial activities of graphene materials. JACS 138(7):2064–2077
35. Venkatesan J, Lowe B, Anil S, Manivasagan P, Kheraif AAA, Kang KH, Kim SK (2015) Seaweed polysaccharides and their potential biomedical applications. Starch-Stärke 67(5–6):381–390
36. Aranaz I, Harris R, Heras A (2010) Chitosan amphiphilic derivatives. Chemistry and applications. Curr Org Chem 14(3):308–330
37. Kim S (2018) Competitive biological activities of chitosan and its derivatives: antimicrobial, antioxidant, anticancer, and anti-inflammatory activities. Int J Polym Sci
38. Nunthanid J, Puttipipatkhachorn S, Yamamoto K, Peck GE (2001) Physical properties and molecular behavior of chitosan films. Drug Dev Ind Pharm 27(2):143–157
39. Zhang H, Neau SH (2001) In vitro degradation of chitosan by a commercial enzyme preparation: effect of molecular weight and degree of deacetylation. Biomaterials 22(12):1653–1658
40. Xu Y, Du Y (2003) Effect of molecular structure of chitosan on protein delivery properties of chitosan nanoparticles. Int J Pharm 250(1):215–226
41. Yuan Y, Chesnutt BM, Haggard WO, Bumgardner JD (2011) Deacetylation of chitosan: material characterization and in vitro evaluation via albumin adsorption and pre-osteoblastic cell cultures. Materials 4(8):1399–1416
42. Whithear KG, Bowtell DD, Ghiocas E, Hughes KL (1983) Evaluation and use of a micro-broth dilution procedure for testing sensitivity of fermentative avian mycoplasmas to antibiotics. Avian Dis 937–949
43. Fernandes JC, Tavaria FK, Soares JC, Ramos ÓS, Monteiro MJ, Pintado ME, Malcata FX (2008) Antimicrobial effects of chitosans and chit oligosaccharides, upon *Staphylococcus aureus* and *Escherichia coli*, in food model systems. Food Microbiol 25(7):922–928
44. Chang SH, Lin HTV, Wu GJ, Tsai GJ (2015) pH Effects on solubility, zeta potential, and correlation between antibacterial activity and molecular weight of chitosan. Carbohyd Polym 134:74–81
45. Andres Y, Giraud L, Gerente C, Le Cloirec P (2007) Antibacterial effects of chitosan powder: mechanisms of action. Environ Technol 28(12):1357–1363
46. Mellegård H, Strand SP, Christensen BE, Granum PE, Hardy SP (2011) Antibacterial activity of chemically defined chitosans: influence of molecular weight, degree of acetylation and test organism. Int J Food Microbiol 148(1):48–54

47. Shamaila S, Zafar N, Riaz S, Sharif R, Nazir J, Naseem S (2016) Gold nanoparticles: an efficient antimicrobial agent against enteric bacterial human pathogen. Nanomaterials 6(4):71
48. Zhang Y, Shareena Dasari TP, Deng H, Yu H (2015) Antimicrobial activity of gold nanoparticles and ionic gold. J Environ Sci Health 33(3):286–327
49. Katas H, Lim CS, Azlan AYHN, Buang F, Busra MFM (2019) Antibacterial activity of biosynthesized gold nanoparticles using biomolecules from *Lignosus rhinocerotis* and chitosan. Saudi Pharm J 27(2):283–292
50. Martinez-Castanon GA, Nino-Martinez N, Martinez-Gutierrez F, Martinez-Mendoza JR, Ruiz F (2008) Synthesis and antibacterial activity of silver nanoparticles with different sizes. J Nanopart Res 10(8):1343–1348
51. Li X, Robinson SM, Gupta A, Saha K, Jiang Z, Moyano DF, Rotello VM (2014) Functional gold nanoparticles as potent antimicrobial agents against multi-drug-resistant bacteria. ACS Nano 8(10):10682–10686
52. Rai A, Prabhune A, Perry CC (2010) Antibiotic mediated synthesis of gold nanoparticles with potent antimicrobial activity and their application in antimicrobial coatings. J Mater Chem 20(32):6789–6798
53. Gupta N, Rai DB, Jangid AK, Kulhari H (2019) Use of nanotechnology in antimicrobial therapy. Nanotechnology 46:143
54. Sportelli M, Izzi M, Volpe A, Clemente M, Picca R, Ancona A, Cioffi N (2018) The pros and cons of the use of laser ablation synthesis for the production of silver nano-antimicrobials. Antibiotics 7(3):67
55. Kalwar K, Shan D (2018) Antimicrobial effect of silver nanoparticles (AgNPs) and their mechanism—a mini review. Micro Nano Lett 13(3):277–280
56. Kalwar K, Hu L, Li DL, Shan D (2018) AgNPs incorporated on deacetylated electrospun cellulose nanofibers and their effect on the antimicrobial activity. Polym Adv Technol 29(1):394–400
57. Carpenter AW, Slomberg DL, Rao KS, Schoenfisch MH (2011) Influence of scaffold size on bactericidal activity of nitric oxide-releasing silica nanoparticles. ACS Nano 5(9):7235–7244
58. Musee N, Thwala M, Nota N (2011) The antibacterial effects of engineered nanomaterials: implications for wastewater treatment plants. J Environ Monit 3(5):1164–1183
59. Hegde K, Brar SK, Verma M, Surampalli RY (2016) Current understandings of toxicity, risks and regulations of engineered nanoparticles with respect to environmental microorganisms. J Nanosci Nanotechnol 1(1):5
60. Andrade-Silva LE, Ferreira-Paim K, Ferreira TB, Vilas-Boas A, Mora DJ, Manzato VM, Araujo NE (2018) Genotypic analysis of clinical and environmental *Cryptococcus neoformans* isolates from Brazil reveals the presence of VNB isolates and a correlation with biological factors. PLoS ONE 13(3):e0193237
61. Chauhan R, Reddy A, Abraham J (2015) Biosynthesis of silver and zinc oxide nanoparticles using *Pichia fermentans* JA2 and their antimicrobial property. Appl Nanosci 5(1):63–71
62. Xie Y, He Y, Irwin PL, Jin T, Shi X (2011) Antibacterial activity and mechanism of action of zinc oxide nanoparticles against *Campylobacter jejuni*. Appl Environ Microbiol 77(7):2325–2331
63. Siddiqi KS, Rahman A, Husen A (2018) Properties of zinc oxide nanoparticles and their activity against microbes. Nanoscale Res Lett 13(1):141
64. Elster C, Fourest E, Baudin F, Larsen K, Cusack S, Ruigrok RW (1994) A small percentage of influenza virus M1 protein contains zinc but zinc does not influence in vitro M1-RNA interaction. J Gen Virol 75(1):37–42
65. Lee SP, Xiao J, Knutson JR, Lewis MS, Han MK (1997) Zn^{2+} promotes the self-association of human immunodeficiency virus type-1 integrase in vitro. Biochemistry 36(1):173–180
66. Xie J, Li P, Li Y, Wang Y, Wei Y (2009) Morphology control of ZnO particles via aqueous solution route at low temperature. Mater Chem Phys 114(2–3):943–947
67. Klingshirn C (2007) ZnO: from basics towards applications. Phys Status Solidi B 244(9):3027–3073

68. Azam A, Ahmed AS, Oves M, Khan MS, Habib SS, Memic A (2012) Antimicrobial activity of metal oxide nanoparticles against gram-positive and gram-negative bacteria: a comparative study. Int J Nanomedicine 7:6003
69. Brayner R, Ferrari-Iliou R, Brivois N, Djediat S, Benedetti MF, Fiévet F (2006) Toxicological impact studies based on *Escherichia coli* bacteria in ultrafine ZnO nanoparticles colloidal medium. Nano Lett 6(4):866–870
70. Stoimenov PK, Klinger RL, Marchin GL, Klabunde KJ (2002) Metal oxide nanoparticles as bactericidal agents. Langmuir 18(17):6679–6686
71. Wahab R, Mishra A, Yun SI, Kim YS, Shin HS (2010) Antibacterial activity of ZnO nanoparticles prepared via non-hydrolytic solution route. Appl Microbiol Biotechnol 87(5):1917–1925
72. Hu X, Cook S, Wang P, Hwang HM (2009) In vitro evaluation of cytotoxicity of engineered metal oxide nanoparticles. Sci Total Environ 407(8):3070–3072
73. Azam A, Ahmed AS, Oves M, Khan MS, Habib SS, Memic A (2012) Antimicrobial activity of metal oxide nanoparticles against gram-positive and gram-negative bacteria: a comparative study. Int J Nanomed 7:6003
74. Beddow J, Stolpe B, Cole P, Lead JR, Sapp M, Lyons BP, Whitby C (2014) Effects of engineered silver nanoparticles on the growth and activity of ecologically important microbes. Environ Microbiol Rep 6(5):448–458
75. Choi O, Deng KK, Kim NJ, Ross L Jr, Surampalli RY, Hu Z (2008) The inhibitory effects of silver nanoparticles, silver ions, and silver chloride colloids on microbial growth. Water Res 42(12):3066–3074
76. Li M, Pokhrel S, Jin X, Mädler L, Damoiseaux R, Hoek EM (2010) Stability, bioavailability, and bacterial toxicity of ZnO and iron-doped ZnO nanoparticles in aquatic media. Environ Sci Technol 45(2):755–761
77. Luo Z, Chen Z, Qiu Z, Li Y, Du Laing G, Liu A, Yan C (2015) Gold and silver nanoparticle effects on ammonia-oxidizing bacteria cultures under ammoxidation. Chemosphere 120:737–742
78. Anaya NM, Solomon F, Oyanedel-Craver V (2016) Effects of dysprosium oxide nanoparticles on *Escherichia coli*. Environ Sci Nano 3(1):67–73
79. Liu LY, Sun L, Zhong ZT, Zhu J, Song HY (2016) Effects of titanium dioxide nanoparticles on intestinal commensal bacteria. Nucl Sci Tech 27(1):5
80. Hang MN, Gunsolus IL, Wayland H, Melby ES, Mensch AC, Hurley KR, Hamers RJ (2016) Impact of nanoscale lithium nickel manganese cobalt oxide (NMC) on the bacterium *Shewanella oneidensis* MR-1. Chem Mater 28(4):1092–1100
81. Yuan Z, Li J, Cui L, Xu B, Zhang H, Yu CP (2013) Interaction of silver nanoparticles with pure nitrifying bacteria. Chemosphere 90:1404–1411
82. Jiang C, Xu X, Meghary M, Naidu R, Chen Z (2015) Inhibition or promotion of biodegradation of nitrate by Paracoccus sp. in the presence of nanoscale zero-valent iron. Sci Tot Environ 530–531:241–246
83. Sharma D, Rajput J, Kaith BS, Kaur M, Sharma S (2010) Synthesis of ZnO nanoparticles and study of their antibacterial and antifungal properties. J Thin Solid Films 519:1224–1229
84. He L, Liu Y, Mustapha A, Lin M (2011a) Antifungal activity of zinc oxide nanoparticles against *Botrytis cinerea* and *Penicillium expansum*. Microbial Res 166:207–215
85. He SY, Feng YZ, Ren HX, Zhang Y, Ning G, Lin XG (2011) The impact of iron oxide magnetic nanoparticles on the soil bacterial community. J Soil Sediment 11:1408–1417
86. Warheit DB, Borm PJ, Hennes C, Lademann J (2007) Testing strategies to establish the safety of nanomaterials: conclusions of an ECETOC workshop. Inhal Toxicol 19:631–643
87. Lovern SB, Klaper R (2006) *Daphnia magna* mortality when exposed to titanium dioxide and fullerene (C-60) nanoparticles. Environ Toxicol Chem 25:1132–1137
88. Baun A, Sørensen SN, Rasmussen RF, Hartmann NB, Koch CB (2008) Toxicity and bioaccumulation of xenobiotic organic compounds in the presence of aqueous suspensions of aggregates of nano-C60. Das SK, Das AR, Guha AK (2009) Gold nanoparticles: microbial synthesis and application in water hygiene management. Langmuir 25:8192–8199

89. Roberts AP, Mount AS, Seda B, Souther J, Quio R, Lin S, Ke PC, Rao AM, Klaine SJ (2007) In vivo biomodification of lipid-coated carbon nanotubes by *Daphnia magna*. Environ Sci Technol 41:3025–3029
90. Gagné F, Auclair J, Turcotte P, Fournier M, Gagnona C, Sauvé S, Blaise C (2008) Ecotoxicity of CdTe quantum dots to freshwater mussels: impacts on immune system, oxidative stress and genotoxicity. Aquat Toxicol 86:333–340
91. Petersen EJ, Huang Q, Weber WJ (2008) Ecological uptake and depuration of carbon nanotubes by *Lumbriculus variegatus*. Environ Health Perspect 113:1–32
92. Das SK, Das AR, Guha AK (2009) Gold nanoparticles: microbial synthesis and application in water hygiene management. Langmuir 25:8192–8199
93. Nowack B, Krug HF, Height M (2011) 120 years of nanosilver history: implications for policy makers. Environ Sci Technol 45(4):1177–1183
94. Wang C, Liu L-L, Zhang A-T, Xie P, Lu J-J, Zou X-T (2012) Antibacterial effects of zinc oxide nanoparticles on *Escherichia coli* K88. Afr J Biotechnol 11:10248–10254
95. Liu Y, He L, Mustapha A, Li H, Hu ZQ, Lin M (2009) Antibacterial activities of zinc oxide nanoparticles against *Escherichia coli* O157:H7. J Appl Microbiol 107:1193–1201
96. Soderberg TA, Sunzel B, Holm S, Elmros T, Hallmans G, Sjoberg S (1990) Antibacterial effect of zinc oxide in vitro. Scand J Plast Reconstr Surg Hand Surg 24:193–197
97. Hernandez-Sierra J, Ruiz F, Pena D, Martinez-Gutierrez F, Martinez AE, Guillen AJ, Tapia-Perez H, Castanon GM (2008) The antimicrobial sensitivity of *Streptococcus mutans* to nanoparticles of silver, zinc oxide, and gold. Nanomed-Nanotechnol 4:237–240
98. Rousk J, Ackermann K, Curling SF, Jones DL (2012) Comparative toxicity of nanoparticulate CuO and ZnO to soil bacterial communities. PLoS ONE 7(3):e34197
99. Durenkamp M, Pawlett M, Ritz K, Harris JA, Neal AL, McGrath SP (2016) Nanoparticles within WWTP sludge have minimal impact on leachate quality and soil microbial community structure and function. Environ Pollut 211:399–405
100. Read DS, Matzke M, Gweon HS, Newbold LK, Heggelund L, Ortiz MD, Lahive E, Spurgeon D, Svendsen C (2016) Soil pH effects on the interactions between dissolved zinc, non-nano- and nano-ZnO with soil bacterial communities. Environ Sci Pollut Res 23:4120–4128
101. Tripathi DK, Tripathi A, Singh S, Singh Y, Vishwakarma K, Yadav G, Dubey NK (2017) Uptake, accumulation and toxicity of silver nanoparticle in autotrophic plants, and heterotrophic microbes: a concentric review. Front Microbiol 8:7
102. Strigul N, Vaccari L, Galdun C, Wazne M, Liu X, Christodoulatos C, Jasinkiewicz K (2009) Acute toxicity of boron, titanium dioxide, and aluminum nanoparticles to *Daphnia magna* and *Vibrio fischeri*. Desalination 248:771–782
103. Barrena R, Casals E, Colon J, Font X, Sanchez A, Puntes V (2009) Evaluation of the ecotoxicity of model nanoparticles. Chemosphere 75:850–857
104. Zhai Y, Hunting ER, Wouters M, Peijnenburg WJGM, Vijver MG (2016) Silver nanoparticles, ions, and shape governing soil microbial functional diversity: nano shapes micro. Front Microbiol 7:1123–1132
105. Simonin M, Martins JMF, Uzu G, Vince E, Richaume A (2016) Combined study of titanium dioxide nanoparticle transport and toxicity on microbial nitrifying communities under single and repeated exposures in soil columns. Environ Sci Technol 50:10693–10699
106. Crane M, Handy RD, Garrod J, Owen R (2008) Ecotoxicity test methods and environmental hazard assessment for engineered nanoparticles. Ecotoxicology 17(5):421
107. Keller AA, Mcferran S, Lazareva A, Suh S (2013) Global life cycle releases of engineered nanomaterials. J Nanopart Res 15:1692
108. Baptista MS, Miller RJ, Halewood ER, Hanna SK, Almeida CMR, Vasconcelos VM, Keller AA, Lenihan HS (2015) Impacts of silver nanoparticles on a natural estuarine plankton community. Environ Sci Technol 49:12968–12974
109. Doran JW, Parkin TB (1996) Quantitative indicators of soil quality: a minimum data set. SSSA Spec Publ 49:25–38
110. Judy JD, Kirby JK, Creamer C, McLaughlin MJ, Fubiger C, Wright C, Cavagnaro TR, Bertsch PM (2015) Effect of silver sulfide nanomaterials on mycorrhizal colonization of tomato plants and soil microbial communities on biosolid-amended soil. Environ Pollut 206:256–263

111. Godwin HA, Chopra K, Bradley KA, Cohen Y, Harthorn BH, Hoek EM, Holden P, Keller AA, Lenihan HS, Nisbet RM (2009) The university of california center for the environmental implications of nanotechnology. Environ Sci Technol 43:6453–6457
112. Hristozov D, Malsch I (2009) Hazards and risks of engineered nanoparticles for the environment and human health. Sustainability 1:1161–1194
113. Simonin M, Guyonnet JP, Martins JM, Ginot M, Richaume A (2015) Influence of soil properties on the toxicity of TiO_2 nanoparticles on carbon mineralization and bacterial abundance. J Hazard Mater 283:529–535
114. Xu C, Peng C, Sun L, Zhang S, Huang H, Chen Y, Shi J (2015) Distinctive effects of TiO_2 and CuO nanoparticles on soil microbes and their community structures in flooded paddy soil. Soil Biol Biochem 86:24–33
115. Kizilkaya R, Bayrakli B (2005) Effects of N-enriched sewage sludge on soil enzyme activities. Appl Soil Ecol 30:192–202
116. Simonin M, Richaume A, Guyonnet JP, Dubost A, Martins JM, Pommier T (2016) Titanium dioxide nanoparticles strongly impact soil microbial function by affecting archaeal nitrifiers. Sci Rep 6:33643
117. Aengenheister L, Dugershaw BB, Manser P, Wichser A, Schoenenberger R, Wick P, Hesler M, Kohl Y, Straskraba S, Suter MJ, Buerki-Thurnherr T (2019) Investigating the accumulation and translocation of titanium dioxide nanoparticles with different surface modifications in static and dynamic human placental transfer models. Eur J Pharm Biopharm 142:488–497
118. Lahive E, Matzke M, Durenkamp M, Lawlor AJ, Thacker SA, Pereira MG, Spurgeon DJ, Unrine JM, Svendsen C, Lofts S (2017) Sewage sludge treated with metal nanomaterials inhibits earthworm reproduction more strongly than sludge treated with metal metals in bulk/salt forms. Environ Sci-Nano 4(1):78–88
119. Asadishad B, Chahal S, Akbari A, Cianciarelli V, Azodi M, Ghoshal S, Tufenkji N (2018) Amendment of agricultural soil with metal nanoparticles: effects on soil enzyme activity and microbial community composition. Environ Sci Technol 52:1908–1918
120. Loureiro S, Tourinho PS, Cornelis G, Van Den Brink NW, Díez-Ortiz M, Vázquez-Campos S, Van Gestel CA (2018) Nanomaterials as soil pollutants. In: Soil pollution. Academic Press, pp 161–190
121. Ma R, Levard C, Judy JD, Unrine JM, Durenkamp M, Martin B, Lowry GV (2013) Fate of zinc oxide and silver nanoparticles in a pilot wastewater treatment plant and in processed biosolids. Environ Sci Technol 48:104–112
122. El-Temsah YS, Joner EJ (2012) Impact of Fe and Ag nanoparticles on seed germination and differences in bioavailability during exposure in aqueous suspension and soil. Environ Toxicol 27(1):42–49
123. Cox A, Venkatachalam P, Sahi S, Sharma N (2016) Silver and titanium dioxide nanoparticle toxicity in plants: a review of current research. Plant Physiol Biochem 107:147–163
124. Nair PM, Chung IM (2014) Assessment of silver nanoparticle-induced physiological and molecular changes in *Arabidopsis thaliana*. Environ Sci Pollut Res Int 21(14):8858–8869
125. Nair PM, Chung IM (2015) Physiological and molecular level studies on the toxicity of silver nanoparticles in germinating seedlings of mung bean (*Vigna radiata* L.). Acta Physiol Plant 37(1):1719
126. You T, Liu D, Chen J, Yang Z, Dou R, Gao X, Wang L (2018) Effects of metal oxide nanoparticles on soil enzyme activities and bacterial communities in two different soil types. J Soil Sediment 18:211–221
127. Zhang M, Yang J, Cai Z, Feng Y, Wang Y, Zhang D, Pan X (2019) Detection of engineered nanoparticles in aquatic environments: current status and challenges in enrichment, separation, and analysis. Environ Sci-Nano 6(3):709–735
128. Peng C, Zhang W, Gao H, Li Y, Tong X, Li K, Chen Y (2017) Behavior and potential impacts of metal-based engineered nanoparticles in aquatic environments. Nanomaterials 7:21–25
129. Miao AJ, Zhang XY, Luo Z, Chen CS, Chin WC, Santschi PH, Quigg A (2010) Zinc oxide–engineered nanoparticles: dissolution and toxicity to marine phytoplankton. Environ Toxicol Chem 29(12):2814–2822

130. Majedi SM, Kelly BC, Lee HK (2014) Role of combinatorial environmental factors in the behavior and fate of ZnO nanoparticles in aqueous systems: a multiparametric analysis. J Hazard Mater 264:370–379
131. Griffitt RJ, Luo J, Gao J, Bonzongo JC, Barber DS (2008) Effects of particle composition and species on toxicity of metallic nanomaterials in aquatic organisms. Environ Toxicol Chem 27(9):1972–1978
132. Qin YK, Guo XP, Tou FY, Pan H, Feng JN, Xu J, Chen B, Liu M, Yang Y (2017) Cytotoxicity of TiO_2 nanoparticles toward *Escherichia coli* in an aquatic environment: effects of nanoparticle structural oxygen deficiency and aqueous salinity. Environ Sci Nano 4(5):1178–1188
133. Aruoja V, Dubourguier HC, Kasemets K, Kahru A (2009) Toxicity of nanoparticles of CuO, ZnO and TiO2 to microalgae *Pseudokirchneriella subcapitata*. Sci Total Environ 407:1461–1468
134. Zhang L, Li J, Yang K, Liu J, Lin D (2016) Physicochemical transformation and algal toxicity of engineered nanoparticles in surface water samples. Environ Pollut 211:132–140
135. Griffitt RJ, Luo J, Gao J, Bonzongo JC, Barber DS (2008) Effects of particle composition and species on toxicity of metallic nanomaterials in aquatic organisms. Environ Toxicol Chem 27:1972–1978
136. Vicario-Parés U, Lacave JM, Reip P, Cajaraville MP, Orbea A (2018) Cellular and molecular responses of adult zebrafish after exposure to CuO nanoparticles or ionic copper. Ecotoxicology 27:89–101
137. Sharma VK (2009) Aggregation and toxicity of titanium dioxide nanoparticles in aquatic environment—a review. J Environ Sci Health A 44(14):1485–1495
138. Dimkpa CO, Calder A, Gajjar P, Merugu S, Huang W, Britt DW, McLean JE, Johnson WP, Anderson AJ (2011) Interaction of silver nanoparticles with an environmentally beneficial bacterium, *Pseudomonas chlororaphis*. J Hazard Mater 188(1–3):428–435
139. Adeleye AS, Conway JR, Perez T, Rutten P, Keller AA (2014) Influence of extracellular polymeric substances on the long-term fate, dissolution, and speciation of copper-based nanoparticles. Environ Sci Technol 48(21):12561–12568
140. Ali D, Alarifi S, Kumar S, Ahamed M, Siddiqui MA (2012) Oxidative stress and genotoxic effect of zinc oxide nanoparticles in freshwater snail *Lymnaea luteola* L. Aquat Toxicol 124:83–90
141. Mahaye N, Thwala M, Cowan DA, Musee N (2017) Genotoxicity of metal based engineered nanoparticles in aquatic organisms: a review. Mutat Res-Rev Mutat 773:134–160
142. John AC, Küpper M, Manders-Groot AM, Debray B, Lacome JM, Kuhlbusch TA (2017) Emissions and possible environmental implication of engineered nanomaterials (ENMs) in the atmosphere. Atmosphere 8(5):84
143. Nazaries L, Pan Y, Bodrossy L, Baggs EM, Millard P, Murrell JC, Singh BK (2013) Evidence of microbial regulation of biogeochemical cycles from a study on methane flux and land use change. Appl Environ Microbiol 79(13):4031–4040
144. Rousk J, Bengtson P (2014) Microbial regulation of global biogeochemical cycles. Front Microbiol 5:103
145. Philippot L, Raaijmakers JM, Lemanceau P, van der Putten WH (2013) Going back to the roots: the microbial ecology of the rhizosphere. Nat Rev Microbiol 11:789–799
146. Hegde K, Brar SK, Verma M, Surampalli RY (2016) Current understandings of toxicity, risks and regulations of engineered nanoparticles with respect to environmental microorganisms. Nanotechnol Environ Eng 1:5–11
147. Dimkpa CO, Zeng J, McLean JE, Britt DW, Zhan J, Anderson AJ (2012) Production of indole-3-acetic acid via the indole-3-acetamide pathway in the plant-beneficial bacterium *Pseudomonas chlororaphis* O6 is inhibited by ZnO nanoparticles but enhanced by CuO nanoparticles. Appl Environ Microbiol 78(5):1404–1410
148. Shen Z, Chen Z, Hou Z, Li T, Lu X (2015) Ecotoxicological effect of zinc oxide nanoparticles on soil microorganisms. Front Environ Sci Eng 9(5):912–918
149. Kucerka N, Papp-Szabo E, Nieh MP, Harroun TA, Schooling SR, Pencer J, Katsaras J (2008) Effect of cations on the structure of bilayers formed by lipopolysaccharides isolated from *Pseudomonas aeruginosa* PAO1. J Phys Chem B 112(27):8057–8062

150. Eduok S, Hendry C, Ferguson R, Martin B, Villa R, Jefferson B, Coulon F (2015) Insights into the effect of mixed engineered nanoparticles on activated sludge performance. FEMS Microbiol Ecol 91(7)
151. Lecoanet HF, Wiesner MR (2004) Velocity effects on fullerene and oxide nanoparticle deposition in porous media. Environ Sci Technol 38(16):4377–4382
152. Yavuz CT, Mayo JT, William WY, Prakash A, Falkner JC, Yean S, Cong L, Shipley HJ, Kan A, Tomson M, Natelson D (2006) Low-field magnetic separation of monodisperse Fe_3O_4 nanocrystals. Science 314(5801):964–967
153. Mitrano DM, Motellier S, Clavaguera S, Nowack B (2015) Review of nanomaterials aging and transformation through the life cycle of nano-enhanced products. Environ Int 77:132–147
154. Taylor AA, Marcus IM, Guysi RL, Walker SL (2015) Metal oxide nanoparticles induce minimal phenotypic changes in a model colon gut microbiota. Environ Eng Sci 32:602–612
155. Frenk S, Ben-Moshe T, Dror I, Berkowitz B, Minz D (2013) Effect of metal oxide nanoparticles on microbial community structure and function in two different soil types. PLoS ONE 8(12):e84441
156. Chen KL, Elimelech M (2007) Influence of humic acid on the aggregation kinetics of fullerene (C-60) nanoparticles in monovalent and divalent electrolytic solutions. J Colloid Interface Sci 309:126–134
157. Li F, Wu J, Qin Q, Li Z, Huang X (2010) Controllable synthesis, optical and photocatalytical properties of CuS nanomaterials with hierarchical structures. Powder Technol 198:267–274
158. Sondi I, Salopek-Sondi B (2004) Silver nanoparticles as antimicrobial agent: a case study on *E. coli* as a model for gram-negative bacteria. J Colloid Interface Sci 275:177–182
159. Jin T, Sun D, Su JY, Zhang H, Sue HJ (2009) Antimicrobial efficacy of zinc oxide quantum dots against *Listeria monocytogenes*, *Salmonella enteritidis* and *Escherichia coli* 0157:H7. J Food Sci 74:46–52
160. Ajitha B, Reddy YA, Reddy PS, Jeon HJ, Ahn CW (2016) Role of capping agents in controlling silver nanoparticles size, antibacterial activity and potential application as optical hydrogen peroxide sensor. RSC Adv 6(42):36171–36179
161. Rossi LM, Fiorio JL, Garcia MA, Ferraz CP (2018) The role and fate of capping ligands in colloidally prepared metal nanoparticle catalysts. Dalton Trans 47(17):5889–5915
162. Wang F, Yao J, Liu H, Liu R, Chen H, Yi Z, Yu Q, Ma L, Xing B (2015) Cu and Cr enhanced the effect of various carbon nanotubes on microbial communities in an aquatic environment. J Hazard Mater 137–145
163. García A, Delgado L, Torà JA, Casals E, González E, Puntes V, Font X, Carrera J, Sánchez A (2012) Effect of cerium dioxide, titanium dioxide, silver, and gold nanoparticles on the activity of microbial communities intended in wastewater treatment. J Hazard Mater 199:64–72