Juan Rodríguez-Hernández

Polymers against Microorganisms

On the Race to Efficient Antimicrobial Materials

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Preface

Bacterial contamination is still an unresolved problem present in cases in which a biomaterial is required. This is an issue independent of the biomaterial considered and is particularly serious in those cases in which long-term implants are employed. In this context, polymers have been proposed as interesting candidates to improve the biomaterial performance in order to prevent microbial contamination. Different previous books have been published focusing their efforts on one of the aspects of antimicrobial polymers: the synthesis, in the biology of the microorganisms in contact with synthetic materials or related to their final use (e.g., food packaging). This book aims to present a complete overview of this rapidly evolving field providing a concise, clear, and precise image of the most important aspects involved in the use of polymers to combat microorganisms.

As will be depicted throughout this book, polymers' mode of action relies on physiochemical parameters such as hydrophobicity and cationic charge, rather than specific receptor-mediated interactions, so the activity of the polymers can be modulated by tuning key structural parameters. Taking into account the mechanism of action, polymers exhibit important advantages that have motivated their investigation as antibacterial materials. These include that polymers do not provide toxicity to the environment, do not develop resistance, and have an enhanced antimicrobial action. Other important advantages are their versatility; polymers are easy to process and cheap.

I hope that this text will be helpful for readers with very different backgrounds, ranging from chemists, biochemists, materials scientists, and engineers, who aim to have a general and complete overview of the use of polymers in the preparation of antimicrobial materials. This book is not presented as a manual and will not provide answers to all possible questions about polymers with antimicrobial properties. On the contrary, this book is intended to provide an introductory view highlighting important aspects including synthesis, surface functionalization and structuration, and the extension of these important aspects to the preparation of antimicrobial fibers, hydrogels, or membranes among others.

This text, devoted to the recent developments and ongoing works concerning the use of polymers as antifouling and antimicrobials for different applications, is organized as follows. The first part of this book (Chaps. 2 and 3) describes the basics of bacterial infections and the main functional groups incorporated into polymeric structures to avoid microorganism contamination. Chapter 4 depicts the use of nanostructured polymer assemblies in solution as antimicrobials.

The design and fabrication of polymer surfaces is analyzed in Chaps. 5 and 6. Chapter 5 discusses the alternatives to modify the surface chemical composition in order to introduce both antifouling and/or antimicrobial functional groups. Chapter 6 concerns those approaches that resort to both the modification of the surface topography and those that combine surface functionalization and patterning to remove bacterial contamination and biofilm formation.

Chapters 7, 8, and 9 are devoted to the use of antimicrobial polymers for the elaboration of three different materials. The approaches developed for the fabrication of nano- and microstructured fibers are depicted in Chap. 7. In Chapter 8, the synthesis and modification of hydrogels to improve the bacterial adhesion and to introduce antimicrobial moieties are described. Finally, Chap. 9 focuses on the elaboration of membranes with enhanced antifouling properties.

The last part of this book will analyze the eventual environmental concerns as well as safety issues related to the use of nanoparticles. The last chapter will summarize the future trends on the development of more sophisticated and effective antimicrobial polymer systems.

Madrid, Spain Juan Rodríguez-Hernández

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Contents

Chapter 1
Polymers Against Microorganisms

Abstract The significant advances on the control and prevention of infectious diseases carried out during the first decades of the twentieth century produced an optimist sensation about the possibility to completely eradicate any illness. But this optimistic vision rapidly changed as a result of the reemerging of new and in some cases antimicrobial-resistant infections. Examples of the novel/old/appearing/reappearing infectious diseases include the Ebola virus, HIV, or Legionnaire's disease that are still a public health problem in the twenty-first century.

Within this context, two main aspects have deserved particular attention during the last decades. On the one hand, food-borne diseases are directly related to the emergence of microbial diseases. On the other hand, the emergence of antimicrobial resistance, recognized soon after the discovery of penicillin, has followed the introduction of most every new drug. As will be depicted in this chapter, synthetic macromolecular antimicrobials have emerged as a highly promising class of therapeutics with immense potential for combating multidrug-resistant microbes. In effect, the polymers mode of action relies on physiochemical parameters such as hydrophobicity and cationic charge, rather than specific receptor-mediated interactions, the activity of the polymers can be modulated by tuning key structural parameters. Taking into account the action mechanism, polymers exhibit in comparison with other materials, important advantages that have motivated their investigation as antibacterial materials. These include that polymers do not provide toxicity to the environment, do not develop resistance, and have an enhanced antimicrobial action.

Keywords Bacterial resistance • Infectious disease • Antibiotics • Implants • Antimicrobial polymers

1.1 Infectious Diseases: Historical Context

The significant advances on the control and prevention of infectious diseases carried out during the first decades of the twentieth century produced an optimist sensation about the possibility to completely eradicate any illness [1]. Prominent scientists of that time such as Henry Sigerist [2] and later others including William H. Stewart [3] anticipated that those advances will be the key to definitely prevent infection.

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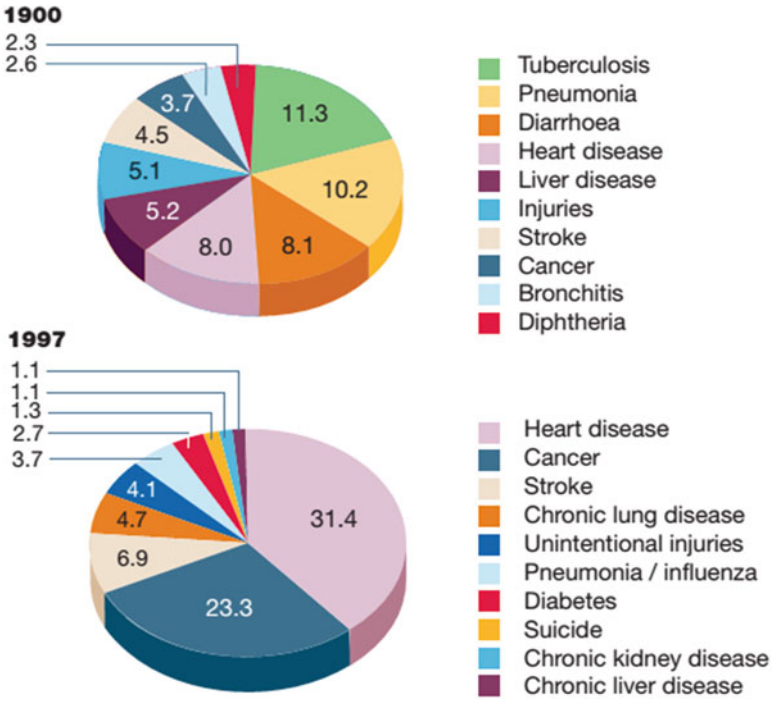

Fig. 1.1 Leading causes of deaths in the USA in 1900 and 1997. Reproduced with permission from [1]

Pioneer and priori successful studies carried out by Sigerist and coworkers during the first three decades concluded in an extermination of many illnesses and control of many others. Later developments we carried out on the fabrication of novel antibiotics. These antibiotics were successfully employed between 1940 and 1960 and further developed by pharmaceutical companies during the following decades. However, in the 1980s the tendency varied and pharmaceutical companies started to reduce the development of new drugs or redirecting it away from antibiotics [1, 4, 5].

But this optimistic vision rapidly changed as a result of the reemerging of new and in some cases antimicrobial-resistant infections. Examples of the novel/old/appearing/reappearing infectious diseases include the Ebola virus, HIV, or Legionnaire's disease that are still a public health problem in the twenty-first century. The evolution of the infectious disease patterns has been thoroughly described by Cohen [1]. As mentioned in its review, at the beginning of the twentieth century, infectious diseases were the leading cause of death worldwide. In particular, in the USA, only three diseases (tuberculosis, diarrhoeal disease, and pneumonia) were the cause of 30 % of deaths (Fig. 1.1).

However, by the end of the twentieth century, in most of the developed world, mortality from infectious diseases had been replaced by mortality from chronic

illnesses such as heart disease, cancer, and stroke. This situation enhanced the average life span that had increased by about 60 % to more than 76 years [6].

While this is, a priori, true for the already developed countries, those under development do still have a serious problem with infectious diseases which are still the major cause of morbidity and mortality. According to the World Health Organization, the infectious diseases caused over 13 million deaths that correspond to a quarter of the deaths worldwide [7]. In particular, three diseases are the most common: pneumonia (3.5 million), diarrhoeal disease (2.2 million) and tuberculosis (1.5 million) [1]. Interestingly, these diseases were common on the developed world at the beginning of the twentieth century.

However, as depicted by Cohen [1] in both developed and developing worlds exhibit new microorganisms and infectious diseases have been recognized. These include toxic shock syndrome, Lyme disease, HIV, Helicobacter pylori, Nipah virus, flesh-eating bacteria, or Legionnaire's disease just to mention a few of them. Moreover, some of these infectious diseases are nowadays the origin of other chronic illnesses. For instance, Helicobacter pylori has been evidenced to be at the origin of peptic ulcers.

It is also important to note that new infectious agents had the potential for rapid international spread. This is for instance the case of Ebola or Marburg virus. Other, on the contrary, dengue fever and in spite of their apparently easier control they were reemerging. This is the case of yellow fever or malaria.

In effect, in addition to the appearance of new microorganisms, the reemerging of old infections as a result of resistance to antimicrobial agents is currently a serious global problem. This involves both developing and developed countries. For instance, in the USA from 1981 to 1995, this increase was at a rate of 4.8 % per year from 36 to 63 deaths per 100,000 [8].

Within this context, two main aspects have deserved particular attention during the last decades. On the one hand, food-borne diseases are directly related to the emergence of microbial diseases. According to the IOM [9]: "The potential for foods to be involved in the emergence or reemergence of microbial threats to health is high, in large part because there are many points at which food safety can be compromised." On the other hand, the emergence of antimicrobial resistance, recognized soon after the discovery of penicillin, has followed the introduction of every new drug. As a result, the IOM [9] reported that: "Microbes that once were easily controlled by antimicrobial drugs are, more and more often, causing infections that no longer respond to treatment with these drugs." The seriousness of this situation has increased during the twenty-first century and today antimicrobial resistance is a serious problem. Some examples of antimicrobial-resistant microbes are depicted in Table 1.1.

As mentioned above, the effect of bacterial infections significantly decreased with penicillin that became available for use in the early 1940s. In that and the following decades, small molecular weight antibiotics were used as efficient antimicrobial agents. As has been clearly explained by Ganewatta et al. [10], the targets of these antimicrobial molecules typically involved cell membranes, biosynthetic pathways, 60S ribosomes, cell wall, or genetic materials (Fig. 1.2).

Table 1.1 Antimicrobial-resistant microbes affecting treatment and control of infectious diseases in the twenty-first century

Hospital-acquired infections
Methicillin-resistant staphylococci
Vancomycin-resistant staphylococci
Vancomycin-resistant enterococci
ESC-resistant Gram-negative bacteria
Azole-resistant *Candida*
Community-acquired infections
Multidrug-resistant pneumococci
FQ- and ESC-resistant *Salmonella* (including *S. typhi*)
Multidrug-resistant *Shigella* (including *Shig. dysenteriae*)
FQ-resistant gonococci
Multidrug-resistant *M. tuberculosis*
Drug-resistant malaria
Drug-resistant HIV

Reproduced with permission from [1]
ESC extended-spectrum cephalosporin (e.g., ceftriaxone or cefotaxine), *FQ* fluoroquinolone (e.g., ciprofloxacin)

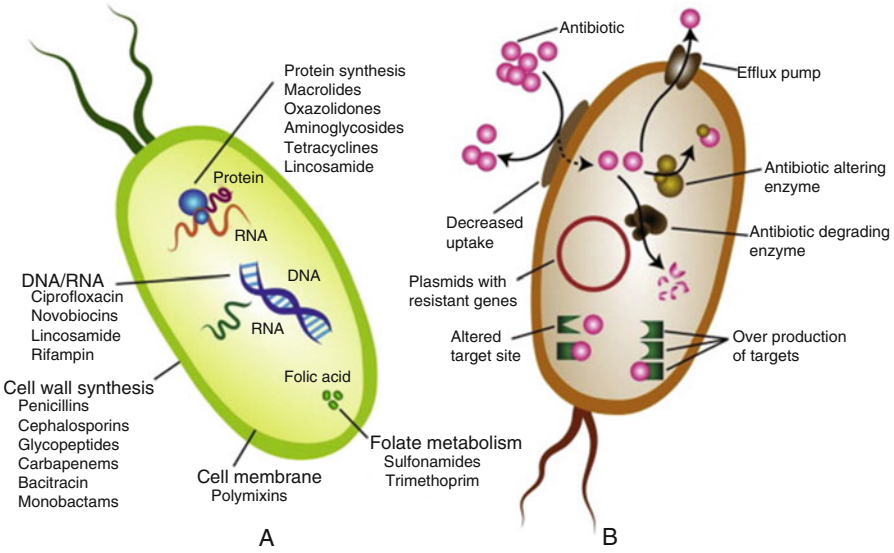

Fig. 1.2 Schematic representation of antibiotic action in bacterial cells (**a**) and the resulting mechanisms developed by bacteria for antibiotic resistance (**b**). Reproduced with permission from [10]

However, bacteria rapidly responded by exhibiting various forms of resistance. It is worth mentioning that some species of bacteria are innately resistant to one or more classes of antimicrobial agents. In such cases, all strains of that bacterial species are likewise resistant to all the members of those antibacterial classes. However, of greater concern are cases of acquired resistance, where initially susceptible populations of bacteria become resistant to an antibacterial agent and proliferate and spread under the selective pressure of use of that agent [11].

The level and complexity of the resistance mechanisms in bacteria has been developed with the usage of antibiotics and in spite of the large amount of work devoted to develop new antibacterial agents, bacteria evolve equally with novel and smarter mechanisms [12–14].

For example, Gram-positive *Staphylococcus aureus* has evolved from penicillin-resistant phenotypes into a methicillin-resistant strain (MRSA), which has become a global epidemic. Out of the wide range of antibiotic ammunitions in clinics, the MRSA strain is known to be only susceptible toward vancomycin treatment. More worrisome to this is the fact that as soon as vancomycin was used to treat MRSA infections, vancomycin-resistant *S. aureus* was identified in a controlled healthcare setting. It is apparent that there is an urgent need for new antimicrobial agents that are not easily susceptible to resistance.

Bacterial resistance is a major concern for different reasons as reported by Tenover [11]. First of all, healthcare institutions may be a commonplace for resistant bacteria. These can include *Klebsiella pneumoniae*, *staphylococci*, *enterococci*, and *Pseudomonas* spp. [15–19]. In addition, bacterial resistance finally leads to treatment failure. Inadequate antibacterial therapies are, in turn, associated with increased mortality rates in patients with bloodstream infections due to resistant *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *K pneumoniae*, *Escherichia coli*, *Enterobacter* spp., *coagulase-negative staphylococci*, and *enterococci* [20, 21].

Three additional aspects require also consideration. First of all, the use of prolonged therapy employing antimicrobial agents, such as vancomycin or linezolid, can induce the development of low-level resistance that compromises therapy. Secondly, the spread of resistant bacteria in a particular environment poses additional difficulties to control the infection a task that is complicated by the increased mobility of our population. Actually, resistant bacteria can spread leading to broader infection-control problems. Examples of clinically important bacteria increasingly observed include methicillin-resistant *S. aureus* (MRSA) [22] and extended-spectrum-lactamase (ESBL)-producing *E. coli* [23, 24]. As a result of the spreading of resistant bacteria, infected individuals, including children, often lack identifiable risk factors for MRSA, and appear to have acquired their infections in a variety of community settings [25, 26].

Finally, the third important aspect in bacterial resistance is related to the associated costs. Although its full economic impact remains to be determined, antibacterial drug resistance places an added burden on healthcare costs [27].

In conclusion, the emergence of antibiotic resistance has mitigated most of the benefits of using antibiotics. In this context, as will be depicted throughout this book, antibacterial polymers exhibit interesting properties that allow them to avoid antibiotic resistance.

1.1.1 Mechanisms of Resistance to Antibacterial Agents

As depicted in Fig. 1.2, bacteria may manifest resistance to antibacterial drugs through four main mechanisms [11]:

- The first mechanism involves the acquisition of genes encoding enzymes by the organism. These enzymes (for instance, lactamases) are able to destroy the antibacterial agent thus avoiding their activity.
- The second alternative involves the formation of efflux pumps in the bacteria that extrude the antibacterial agent from the cell before it can reach its target site.
- The third possible mechanism resort to the acquisition by bacteria of genes for a metabolic pathway which finally leads to modified bacterial cell walls in which either the binding site of the antimicrobial agent has been removed, or mutations acquired by the bacteria limit the access of antimicrobial agents to the intracellular target site.
- Finally, as reported by Tenover et al. [11] the last mechanism can occur through one or combining several genetic mechanisms, including conjugation, transformation, or transduction. Through genetic exchange mechanisms, many bacteria have become resistant to multiple classes of antibacterial agents, and these bacteria with multidrug resistance (defined as resistance to three antibacterial drug classes) have become a cause for serious concern, particularly in hospitals and other healthcare institutions where they tend to occur most commonly.

As has been mentioned one of the most important mechanism developed in bacteria to acquire resistance to one or more antimicrobial agents is via new mutations [28]. Four different causes may be at the origin of resistance: (1) upregulating the production of enzymes that inactivate the antimicrobial agent (e.g., erythromycin ribosomal methylase in *staphylococci*), (2) altering the target protein to which the antibacterial agent binds by modifying or eliminating the binding site (e.g., change in penicillin-binding protein 2b in pneumococci, which results in penicillin resistance), (3) upregulating pumps that expel the drug from the cell (efflux of fluoroquinolones in *S. aureus*), or (4) downregulating or altering an outer membrane protein channel that the drug requires for cell entry (e.g., OmpF in *E. coli*) [28].

As a result of these processes, two types of bacterial evolution have been identified. On the one hand, *vertical evolution* is related to the acquired resistance that develops due to chromosomal mutation and selection. In all of these cases, strains of bacteria carrying resistance-conferring mutations are selected by antimicrobial use, which kills the susceptible strains but allows the newly resistant strains to survive and grow.

Horizontal evolution occurs when the resistance is developed through the acquisition of new genetic material from other resistant organisms. This evolution can occur between strains of the same species or between different bacterial species or genera. Mechanisms of genetic exchange include conjugation, transduction, and transformation [28].

In summary, as reported by McManus et al. [28] mutation and selection, together with the mechanisms of genetic exchange, enable many bacterial species to adapt quickly to the introduction of antibacterial agents into their environment. Although a single mutation in a key bacterial gene may only slightly reduce the susceptibility of the host bacteria to that antibacterial agent, it may be just enough to allow its initial survival until it acquires additional mutations or additional genetic information resulting in full-fledged resistance to the antibacterial agent.

1.2 Implant-Associated Infections

A today's crucial issue in materials applications for biorelated purposes concerns the contamination by microorganisms and in particular bacteria. In effect, this problem affects many different areas ranging from such as medical devices, healthcare products, water purification systems, hospitals, dental office equipment, food packaging, food storage, household sanitation, just to mention a few of them [29].

Bacterial contamination is still a common unresolved problem present in the major cases in which a biomaterial is required. While this is a general problem present independently of the biomaterial considered, it is even more serious in those cases in which long-term implants are employed. For instance, long-term catheters can produce implant-associated infections. Particularly critical are those cases in which the infections become resistant to antibiotics (those cases in which biofilm is already produced), and the implant need to be removed. Depending on the implant and the infection produced by the bacteria, the situation can be even critical since the antibiotics cannot be effectively delivered. The impact of implant failures on the entire population and on the costs for the national health systems is enormous. This impact is particularly significant for septic failures, when microbial infections develop on biomaterial surfaces. Following an initial colonization, bacterial biofilms develop and establish on contaminated surfaces, critically compromising the functionality and performance of the implant itself, recruiting inflammatory cells, affecting the integration in the surrounding tissues, but also posing the patient at serious risk of systemic infections, septicemia when not even death. More important, once a mature bacterial biofilm has established, conventional medical therapies based on systemic antibiotics are not efficacious and implant removal often represents the only chance to eradicate the infection.

While this is true, biomedical devices are an essential aspect of the human healthcare system. Over the past three decades, the number of artificial hip and knee implants has increased markedly, and stents, heart valves, vascular grafts, and other implants devices have been used widely to save lives and to restore the quality of life for many people. For instance, according to the Freedonia Group, the demand in the USA for implantable medical devices is projected to rise 7.7 % annually to $52B in 2015 [30]. Polymers are likely to be the fastest growing category among all segments during 2013–2019, owing to rising applications of these biomaterials and various benefits over metals that include elasticity, flexibility, biocompatibility,

bio-inertness, and longevity. The usage of polymers such as polyurethanes and polytetrafluoroethylene (PTFE) is being popular for synthetic vascular grafts, whereas the conventional use of polymers in ophthalmology is estimated to grow with increasing numbers of ophthalmic disorders and continuous demand from geriatrics.

In order to reduce the risk of infection in implantable materials, different strategies have been proposed. On the one hand, a large effort has been done in terms of prevention. However, a point has currently been reached where significant efforts to tighten asepsis control result in just a relatively low advantage in terms of reduction in the rate of infections. At present, there is not a single strategy based on prevention that could totally eliminate the incidence of infections associated to biomaterials. On the other hand, along with all these preventive measures that are currently applied, an important strategy that has progressively been gaining ground over the years is the use of biomaterials that are less susceptible or even resistant to bacterial infections. Such biomaterials include, among others, materials with self-sterilizing (or, more appropriately, self-disinfecting) surfaces. Typically, such surfaces have been designed to contain antimicrobial drugs that are then delivered locally. In some cases, this strategy has been proven useful to clear and eradicate preexisting infections. Although chemically based bactericidal mechanisms are known to be effective, the duration and specificity of any particular chemical antibacterial mechanism is still a limitation. As alternative to these approaches, several groups have described bactericidal surfaces that have the capability of killing any bacteria that come into contact with them. These studies are based both on novel functional groups and also the surface structure (nano- or microscale features).

1.3 The Use of Macromolecules as Antimicrobials

In contrast to low-molecular weight molecules employed as antimicrobials, more recently synthetic macromolecular antimicrobials have emerged as a highly promising class of therapeutics with immense potential for combating multidrug-resistant microbes [14]. The first macromolecular systems explored involved the use of antimicrobial peptides (AMPs). AMPs were discovered by Boman et al. [31] studying how multicellular organisms naturally defend themselves against infections from opportunistic pathogens. In particular, they found AMPs from the humoral immune system of silk moths (Hyalophora cecropia). According to their findings, these peptides were able to kill a broad range of pathogenic microbes, including Gram-negative bacteria, Gram-positive bacteria, and fungi. Since this pioneer work, a large amount of AMPs have been developed, and today an antimicrobial peptide database (APD) has been established based on an extensive literature search [32].

In general, AMPs share common features since they are generally amphiphilic, with nonpolar amino acids that constitute a hydrophobic side chain environment (e.g., tryptophan) combined with cationic amino acids such as lysine. As a result of this structure, they usually interact preferentially with microbial membranes using

electrostatic interaction between cationic charge of AMPs and anionic charge on the membrane surface. Upon contact with the membrane, AMPs form a secondary structure, allowing insertion of hydrophobic components into membrane lipid domains, disrupting membrane structure. These AMPs offer an alternative solution in the fight against multidrug-resistant pathogens, while at the same time reduce the possibility of developing new strains of drug-resistant pathogens due to the physical nature of membrane disruption [33–35].

Around the same time AMPs were discovered, a new field, antimicrobial polymers, designed to mimic the salient structural features of host defense peptides, were emerging class of materials with potential for applications to combat infectious disease [36]. As highlighted by Engler et al. [14] inspired by the efficacy and notable versatility of AMPs in combating various pathogenic microbes combined with knowledge of polymer disinfectants, chemists, and material scientists have devised a number of strategies to develop synthetic peptides and polymers that mimic the amphiphilicity and antimicrobial functions of AMPs. These synthetic macromolecules widen the antimicrobial spectrum, allowing in vivo applications [37, 38]. In particular, nanostructured antimicrobials have recently received increased attention because nanostructure formation increases local charge density, enhancing antimicrobial activity [37].

As will be depicted throughout this book, polymers mode of action relies on physiochemical parameters such as hydrophobicity and cationic charge, rather than specific receptor-mediated interactions, the activity of the polymers can be modulated by tuning key structural parameters. Taking into account the action mechanism, polymers exhibit in comparison with other materials, important advantages that have motivated their investigation as antibacterial materials. These include that polymers do not provide toxicity to the environment, do not develop resistance, and have an enhanced antimicrobial action. Other important advantages are their versatility; polymers are easy to process and cheap.

1.4 About This Book

This book is devoted to the recent developments in the use of polymers as antifouling and antimicrobials for different applications. The first part of this book (Chaps. 2 and 3) describes the basics of bacterial infections and the main functional groups incorporated into polymeric structures to avoid microorganism contamination. Chapter 4 depicts the use of nanostructured polymer assemblies in solution as antimicrobials.

The design and fabrication of polymer surfaces is analyzed in Chaps. 5 and 6. Chapter 5 discusses the alternatives to modify the surface chemical composition in order to introduce both antifouling and/or antimicrobial functional groups. Chapter 6 concerns those approaches that resort both to the modification of the surface topography and those that combine surface functionalization and patterning to remove bacterial contamination and biofilm formation.

Chapters 7, 8, and 9 are devoted to the use of antimicrobial polymers for the elaboration of three different materials. First, the approaches developed for the fabrication of nano- and microstructured fibers are depicted in Chap. 7. Second, the synthesis and modification and hydrogels to improve the bacterial adhesion as well as to introduce antimicrobial moieties is described (Chap. 8). Finally, Chap. 9 focuses on the elaboration of membranes with enhanced antifouling properties.

The last part of this book will analyze the eventual environmental concerns as well as safety issues related mainly to the use of nanoparticles. The last chapter will summarize the future trends on the development of more sophisticated and effective antimicrobial polymer systems.

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Chapter 2
Bacterial Infections: Few Concepts

Abstract A principal challenge defying current medicine in the twenty-first century is the large occurrence of antibiotic resistance, as well as, the risk posed by drug-resistant superbugs. In spite of this, progresses on the development of novel antibiotics to combat this problem are quite limited. It appears necessary to carry out a more concerted effort to advance in the discovery of novel therapeutic agents with excellent activity and unique mechanisms of action to overcome the problem of drug resistance. In this context, macromolecular antimicrobials with a different interaction with bacteria may offer an interesting alternative to current strategies in order to successfully prevent resistance. Furthermore, biofilm-forming bacteria are recognized to be gradually resistant to the action of antibiotics and are a leading cause of mortality or morbidity in nosocomial infections.

This chapter will, thus, describe the bacterial structure and summarize the mechanisms involved in the interaction between antibiotics and bacteria as well as the resistance mechanisms developed. In addition, the proposed models of interaction between macromolecular antimicrobials and bacteria will be analyzed.

The second part of this chapter is devoted to implant-associated infections produced by the formation of a biofilms at the surface of biomaterials. More precisely, the steps involved in biofilm formation and its particular properties that reduce the antimicrobial activity will be discussed. Finally, preliminary concepts on the use of polymers to overcome this limitation are depicted.

Keywords Bacterial structure • Antimicrobial mechanisms • Macromolecular antimicrobials • Pore-forming mechanism • Bacterial adhesion • Biofilm formation

2.1 Introduction

A principal challenge defying current medicine in the twenty-first century is the large occurrence of antibiotic resistance, as well as, the risk posed by drug-resistant superbugs. In spite of this, progresses on the development of novel antibiotics to combat this problem are quite limited. It appears necessary to carry out a more concerted effort to advance in the discovery of novel therapeutic agents with excellent activity and unique mechanisms of action to overcome the problem of drug

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resistance. In this context, macromolecular antimicrobials with a different interaction with bacteria may offer an interesting alternative to current strategies in order to successfully prevent resistance. Furthermore, biofilm-forming bacteria are recognized to be gradually resistant to the action of antibiotics and are a leading cause of mortality or morbidity in nosocomial infections [1].

This chapter will, thus, describe the bacterial structure and summarize the mechanisms involved in the interaction between antibiotics and bacteria as well as the resistance mechanisms developed. In addition, the proposed models of interaction between macromolecular antimicrobials and bacteria will be analyzed.

The second part of this chapter is devoted to implant-associated infections produced by formation of a biofilms at the surface of biomaterials. More precisely, the steps involved in biofilm formation and its particular properties that reduce the antimicrobial activity will be discussed. Finally, preliminary concepts on the use of polymers to overcome this limitation are depicted.

2.2 Bacterial Structure

Bacteria comprise a cytoplasm and a membrane and finally a cell wall. On the one hand, the cytoplasm does not have any organized organelles and is formed exclusively by ribosomes and DNA [2]. The membrane of bacterial cells share common features with those membranes found in mammalian cells, i.e., they are formed by a phospholipid bilayer. In both cases and in general biological membranes comprise five major biomolecules: phosphatidyl glycerol (PG), phosphatidyl ethanolamine (PhE), phosphatidylcholine (PhC), phosphatidyl serine (PhS), and sphingomyelin (SfgM). These biomolecules provide the surface charge present at the cell surface. More precisely, at physiological pH, whereas PhS and PhG are negatively charged, PhC, PhE and SfgM form zwitterionic species. In addition to these common biomolecules, bacterial cells present some structural differences that required to be analyzed in order to understand the antimicrobial properties of polymers [3].

The main differences between the plasma membranes in mammalian and microbial cells rely on their composition and their structure. Illustrative examples of a mammalian cell membrane and microbial membranes of Gram-negative bacteria, Gram-positive bacteria as well as Yeast are depicted in Fig. 2.1.

The first key difference between the two relies on the distribution of the negatively charged biomolecules. In mammalian cells, the outer monolayer of the membrane is often constructed from PhC and SfgM. Therefore, the negative charge provided by PS is concentrated in the inner part of the membrane. Microbial cell membranes possess, on the contrary, negative charges in both sides of the membrane as a result of a homogeneous distribution of PhS. As will be depicted later, this characteristic will be crucial for the design of selective antimicrobial polymers [4].

The second major difference concerns the additional components present in the cell wall of microbial membranes. The cell wall composition depends on the microbial strain. Therefore, antimicrobials would not behave equally to all bacteria but

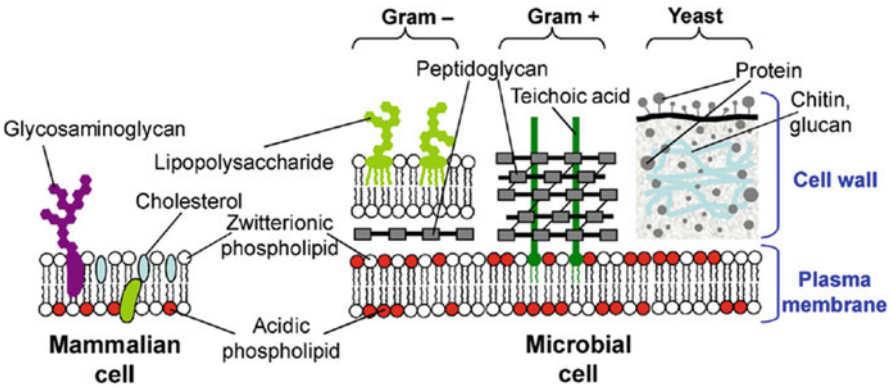

Fig. 2.1 Cell envelope structure and its effect on the antimicrobial selectivity. Cross-sectional illustration showing major changes between cell envelope of mammalian cells and various microbial families. Reproduced with permission from [3]

probably may exhibit larger activity of particular species. As depicted in Fig. 2.1, several differences can be observed between the cell wall of Gram positive, Gram-negative, and Yeast. First of all, Gram-positive bacteria have a thicker peptidoglycan layer in comparison to Gram-positive. Moreover, this layer is around 90 % of the cell wall components in Gram-negative while it supposes around 20 % in Gram negative. The yeast family does not possess peptidoglycans on their walls. In this case the membrane is formed by a layer of chitin and glucan cross-linked polymer network and an outer protein layer [5]. Other significant difference between Gram-positive and Gram-negative bacteria is that in Gram-positive bacteria teichoic acids are attached to the membranes and oriented outwardly. Gram-negative do not have teichoic acids and, in contrast to Gram-positive the peptidoglycan layer is embedded in an additional membrane known as outer membrane [6].

Finally, a third difference between mammalian and bacterial cells is associated to the mechanical stability of the membrane. Microbial cell exhibit, in comparison to mammalian cells membranes, enhanced mechanical stability. This characteristic has to be considered in the design of antimicrobial polymers since, while providing excellent antibacterial activity these may result toxic to mammalian cells.

2.3 Interactions Mechanisms of Antimicrobials with Bacteria in Solution

2.3.1 Bacterial Targets of Antibiotics

Antibiotics are usually antibacterial drugs that interfere with one or more of the bacterial crucial routes such as growth or survival. A strong development during the 60–70s leads to the discovery of many diverse classes of antibiotics such as

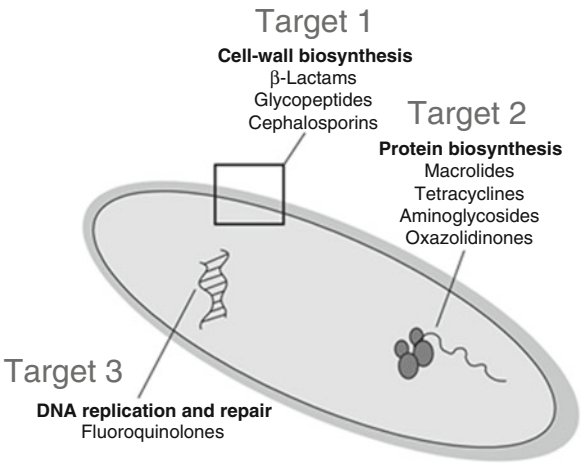

Fig. 2.2 Main targets of antibacterial drugs: Target 1: interaction with the cell wall biosynthesis preventing the cross-linking of peptidoglycan peptide strands. Target 2: blocking the protein biosynthesis at the ribosome in particular those steps involving rRNA and the proteins of the ribosome at the peptidyl transferase center. Target 3: interfering DNA replication. Figure adapted from [7]

vancomycins penicillins, or cephalosporins. As reported in an excellent review by Walsh [7], the antibiotics currently developed have three main targets (Fig. 2.2):

Cell wall biosynthesis: the peptidoglycan layer at the bacterial cell wall confers the stability and strength. This layer is formed by a mesh of peptides and glycans that can be covalent cross-linked. In this context, several antibiotics have been developed to target this layer. Penicillins and cephalosporins inhibit the peptidoglycan to form cross-links thus leading to a weaker wall that predisposes the treated bacteria to a killing lysis of the cell wall layer. An additional family of glycopeptide antibiotics is vancomycin. This antibiotic ties up the peptide substrate [8] and thereby prevents it from reacting with either the transpeptidases or the transglycosylases.

Protein synthesis: many inhibitors of protein synthesis target different steps in ribosome action selective for bacteria. The selectivity is simply reached since the prokaryotic ribosomes are considerably different to those existing in eukaryotic cells. Moreover, taking into account the amount of steps involved in the protein assembly by the ribosome (initiation, elongation, and termination), there are many different processes that can be changed using protein synthesis inhibitors.

DNA replication and repair: ciprofloxacin that belongs to the fluoroquinolones type are antibiotic molecules that target the enzyme responsible for uncoiling the intertwined circles of double-stranded bacterial DNA, i.e., DNA gyrase [9].

2.3.2 Antibiotic Resistance Developed by Bacteria

In spite of the different targets that can be focused to reduce bacterial contamination, bacteria have the ability to find alternatives to overcome the effects of antibiotics. This phenomenon, known as bacterial resistance, appears typically in periods of months for many of the currently available antibiotics thus limiting their use.

Mc Manus [2], Walsh [7], and more recently Blair et al. [10] have reported comprehensive reviews dealing with this aspect. Our discussion herein will be thus limited to mention the most important mechanisms developed by bacteria to survive antibiotics.

2.3.2.1 Mechanism 1: Pump Out the Antibiotic

Taking into account that for antibiotics in order to be active require both enough concentrations but also to approach the selected target and effective way to overcome antibiotic treatments involves the active pumping out of the cell by the so-called efflux pumps. In this case, the drug is pumped out faster than it can diffuse in, so antibiotic concentrations are maintained low and do not affect the protein synthesis [11, 12].

Similar efflux pumps have been observed in different bacterial strains. For instance, efflux pumps have been employed by *staphylococci* to become resistant to the erythromycin class of macrolide antibiotics [11, 13]. Other examples of efflux pumps include FuaABC in *S. maltophilia* [14], KexD in *K. pneumonia* [15], and LmrS in *S. aureus* [16, 17]. It is worth mentioning that some efflux pumps have revealed narrow specificity while others are capable of transporting different substrates, multidrug resistance (MDR) efflux pumps [10].

2.3.2.2 Mechanism 2: Reduce the Permeability of the Cell Membrane

Tamber and Hancock [18] reviewed this alternative mechanism developed by bacteria and concluded that the permeability of the outer membrane can be reduced in order to limit the amount of antibiotic that may enter into the cytoplasm. This can be achieved either by the downregulation of porins or by the replacement of porins with more-selective channels.

2.3.2.3 Mechanism 3: Modification of the Antibiotic Structure

Bacteria are able to develop synthetic routes to chemically modify the chemical structure of the antibiotic employed. Today, a large variety of enzymes have been identified that can damage and alter antibiotics of different classes, comprising β-lactams, aminoglycosides, phenicols, and macrolides [10]. One of these alternatives involves the inactivation of the antibiotic by *hydrolysis*. This is the case of the hydrolytic deactivation of β-lactam rings present both in penicillins and cephalosporins. Bacteria generate a hydrolytic enzyme known as β-lactamase. The closed β-lactam rings participate in the acylation and irreversible modification of the cell membrane cross-linking. The hydrolysis reaction resulted in an aperture of the ring inactivating the antibiotic [19].

A second alternative to modify the antibiotic structure and therefore deactivate them involves the incorporation of chemical groups. This is the case of antibiotics that are not affected by β-lactams such as aminoglycosides. The principle of this

strategy relies on the fact that the addition of chemical groups to particular positions on the antibiotic molecule by bacterial enzymes prevents the antibiotic from binding to the target protein. In particular, aminoglycosides with chemical substituents are unable to bind to the RNA targets in the ribosome [20]. Chemical groups that have been transferred, include acyl, phosphate, nucleotidyl, and ribitoyl groups [21].

2.3.2.4 Mechanism 4: Changing the Target Structure

According to Blair et al. [10], two different alternatives can be employed to alter the structure of the antibiotic target thus conferring bacterial resistance. On the one hand, bacteria can react to the antibiotic by modifying the structure of the antibiotic targets for instance by mutation. On the other hand, it can chemically modify the targets to protect them from the antibiotic (for instance, by methylation processes).

Typically, antibiotics exhibit a high specific binding for a particular target. As a result, the antibiotic modifies and reduces the normal activity of the target. Bacteria react to this situation by introducing changes on target structure in order to prevent the antibiotic binding. Moreover, the changes introduced by bacteria still allow these targets to carry out its normal function. These processes generally involve genetic modifications, i.e., mutations. A single point mutation in the gene encoding may allow bacteria to provide resistance. Thus, the strains with this new genetic information can then proliferate. For instance, *S. aureus* able to incorporate the *mecA* (gene that encodes a PBP2′ protein with low affinity for all β-lactam antibiotics) offers the molecular base for the Methicillin-resistant *S. aureus* (MRSA) phenotype [22, 23] that is now widely disseminated.

In addition to the above-mentioned strategy, targets can be modified to protect them from antibiotics without the use of genetic mutation processes. For instance, Long et al. [24] identified that the chloramphenicol–florfenicol resistance (cfr) methyltransferase, which precisely methylates A2503 in the 23S rRNA; as a result, this provides resistance to a widespread variety of drugs that have targets nearby this position, including phenicols, streptogramins, pleuromutilins, lincosamides, and oxazolidonones (including linezolid).

In Table 2.1 are summarized few examples of the most extended antibiotics employed nowadays, their target and mode of action and finally the resistance mechanism developed by bacteria.

2.3.3 Macromolecular Antimicrobials

In view of all the mechanisms developed by bacteria to overcome the effect of antibiotics, there is an urgent need of novel antimicrobials [25]. In this context, as will be depicted throughout this book, synthetic polymers are currently being investigated as new molecular platforms to create alternative antimicrobial agents that could be active against drug-resistant bacteria [3, 26–28]. The versatility of the polymer chemistry allows for the fabrication of a variety of polymers with variable

Table 2.1 Targets, mode of action, and mechanisms of resistance of the main classes of antibacterial drugs

Antibiotic	Target	Mode of action	Resistance mechanism
Cell wall			
β-Lactams	Transpeptidases/ transglycosylases (PBPs)	Blockade of cross-linking enzymes in peptidoglycan layer of cell walls	β-Lactamases, PBP mutants
Vancomycin	D-Ala-D-Ala termini of peptidoglycan and of lipid II	Sequestration of substrate required for cross-linking	Reprogramming of D-Ala-D-Ala to D-Ala-D-Lac or D-Ala-D-Ser
Protein synthesis			
Macrolides of the erythromycin class	Peptidyl transferase, center of the ribosome	Blockade of protein synthesis	rRNA methylation, drug efflux
Tetracyclines	Peptidyl transferase	Blockade of protein synthesis	Drug efflux
Aminoglycosides	Peptidyl transferase	Blockade of protein synthesis	Blockade of protein synthesis
Oxazolidinones	Peptidyl transferase	Blockade of protein synthesis	Unknown
DNA replication/repair			
Fluoroquinolones	DNA gyrase	Blockade of DNA replication	Gyrase mutations to drug resistance

Reproduced with permission from [7]

backbones and functionalities that have been utilized to prepare antimicrobial polymers. Interestingly, some polymers, in particular bearing cationic groups, with high efficacy have been reported [29–32]. In addition, to the excellent activity against a broad spectrum of bacteria these polymers have shown low propensity for resistance development in bacteria [33]. This is, at least partially, due to the interaction mechanism of polymers with bacterial cells.

The main strategy for designing antimicrobial polymers has been determined taking into account the structural features of the cell membrane of bacterial cells. As has been depicted, the most important characteristic of the outer envelope of the cells is a net negative charge. As a result, considering as the target site the cytoplasmic membrane (so-called membrane active agents) antimicrobial polymers have been mainly designed as cationic hydrophilic–hydrophobic macromolecular systems [34, 35]. It is expected that macromolecular antimicrobials reduce the tendency of microbes developing resistance since they act on the microbial cell membrane and physically damage the membrane structure.

One of the pioneer works in attempting to correlate the structure of the antimicrobial polymer and the mechanism of interaction with bacteria was reported by Gilbert and coworkers [36–38] These groups investigated the mechanism of interaction

between a cationic polyelectrolyte salt polyhexamethylene biguanide chloride (PHMB) with *Escherichia coli*. In these pioneer works, the authors proposed a sequence of events during PHMB interaction with the cell envelope of *E. coli* was proposed as follows: (1) fast attraction of PHMB toward the negatively charged bacterial cell surface, with strong and specific adsorption to phosphate-containing molecules; (2) the structure of the outer membrane is impaired, and PHMB is attracted to the inner part of the membrane; (3) interaction between PHMB and phospholipids occurs, with an increase in inner membrane permeability to K^+ loss together with bacteriostasis; and (4) complete loss of membrane function. This fourth step occurs by precipitation of intracellular elements and finally a bactericidal effect [36–39].

More recently, Wimley [40] and Chan et al. [41] proposed different AMP-induced membrane disruption mechanisms. Wimley described the interaction macromolecule—bacteria by two main possibilities, i.e., **pore-forming** and **non-pore-forming** mechanisms. In the **pore-forming** mechanism, also known as transmembrane mechanism, the AMPs are inserted into the bacterial membrane thus forming aqueous pores across the membrane. The pore-formers AMPs induce the formation of stable pores in the outer envelope of the cells and disturb the homeostasis of the cell metabolism, eventually resulting in cell death. As depicted in Fig. 2.3, there are two main models, i.e., *barrel-stave* pore and toroidal pore model. The difference between the two relies on the fact that in the barrel-stave model, specific peptide–peptide interactions form the original approach for pore formation, which renders small nanopores (1–2 nm in diameter) [42]. On the other hand, the toroidal model does not involve specific peptide–peptide interactions and the role of the AMPs is to alter the curvature of the membrane. In this case, the diameter of the pores formed are larger (3–10 nm) in comparison with the barrel-stave model [43–45].

The second alternative for macromolecules (herein AMPs) to interact with bacteria is based on the **non-pore-forming** mechanism (Fig. 2.3c, d). In this case, AMPs interact in a parallel manner on the surface of microbial cells. Also two alternative models have been described for this mechanisms, i.e., the carpet model and the detergent model. Shai et al. [46] proposed that AMPs are active only on the bacterial membrane by forming a *carpet* on the bilayer surface that finally leads to large defects (larger than 10 nm pore size) on the bacterial membrane. Finally, the *detergent* model in which the AMPs induce a massive collapse of membrane integrity has also been employed to explain the antimicrobial mechanism of AMPs [41].

According to Chan et al. [41] in addition to the above depicted mechanisms, two other lesser known models, the molecular electroporation [47] or the sinking raft model [48, 49], can be important to explain the interaction mechanisms of antimicrobial peptides with bacteria. As shown in Fig. 2.4a, on the one hand, in the molecular electroporation model, the cationic peptides establish interactions with the membrane of the bacteria and generate an electrical potential difference across the membrane that finally forms upon reaching a critical potential value [47, 50]. On the other hand, the sinking raft model (Fig. 2.4b) suggests that an imbalance produced upon binding of the peptides to the membrane leads to an increase in the membrane curvature. Moreover, peptides are able to associate and penetrate inside the membrane producing transient pores [49].

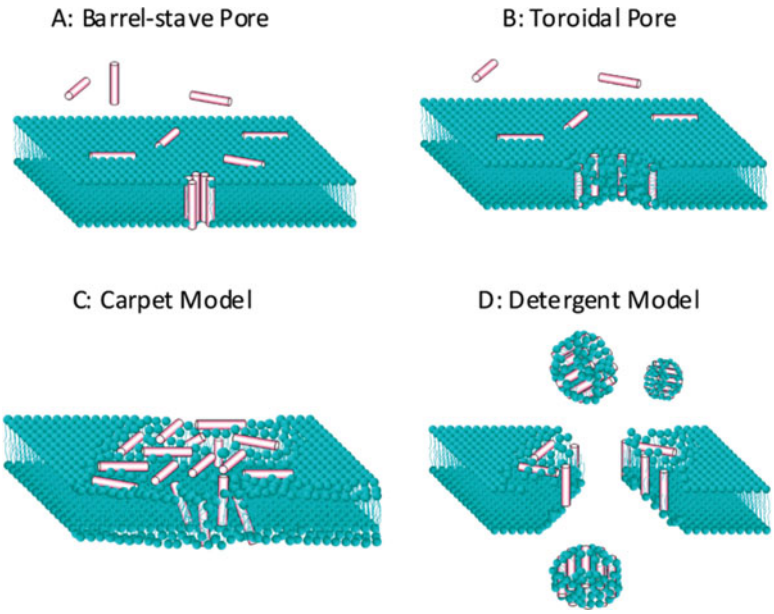

Fig. 2.3 Commonly cited models for antimicrobial peptide activity. Barrel-stave and toroidal pores are membrane-spanning aqueous channels. Antimicrobial peptides are described with the carpet model. Such peptides permeabilize membranes by "carpeting" the bilayer with peptides. At high concentrations, carpet model peptides can behave more like detergents. Reproduced with permission from [40]

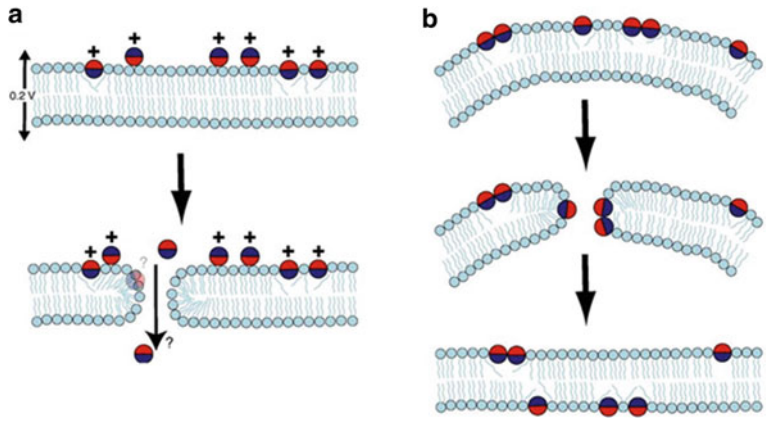

Fig. 2.4 (**a**) The molecular electroporation model and (**b**) the sinking raft model (adapted with permission from [41])

2.4 Biomaterials Surface: Device-Associated Infections

Microorganisms normally attach to both living and inert surfaces, including those of indwelling medical devices, finally leading to biofilm formation made up of extracellular polymers. In this state, microorganisms are highly resistant to antimicrobial cure and are strongly bonded to the surface. Therefore, a today's crucial issue in polymeric materials uses for biorelated applications involves the contamination by microorganisms and in particular bacteria. This problem affects many different areas ranging from such as medical devices, healthcare products, water purification structures, clinics, dental office tools, food storage, household sanitation, or food packaging just to mention a few of them [28, 51]. Moreover, applications free of bacteria surfaces include: dentistry (surface of acrylic resins) [52], implants [53], intraoral materials [54].

Bacterial contamination is still a common unresolved problem present in the major cases in which a biomaterial is required. While this is a general problem present independently of the biomaterial considered, it is even more serious in those cases in which durable implants are used. For instance, long-term catheters can produce implant-associated infections. Particularly critical are those cases in which the infections become resistant to antibiotics (those cases in which biofilm is already produced), and the implant need to be removed. Depending on the implant and the infection created by the microorganism can be even critical since the antibiotics cannot be effectively delivered. The impact of implant failures on the entire population and on the costs for the national health systems is enormous. This effect is above all significant for septic failures, when microbial infections grow on biomaterial surfaces. Subsequently to an initial occupation, bacterial biofilms establish on contaminated surfaces, critically compromising the performance of the implant itself, recruiting inflammatory cells, affecting the integration in the neighboring tissues, but in addition exposing the patient to a serious risk of general infections, septicemia, and in some cases, decease. Moreover, once the bacterial biofilm has been formed, conventional medical therapies based on universal antibiotics are not efficient and implant removal often represents the only chance to eradicate the infection. Thus, to better know and control biofilms in the case of indwelling medical devices, researchers should develop consistent sampling and measurement methods, study the role of biofilms in antimicrobial drug resistance, and establish the relationship between biofilm infection and patient contamination [55].

While this is true, biomedical devices are a vital part of the human healthcare system. For instance, the quantity of artificial hip and knee implants has improved significantly during the last decades, and heart valves, stents, vascular grafts, and other implants devices have been used widely to protect lives and to reestablish the quality of life for many people. For instance, according to the Freedonia Group, the demand in the USA for implantable medical devices is projected to rise 7.7 % annually to $52B in 2015 [56]. Polymers are expected to be the fastest increasing class of materials between 2013 and 2019, mainly due to the rising applications of these biomaterials and numerous advantages over metals that include longevity, elasticity,

flexibility, biocompatibility, and bio-inertness. The use of polymers, for instance, polyurethanes (PUR) and polytetrafluoroethylene (PTFE), is being popular for synthetic vascular grafts, whereas the extended use of polymers in ophthalmology is estimated to grow accordingly with the increasing amounts of ophthalmic illnesses and continuous demand from geriatrics.

2.4.1 Adhesion, Adherence, and Attachment

Before proceeding to analyze the factors involved in the bacterial adhesion to solid substrates (such as living tissues or biomaterials) it is worth analyzing few concepts that will be later employed and have in ambiguously employed in the literature. As described by An and Friedman [57] bacterial adhesion refers to a situation in which bacteria are strongly adhered to the biomaterial surface by physicochemical interactions. These are the result of an initial reversible physical contact and a subsequent irreversible chemical and cellular adherence. Therefore, an energy has been employed to form interactions between the bacteria and surfaces.

According to these authors, adherence should be applied to describe the initial process of bacterial attachment directly to a surface. This term has been employed in a less scientific environment to refer bacterial adhesion. Finally, attachment is associated to the initial stage of bacterial adhesion which are reversible and, thus, refers more to physical contact than to chemical and/or cellular interactions.

2.4.2 Bacterial Adhesion to Biomaterials Surfaces

The first step in the pathogenesis of foreign-body-related infections is the bacterial adhesion that, in general, leads to colonization. Moreover, the early phases of microbial adhesion on biomaterial surfaces that will lead to biofilm formation depend on the contamination route followed by the microorganism. On the one hand, contamination may occur in a dry state by direct transfer from a contaminated material. On the other hand, contamination is produced by either airborne bacteria or by the contact with physiological fluids in wet conditions. As reported by Campoccia et al. [58] contamination by airborne bacteria or by contamination transfer can be reduced or completely avoided by implementing aseptic procedures and by precisely controlling the manipulation protocols of sterile devices [59].

More complicated to prevent are those infections produced by contaminations transferred from liquid carriers. These include physiological fluids, such as blood and serum, or artificial low protein content solutions including saliva or urine. As will be depicted, in this case, bacterial adhesion cannot be prevented by using aseptic protocols. However, there are a number of variables that are involved in the bacterial adhesion that can be identified and applied to reduce contamination. These

parameters are the type of pathogen the physiological fluid involved but also several parameters related to the biomaterial interface [60].

In one of the first reviews devoted to this topic, An et al. [57] reported that the bacterial adhesion phenomenon is a two-phase process. The phase one concerns an initial, instantaneous, and reversible physical adhesion of bacteria to biomaterial surfaces. In phase two, a time-dependent and irreversible molecular and cellular phase are formed. These two phase approach was first proposed by Marshall and colleagues [61, 62] but has been accepted by the majority of researchers [63]. The most prominent results of the analysis of the process leading to bacterial adhesion and biofilm formation on biomaterial surfaces have been recently reviewed among others by Arciola et al. [64, 65], describing the possible implications for the development of biofilm-resistant materials.

These reports indicated that bacterial adhesion on biomaterial surfaces take place through multiple mechanisms, were certain are affect all microbial species, while others are species-specific or even strain-type specific [58]. Mechanisms that involve different bacterial species without any specificity finally leads to passive adsorption of the bacterial cells at the surface of the polymeric material by means of physicochemical surface interactions and are usually observed in the initial adhesion stages. On the other hand, strain-specific adhesion, also known as active mechanisms of adhesion are mediated by bacterial structures termed bacterial adhesins [66, 67].

2.4.2.1 Phase One in Bacterial Adhesion

As mentioned above, the initial interactions between bacteria and a solid surface are nonspecific in nature. In this phase, bacteria are, therefore, passively adsorbed onto the material surfaces [65]. These bacteria–surface interactions are established by different forces including hydrophobic, electrostatic, Van der Waals forces as well as hydrogen bonding [58]. In particular, bacterial behavior is strongly influenced by surface hydrophobicity as well as the electrostatic charge. As a result, both functional groups and chemicophysical properties displayed by the biomaterial surface that will interact with those of the bacterial cells determine the kinetics of microbial adhesion.

A large amount of different factors such as surface morphometry or environmental conditions play additionally a key role on these initial stages (Table 2.2). Even fluid flow rate has however been observed to have a direct influence on the bacterial adhesion kinetics [11].

2.4.2.2 Phase Two in Bacterial Adhesion

In addition to passive bacterial adsorption that spontaneously occurs on almost all biomaterial surfaces, active stable anchorage of the bacterial cells can be established by adhesins [65]. Adhesins are able to bind of host proteins previously adsorbed onto the biomaterial surface. As depicted by Montanaro et al. [68] and

Table 2.2 Variables influencing bacterial adhesion and colonization on biomaterial surfaces

Surface morphometry	Macroporosity
	Microporosity
	Micro-roughness
	Nano-roughness
Physicochemical properties	Surface energy
	Hydrophylicity/superhydrophylicity
	Hydrophobicity/superhydrophobicity
	Hydrophobic functional groups
	Polar functional groups
	Charged functional groups
	Functional groups with specific activities
	Degree of hydration
Environmental conditions	Electrolytes
	pH
	Temperature
	Host proteins/host adhesins
	Shear rate/fluid viscosity
	Fluid flow rate
Pathogen	Gram-positive/Gram-negative
	Genus/species
	Bacterial shape
	Surface energy
	Strain type and specific set of expressed adhesins

Reproduced with permission from [58]

Patti et al. [69], host proteins are usually represented by receptorial proteins named "microbial surface components recognizing adhesive matrix molecules" (MSCRAMMs). These host proteins, also named "host adhesins" for their function, include elastin, fibronectin, collagen, fibrinogen, vitronectin, laminin, clumping factor A and B, bone-sialoprotein, IgG. Nevertheless, other still unknown components of the extracellular matrix may also participate in this process.

One of the pioneer studies evidencing that specific proteins mediate the binding to abiotic surfaces was reported by Heilmann et al. [70]. These authors reported that autolysins (enzymes present at the bacterial surface) possess a double function: enzymatic and adhesive and their structure depends on the bacterial strain. For instance, in *S. aureus*, Foster [71] found that the autolysin/adhesin is AtlA, a 137 kDa protein, highly homologous to AtlE. Similarly, in *S. epidermidis*, Heilmann et al. [70] reported that the major autolysin/adhesin is AtlE, a 148 kDa protein, which mediates attachment to polystyrene.

Finally, it is worth mentioning that adhesins can also intervene in the process of bacterial internalization into host cells [58]. The adhesins mentioned above, i.e., AtlA and AtlE, due to the glycine-tryptophane dipeptide repeats, participate both in the surface association and biofilm formation but also they play a key role on staphylococcal internalization by host cells [72].

2.4.3 *Biofilm Formation*

The term biofilm has been defined differently. According to Taraszkiewicz et al. [73] microbial biofilms can be defined as "a structured community of bacterial cells enclosed in a self-produced polymeric matrix that is adherent to an inert or living surface." Donlan [74] defined biofilm as an assemblage of microbial cells that is irreversibly associated (not removed by gentle rinsing) with a surface. Thus, bacterial biofilms are formed when single organisms come together to generate a larger cell community that will be, in turn attached to a surface and covered by polysaccharide membrane.

Biofilms are usually formed on the surface of synthetic materials such as medical devices, catheters, artificial hips, or contact lenses. However, they can be equally built on living tissues. Examples of living tissues that can be covered by biofilms include endocardium, wounds, and the epithelium of the lungs, particularly in cystic fibrosis patients [75, 76]. The biofilm comprises a matrix (in charge of the structural stability but also protection against adverse environmental conditions) mainly formed by polysaccharides, but also by proteins and extracellular microbial DNA. Moreover, in the same biofilm several microbial species bacterial or fungal can simultaneously coexist. This highly organized structure causes a multitude of problems in the medical field, particularly in those cases related to prosthetic devices such as endotracheal tubes or indwelling catheters [77]. Moreover, these infections are very difficult to be eradicated by conventional antibiotic therapy.

The role of the biofilms can be summarized in three different aspects [78]. First of all, biofilms provide intercellular signaling and communications pathways. In addition, biofilms assist bacteria to evade and deceive the immune system, one of the roles of the latter being detecting and eliminating pathogens. Finally, and most importantly, biofilms protect bacteria from antibiotics and other toxins. According to Chandra et al. [79], biofilm formation happens in three main stages:

(a) Biofilms at the early stage
In the first stage, bacterial cells approximate the surface using their flagella or directed by body fluids [80]. The contact is established and a monolayer of cells is positioned at the surface (Fig. 2.5a, b) [81–83]. In this situation, the bacterial cells can be reversibly detached, and more importantly they are susceptible to antibiotics. As will be depicted, biofilm formation limits the success of generally employed antibiotics.

(b) Intermediate stage
The initial reversible interactions between the bacteria and the surface are irreversible in the next step. This stable situation allows the bacterial cells to grow and multiplicate forming small, micrometer size colonies (Fig. 2.5c, d). According to Stephens [80], the biomaterial surface promote physiological adaptations, including secretion of exopolysaccharides (EPSs) to create a protective matrix surrounding the cells [84]. As a result, the colonies are composed

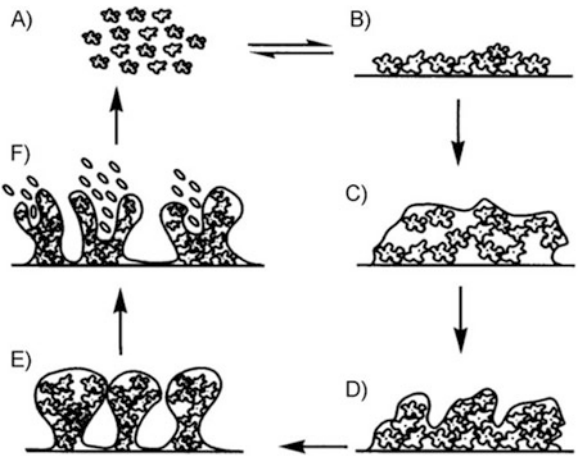

Fig. 2.5 Biofilm growth cycle: (**a**) Planktonic bacteria, (**b**) reversibly attached to a surface suitable for growth, (**c**) bacteria begin secretion of the EPS and attachment becomes irreversible, (**d**) the maturing biofilm begins to take a three-dimensional shape, (**e**) the biofilm fully matures, and a complex architecture is observed, (**f**) bacteria disperse from the biofilm to reinitiate biofilm colonization of a distal surface. Reproduced with permission from [78]

of a mixture of polymeric compounds, mainly polysaccharides (the matrix gives 50–90 % of the organic matter in biofilms) [85]. Nevertheless, the biofilm matrix is a rather complex material [86, 87] formed by:

- Polymers secreted by microorganisms within the biofilm.
- Cell lysis products, i.e., macromolecules including nucleic acids polysaccharides and proteins.
- Absorbed nutrients and metabolites.
- Peptidoglycan, lipids, phospholipids, and other cell components.

A crucial mechanism, critical at this stage, that regulates the biofilm formation is the quorum sensing (QS) [28–31]. The QS mechanism is related to the communication between microbial cells. More precisely, QS mechanism is a process that regulates back and forth the gene expression of those genes required for the formation and maturation of the biofilm. Some studies have evidenced that gene production is activated when a particular bacterial density is achieved and is retarded when the density is low. Hooshangi and Bentley [88] demonstrated that this process is regulated by signaling molecules and identified three well-defined groups in bacteria oligopeptides, acyl homoserine lactones (AHLs), and autoinducer-2 (AI-2).

(c) Mature stage

The final stage involves the formation of a mature biofilm clearly distinguished by the formation of mushroom-shaped colonies (Fig. 2.5e). The mature biofilm can be partially disrupted in order to promote the delivery of microbial cells.

The latter are able to swim to other surface areas and promote the biofilm formation in a non-contaminated zone (Fig. 2.5f).

As a result, in order to fabricate antibacterial/antifouling surfaces one of the key steps is the prevention of bacterial adhesion and thus biofilm formation. These two objectives have been typically pursued using different strategies to modify the surface and render the polymeric material antimicrobial.

2.4.4 Antibiotic Resistance of Bacteria in Biofilms

In the previous paragraph, it has been anticipated that antibiotics are only effective on the initial stages of bacterial adhesion but do not exhibit any influence in mature biofilms [89]. For example, Anderl et al. [90] estimated that a β-lactamase-negative strain (obtained from *K. pneumonia*) had a minimum inhibitory concentration of 2 μg/mL ampicillin in aqueous suspension. The same strain, when grown as a biofilm, was poorly affected (66 % survival) by 4 h treatment with 5000 μg/mL ampicillin, a quantity that eliminated free floating bacteria. Moreover, when bacteria are dispersed from a biofilm they quickly become vulnerable to antibiotics [91, 92]. This fact evidenced that resistance of bacteria in biofilms is not only acquired via mutations or mobile genetic elements [93–95] nor is due to efflux pumps [96]. Therefore, in contrast to the antibiotic resistance mechanisms depicted above such as efflux pumps, modifying enzymes, or target mutations [7] biofilm resistance should additionally be influenced by other processes.

According to Mah and O'Toole [76], three main hypothesis can explain the mechanisms of resistance to antibiotics in bacterial biofilms.

The first hypothesis is related to the slower or incomplete penetration of the antibiotic into the biofilm. While it is true that measurements on the antibiotic penetration revealed that there is no generic barrier to the diffusion of solutes through the biofilm matrix [97, 98], it is also true that, in some cases, if the antibiotic is neutralized in the biofilm, infiltration can be deeply retarded. For example, Anderl et al. [90] demonstrated that ampicillin is able to infiltrate through a biofilm made by a β-lactamase-negative strain of K pneumonia but not a biofilm formed by the β-lactamase-positive wild-type strain of the same microorganism. In the wild strain biofilm, the antibiotic is deactivated in the surface layers more rapidly than it diffuses [99–102].

The second hypothesis is related to the altered chemical microenvironment within the biofilm that can, in some cases change an aerobic environment into anaerobic. Debeer et al. [103] demonstrated that oxygen can be totally consumed in the superficial layers of a biofilm, leading to anaerobic areas in the deep layers of the biofilm. In this context, aminoglycoside antibiotics are clearly less effective against the same microorganism in anaerobic than in aerobic conditions [104]. In addition to the oxygen content, local accumulation of acidic waste products might lead to important pH differences between the bulk fluid and the biofilm interior, which could directly antagonize the action of an antibiotic [105]. Equally, the deple-

tion of a substrate or accumulation of an inhibitive waste product that might cause some bacteria to enter a non-growing state [106]. Finally, variations on the osmotic environment within a biofilm may induce an osmotic stress response. Such a response could contribute to antibiotic resistance by altering the relation of porins in a way that reduces cell envelope permeability to antibiotics [107].

A third and still controversial mechanism of antibiotic resistance is related to the unique characteristics of biofilms that form a highly protected, phenotypic state. This is true for some cases while other findings contradict this model. For instance, newly formed biofilms can show resistance even if their barriers to penetration are too thin to either an antimicrobial agent or metabolic substrates [108, 109] (Fig. 2.6).

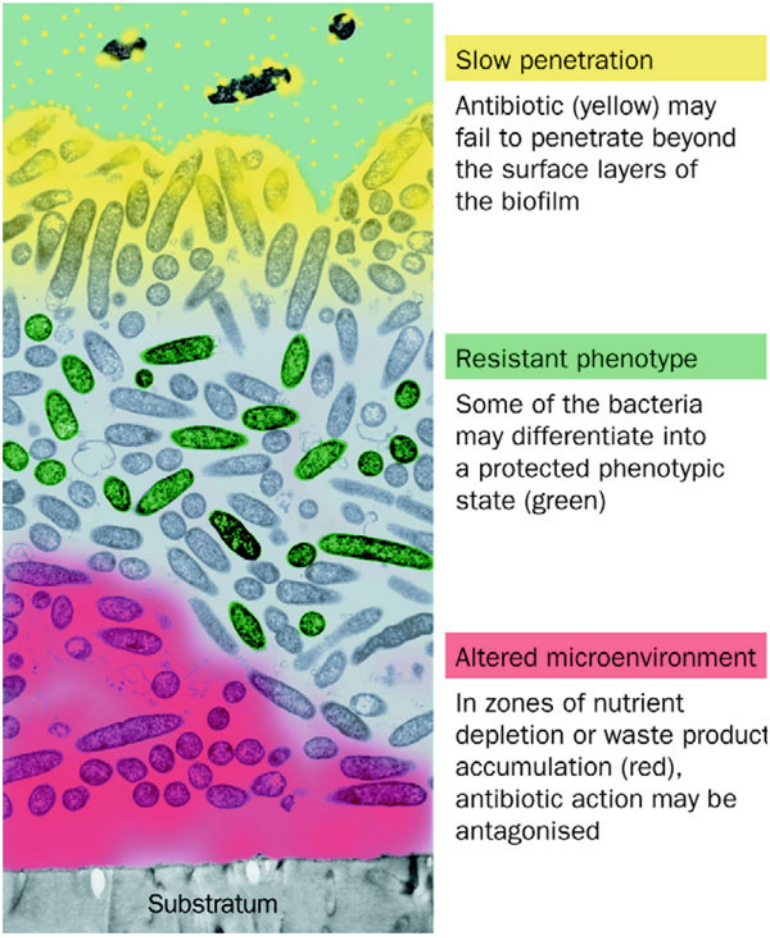

Fig. 2.6 Currently proposed hypotheses for mechanisms of antibiotic resistance in biofilms The attachment surface is shown at the bottom and the aqueous phase containing the antibiotic at the top. Reproduced with permission from [89]

2.4.5 *Approaches Developed to Achieve Polymeric Biomaterials with Antibacterial Properties*

The large amount of requirements that antibacterial biomaterials need to fulfill are very broad. In particular, these depend on the final application and, of course, they should resist microorganism and mainly bacterial infections. The strategies developed to produce antibacterial surfaces and interfaces that will be thoroughly described in Chaps. 5 and 6 are summarized in Fig. 2.7. These include the fabrication of surfaces with low adhesion or bacterial repulsion, the incorporation of compounds with bactericidal activity or the attack to bacterial surviving mechanism (quorum sensing existing between bacteria, the modulation of the host immune system, or the interference with bacteria).

2.4.5.1 Bacteria Repelling and Antiadhesive Surfaces

The first strategy to prevent biofilm formation involves the development of alternatives to prevent bacteria to adhere to the material surface. In this case, a thorough analysis of the contamination route, i.e., whether contamination occurs in a dry state by direct mechanical transfer through contaminated objects and by airborne bacteria or in wet conditions, if contamination occurs by the contact with physiological fluids. Whereas, direct airborne bacteria or mechanical deposition of bacteria can be reduced to a large extent by following strict aseptic procedures during manipulation contamination by the contact with physiological fluids that cannot be completely removed [59].

2.4.5.2 Bioactive Materials with Intrinsically Antibacterial Properties

Surface functionalization with materials exhibiting antibacterial properties is also an extended alternative to reduce bacterial contamination. This functionalization can be achieved by immobilizing antibacterial compounds or delivering biocides.

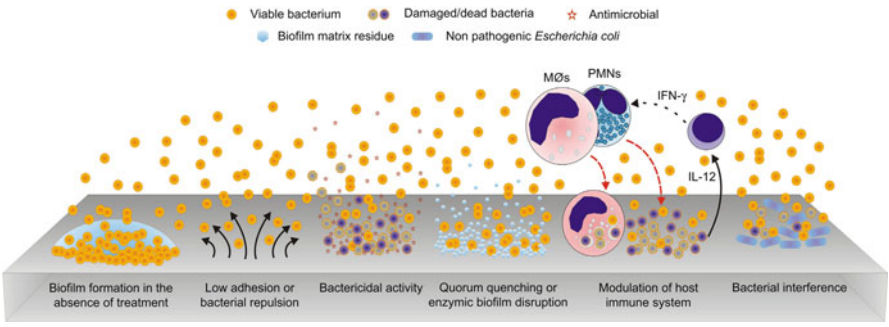

Fig. 2.7 Strategies designated to contrast the establishment of infections on medical devices. Reproduced with permission from [58]

For instance, silver has been described as one of the earliest materials to be intentionally used in surgery for its bactericidal properties. In addition to the material functionalization, bulk materials are described as intrinsically antibacterial when they exhibit an antibacterial action in the absence of modifications, such as loading with bactericidal molecules or coating with active biocides.

2.4.5.3 Materials Incorporating Bioactive Molecules Interfering with the Production of Bacterial Biofilm

Taking advantage of the continuous advances in the understanding of the molecular mechanisms underlying biofilm formation of different bacterial species has opened new alternatives to reduce the contamination on biomaterials surfaces [110, 111].

These approaches rely on the grafting or release of active substances conferring the surface antibiofilm activity [112]. As reviewed by Campoccia et al. [58], these surfaces may be decorated with active substances involved in different mechanisms:

(a) enzymes capable of selectively degrading extracellular polymeric substances of the biofilm (e.g,. Dispersin B, rhDNase I)
(b) bactericidal molecules capable of killing even metabolically quiescent bacterial cells inside biofilms (e.g., lysostaphin, AMPs)
(c) molecules interfering with the Quorum sensing system and inducing biofilm dispersion (e.g., furanones)
(d) molecules downregulating the expression of biofilm extracellular polymeric substances (e.g., *N*-acetylcysteine) or nevertheless reducing the biofilm metabolism (e.g., hamamelitannin)

For a detailed description of the alternatives to reduce bacterial contamination using bioactive compounds, the reader is referred to the following references [113–120].

2.5 Conclusions

In this chapter, we have revised the mechanisms involved in the antiobiotics–bacteria interactions. In contrast to traditional antibiotics to which bacteria can easily develop resistance, macromolecular antimicrobials have emerged as an interesting alternative to overcome this issue. Macromolecules, due to their large molecular weight, associated to particular functional groups (in general positively charged) establish interactions and alter the processes occurring in the cell membrane. As a result, by different mechanisms (following pore or non-pore-forming models) the permeability of the membrane is altered and finally provokes cell apoptosis.

The biofilm formation on the surface of polymeric biomaterials, also a major remaining problem among others in implant-associated infections, has also been considered in this chapter. Resistance in biofilms occurs, in addition to those

depicted for single bacteria, by other mechanisms such as changes on the environment, limiting diffusion or modifying bacteria. As a result, the fabrication of antibacterial surfaces is focusing on reducing or completely avoiding the initial bacterial adhesion.

The following chapters will be devoted to the different methodologies and strategies developed to fabricate polymeric materials with antimicrobial activity in solution and at surfaces of, for instance, planar rigid polymers or soft membranes but also on fibers or topographically structured surfaces.

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Chapter 3
Chemical Approaches to Prepare Antimicrobial Polymers

Abstract Until the early 1980s, low-molecular weight substances were mainly employed for their antimicrobial activity. However, the discovery of antimicrobial peptides (AMPs) carried out by dramatically changed this situation. This group demonstrated that macromolecular peptides were able to kill Gram-positive bacteria, Gram-negative bacteria, and fungi. AMPs have been extensively developed and today an Antimicrobial Peptide Database (APD). Based on this finding and around the same time antimicrobial polymers known under the name "polymer disinfectants" started to be investigated. As a result, studies on syntheses of polymeric biocides have been started to develop a new utilization field of polymer materials from 1980s. In particular, synthetic polymers have been widely investigated as a new molecular platform to create antimicrobial agents that are active against drug-resistant bacteria.

As will be depicted throughout this chapter, a variety of synthetic polymers with different chemical structures have been utilized to prepare antimicrobial polymers, and some polymers with high efficacy have been reported. In addition, a thorough analysis of the chemical characteristics of antimicrobial polymers and the different strategies to prepare them will be provided.

Keywords Antimicrobial macromolecules • Quaternary ammonium • Cationic polymers • *N*-halamine • Antimicrobial peptides

3.1 Introduction

Until the early 1980s, low-molecular weight substances were mainly employed for their antimicrobial activity. However, the discovery of antimicrobial peptides (AMPs) carried out by Boman et al. [1] dramatically changed this situation. This group demonstrated that macromolecular peptides were able to kill Gram-positive bacteria, Gram-negative bacteria, and fungi. AMPs have been extensively developed and today an Antimicrobial Peptide Database (APD) [2].

AMPs share an important characteristic: they are mainly amphiphilic composed by nonpolar side chains formed by peptides such as tryptophan and polar units typically based on cationic amino acids such as lysine.

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Based on this finding and around the same time antimicrobial polymers known under the name "polymer disinfectants" started to be investigated [3]. As a result, studies on syntheses of polymeric biocides have been started to develop a new utilization field of polymer materials from 1980s. In particular, synthetic polymers have been widely investigated as a new molecular platform to create antimicrobial agents that are active against drug-resistant bacteria [4–8].

As will be depicted throughout this chapter, a variety of synthetic polymers with different chemical structures have been utilized to prepare antimicrobial polymers, and some polymers with high efficacy have been reported [8–13].

The chemical structures developed by chemists and material scientists are designed taking into account the membrane structures and proposed membrane disruption mechanisms. As a result, as summarized by Katsumi [14] when designing antimicrobial macromolecules, four requirements must be fulfilled:

(a) It must have sufficient contact with the microbes.
(b) It must have sufficient cationic charge to promote adhesion to the microbial cell envelope.
(c) It must contain a hydrophobic moiety that will attach onto or integrate into the cellular membrane.
(d) Finally, these materials must selectively target and kill microbes without imparting toxicity on mammalian cells, typically measured by hemolytic activity. This selectivity often comes from enhanced long-range electrostatic interaction between antimicrobial macromolecules and microbes in comparison to mammalian cells.

In addition to these conditions related to the antibacterial mechanism, according to Kenawy et al. [15], the ideal antimicrobial polymer should possess the following characteristics:

1. easily and inexpensively synthesized
2. stable in long-term usage and storage at the temperature of its intended application
3. not soluble in water for a water disinfection application
4. does not decompose to and/or emit toxic products
5. should not be toxic or irritating to those who are handling it
6. can be regenerated upon loss of activity
7. biocidal to a broad spectrum of pathogenic microorganisms in brief times of contact

In this chapter, a thorough analysis of the chemical characteristics of antimicrobial polymers and the different strategies to prepare them will be provided.

3.2 Types of Antimicrobial Groups Incorporated in Polymers

It is already well known that both Gram-positive and Gram-negative bacterial cells have a negative net charge on the surface of the cell wall due to the presence of teichoic acids and phospholipids [16, 17]. Considering these particular surface

characteristics, it is well established nowadays that polymers bearing cationic compounds interact better with these cells than any other functional groups. However, as will be analyzed in this section, in addition to cationic polymers, several other functional groups have shown excellent antimicrobial properties.

3.2.1 Quaternary Ammonium/Phosphonium

Quaternary ammonium/phosphonium groups among most explored functionalities that are used in the synthesis of cationic polymers can be employed as efficient antimicrobial materials. In these materials, the positive charge is crucial for their interaction with bacterial. In effect, as has been mentioned in Chap. 2, bacteria contain in their membrane negatively charged groups. Cationic polymers can thus interact and destroy the bacterial membrane. As a result, they are capable of preventing their proliferation. A large amount of experimental work has been carried out using cationic polymers.

As proposed by Muñoz-Bonilla and Fernández-García [7] within this category, we can distinguish between:

(a) Polymers containing aromatic or heterocyclic structures
Cationic polymers with aromatic and heterocyclic structures are generally obtained by chemical modification of either polystyrene (PS) or poly(vinylpyridine)s. However, the most extensively used polymers are based on pyridinium-type functional groups obtained by quaternization of poly(4-vinylpyridine) (P4VP) [18–21]. Aromatic polymers with antimicrobial activity have been equally obtained using imidazole derivatives [22]. Imidazoles are part of biomolecules such as the amino acid histidine and related compounds, biotin, and the imidazole alkaloids. Imidazoles have been employed both uncharged and quaternized to form imidazolium salt groups both having antimicrobial activity [23].

(b) Acrylic and methacrylic polymers
A large number of acrylic and methacrylic antimicrobial polymers have been prepared due to the large number of available monomers, some of them commercial such as 2-(dimethylamino)ethyl methacrylate (DMAEMA). As a result, many different homo- and copolymers have been explored varying structural parameters such as type of charge included in the main chain, hydrophilic/hyrophobi ratio as well as the polymer molecular weight aiming to optimize the final antimicrobial activity [24–26].

(c) Cationic conjugated polyelectrolytes
Conjugated polymers are distinguished by alternating single and double bonds within the backbone eventually functionalized with side groups typically to provide additional properties as well as to enhance their, otherwise, low solubility. Within this group, the most extensively studied polymers are poly(phenylene ethynylene) (PPE)-based cationic conjugated polyelectrolytes. For instance, Whitten and coworkers [27–30] fabricated different PPEs bearing pendant alkylpyridinium groups, which were effective white light-activated biocides [31, 32].

(d) Polysiloxanes
Another interesting group of antimicrobial polymers was obtained using polysiloxanes with quaternary ammonium salt side-chain groups. One of the most important characteristics of these polymers is their high main chain flexibility that enhanced the contact of the biocide groups and the bacteria. In addition, the amphiphilic character obtained upon modification allows for concentrating the active groups at the membrane of the bacteria thus improving the efficiency [33, 34].

(e) Hyperbranched and dendritic polymers
Nonlinear architectures have been equally employed as antimicrobials mainly based on the high density of functional groups they can contain within the polymer structure. The most illustrative example of hyperbranched polymers with antimicrobial activity is polyethylene imine (PEI) that can be modified with both cationic and variable hydrophobic substituents to optimize the antimicrobial activity [35–37]. However, hyperbranched structures exhibit large polydispersities that impedes to systematically rationalize their interaction with the cell membranes. In order to improve this aspect several groups investigated the use of perfectly monodisperse dendritic structures such as poly(ethyleneglycol) diacrylate (PEGDA)-based dendrimers [38], quaternary ammonium-functionalized poly(propyleneimine) (PPI) dendrimers [39, 40], or amine- and ammonium-terminated carbosilane dendrimers [41].

(f) Polymers with quaternary ammonium end groups (e.g., oxazolines)
Polyoxazolines are pseudopeptides usually fabricated by living cationic ring-opening polymerization [42–44]. They exhibit good biocompatibility and can be easily end-functionalized. These types of polymers were employed by Waschinski et al. [45–47] to fabricate different series of poly(oxazoline)s end-functionalized with quaternary ammonium salts. They exhibit excellent antimicrobial properties that, however, depended on the chain length.

One of the pioneer works using quaternary ammonium groups was reported in 1984 by Ikeda et al. [48, 49] who synthesized polyvinylbenzyl ammonium chloride. The authors reported a high antimicrobial activity and hypothesized that the quaternary ammonium functional groups contained in the polymer, kill cells by damaging the negatively charged membrane [16].

In addition to quaternary amine groups, more recently other cationic functional groups have been later investigated. As a result, polymers containing primary, secondary, and tertiary amino groups which when protonated provides a positive charge have also been proposed. For example, Gelman et al. [50], fabricated polystyrene containing tertiary amine groups. In a second step, the authors protonated these groups and evidenced an antibacterial activity similar to that exhibited by quaternary amine groups. Another example was reported by Vigliotta et al. [51] synthesized polymers containing dimethylamino ethyl methacrylate (DMAEMA), a tertiary amine group that also had antimicrobial activity, due to the protonation of the amine group when it was in contact with moisture. While it is true that cationic polymers exhibit excellent antimicrobial properties, it has also been reported that

quaternary ammonium compounds can lead to hemolysis, which is the most harmful side effect of many cationic polymers.

Another alternative to cationic groups based on amines consist on the employment of phosphonium groups. In contrast to quaternary ammonium groups, recent reports suggested that the phosphonium groups are less toxic to mammalian cells and exhibit an enhanced thermal stability [52, 53]. Polymers containing phosphonium groups were employed by Dehelean et al. [54] as antimicrobial polymers. They prepared a copolymer of styrene and divinylbenzene in which they grafted quaternary phosphonium groups. According to their findings, the substituent plays a key role of the activity against bacteria. Thus, polymers with grafted ethyl phosphonium improved their activity with respect to phenyl phosphonium. Ao et al. [55] modified epoxy natural rubber with quaternary phosphonium groups and found and enhanced antibacterial activity of the resulting materials. Finally, Zhao et al. [56] fabricated terpolymers containing polyacrylamide and phosphonium groups. These polymers showed excellent antiviral activity to adenovirus (ADV). Moreover, they demonstrated that an increase in the phosphonium content produced a decrease in the Minimum Inhibitory Concentration (MIC), indicating better antibacterial activity.

3.2.2 N-*Halamine and Other Halogen Containing Polymers*

N-halamine groups have been equally extensively employed as antimicrobial over the past decade thanks to their numerous qualities such as effectiveness toward a broad spectrum of microorganisms, long-term stability, regenerability, safety to humans and environment, and low cost.

N-halamines are composed by one or more nitrogen atoms directly bonded to a halogen atom. The antimicrobial activity is explained by the interaction or transfer of positive halogen atom to a cellular receptor. The next stage is an oxidation reaction. The positive halogen atom inhibits the enzymatic activity of the cell and causes cell death. *N*-halamine compounds containing either N–Cl or N–Br moieties have shown excellent efficacies in inactivating a wide range of microorganisms [57]. *N*-halamine compounds, which contain releasable halogen atoms, have been widely used as disinfecting agents.

Actually, three main approaches of preparation are currently being employed: polymerization, generation by electrochemical route with proteins as monomers and grafting with precursor monomers [58].

Among the pioneer works, Sun and Worley designed *N*-halamine groups containing polymers to achieve long-term storage of antimicrobial chlorine [59]. More recently, other groups employed *N*-halamine as antimicrobial additives for polymeric materials. For instance, Chen et al. [60] prepared a series of 3-alkyl-5,5-dimethylhydantoin derivatives by reacting 5,5-dimethylhydantoin with alkyl bromides with different alkyl chain length (C-2 to C-22). Upon chlorination, the hydration derivatives were transformed into 1-chloro-3-alkyl-5,5-dimethylhydantoins (CADMH). CADMH were used as antimicrobial additives and the authors found

that the presence of as low as 1 % of CADMH could provide the samples with potent antimicrobial functions.

In addition to *N*-halamines, there are other halogen containing polymers with excellent antimicrobial properties. These include fluorine or chloride containing polymers. For instance, polymers bearing fluorine groups have been successfully employed due to the high chemical, thermal stability as well as its extremely low surface energy. Using fluorinated polymers, Guittard et al. [61–64] designed surfactants, called Quaterfluo®, in which perfluoroalkyl chains were introduced in the gemini structure. The antimicrobial activity tests against bacteria (*S. aureus, P. aeruginosa),* yeast (*C. albicans*), and fungus (*A. niger*) evidenced a strong antimicrobial capacity (almost no bacteria were detected after 1 h of contact).

Equally polymers functionalized with chloride groups have shown antimicrobial properties. For instance, Kugel et al. [65] modified acrylate monomers with triclosan (2,4,4′-trichloro-2′-hydroxydiphenylether) which is a well-known antibacterial and antifungal agent. Interestingly, the antimicrobial activity was improved by increasing the amount of triclosan without leaching.

3.2.3 Antimicrobial Peptides and Other Polymers Mimicking Natural Peptides

The antibacterial efficiency of antimicrobial peptides was first reported in the 1980s [1]. Based on this pioneer studies, a large number of AMPs have been discovered and evaluated. In general, AMPs are amphiphilic sequences of 5–50 amino acids with a net positive charge [66]. As a result, similar to cationic polymers, the action mechanism of AMP involves the interaction of these peptides with the negatively charged bacterial cell wall. This finally leads to an increase of the permeability causing cell apoptosis. Based on this principle, four models have been proposed [67]:

(a) Wormhole, aggregates peptide and lipid monolayers continually tempted curve through the pore so both peptides and the heads of the lipid groups.
(b) Added channel, the peptides are inserted into the membrane in aggregates without collapsing the membrane structure.
(c) The folder model in which the peptide molecules covering both sides of the cell membrane as does a detergent.
(d) Barrel stave, the peptides bind to the cell membrane, then the peptides themselves are inserted into the hydrophobic part of the membrane forming a pore, causing leakage of cytoplasmic material and cell death.

Most AMPs are made of natural amino acids and fold into a secondary structure, especially when in contact with microbial cell membrane. These AMPs have been designed and synthesized mainly to idealize the hydrophobic-cationic distribution/balance, to enhance their antimicrobial potency and selectivity, and to reduce their cytotoxicity toward mammalian cells [4].

In addition to natural occurring polypeptides, more recently, a number of non-natural peptides have been described with sequences designed to provide biologically active structures [68, 69]. For instance, facially amphiphilic peptides from amino acids that mimic both the structures and biological functions of natural antimicrobial peptides have been explored by Tew et al. [68]. This group prepared a number of facially amphiphilic acrylamide polymers having the physical and biological properties and functions of this class of antimicrobial peptides.

Most of the current antimicrobial peptides are made of α-amino acids and fold into a secondary structure, especially when they are in contact with cell membranes [70–73]. In addition to the secondary structure, AMPs are designed and made primarily to optimize the balance of hydrophobic-cationic distribution in order to improve their antimicrobial potency and selectivity against bacteria thus reducing their toxicity to mammalian cells [73–75].

In addition to α-peptides, also β-peptides have been used in order to analyze the role of the secondary structure on the antimicrobial activity. Similar to α-helical folding of AMPs, helices formed by β-peptides are relatively rigid and are often exploited to exhibit antimicrobial activity through disruption of the integrity of the microbial membrane. However, β-peptides can fold in different types of helix, depending on the local torsional stress and long-range interactions between backbone, side chains or side chains and backbone [76]. This feature, together with the cationic-hydrophobic balance, has been considered an important parameter affecting antimicrobial activity and selectivity.

Interestingly, the variation of structural parameters and thus the hydrophobic/hydrophilic balance in AMPs can be obtained by two different approaches:

(a) The first approach involves variations on the structure by varying the type of amino acids incorporated having different hydrophobic or cationic trend types [77].
The role of the amino acid configuration has been studied by Blondelle and Houghten [78]. These authors compared the patterns of helical peptides with the same amino acid composition and primarily presenting two different configurations. They investigated in greater detail the biological activities of model peptides composed of leucine and lysine residues. In particular, we have systematically examined the biological activities of leucine or lysine substitution analogues of Ac-LKLLKKLLKKLKKLLKKL-NH_2 which exhibited potent antimicrobial activities against both Gram-positive and Gram-negative bacteria. They evidenced that a large number of contiguous hydrophobic residues in an amphipathic peptide appear to be necessary for significant hemolysis to occur. Thus, shortening the hydrophobic region upon omitting any of the leucine residues or reducing the length of the peptide yielded a decrease in hemolytic activity. Replacement of individual leucine residues for lysine resulted in a dramatic decrease in hemolytic activity. Furthermore, Wiradharma et al. [77] studied the role of the variation of amino acid residues from the hydrophobic amino acids alanine, phenylalanine, and leucine and charged amino acids arginine and lysine. Their results revealed that AMPs of lysine and leucine resulted in the most selective antimicrobial activity.

(b) The second approach to provide a correct balance of cationic charge and hydrophobicity that impacts the activity and selectivity is related to the amino acid distribution. As depicted in Fig. 3.1 different configurations, i.e., sparse or secreted of facial amphiphilicity can be obtained which, in turn, maybe an important parameter to design antimicrobial helical structures [73, 79].

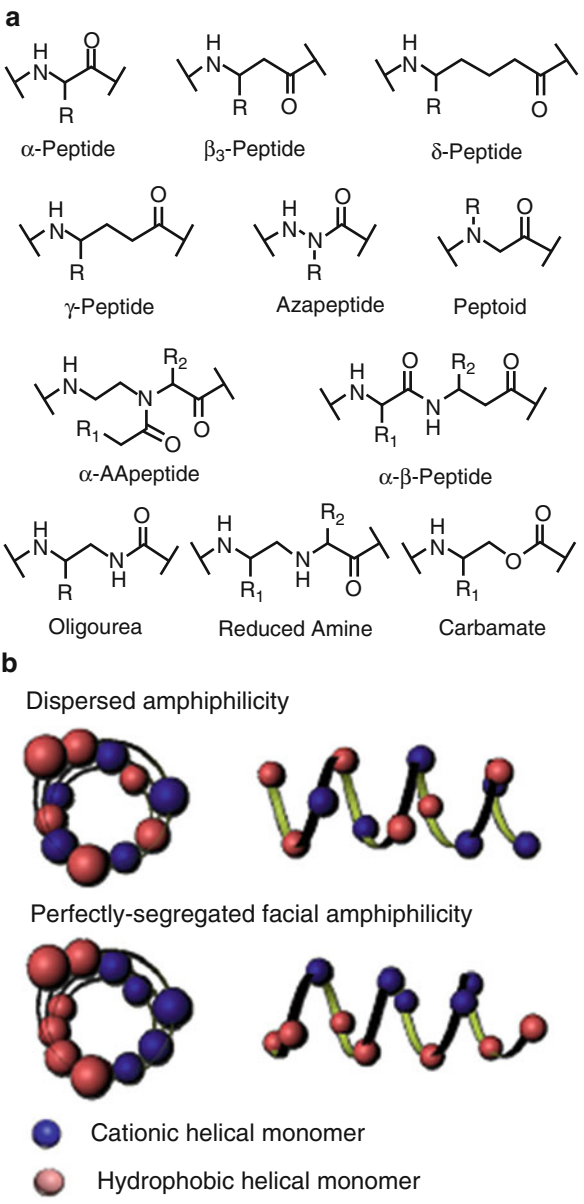


Fig. 3.1 (**a**) Macromolecular backbones able to fold into helical structures; (**b**) facial amphiphilicity either dispersed or perfectly segregated in helical structures. Reproduced with permission from [4]

Using a phospholipid bilayer membrane model, Ianoul et al. [80] showed that perfectly facial amphiphilic helical peptides interact more readily with the negatively charged dipalmitoylphosphatidylglycerol (DPPG) vesicles than with the zwitter-ionic 1,2-dipalmitoyl-sn-phosphocholine (DPPC) bilayer.

A similar approach was also shown by Zeletsky et al. [79] with peptides from nonnaturally occurring amino acids. In their report, a method based on the rational and systematic modulation of macroscopic structural characteristics on a template originating from a large number of natural, cell-lytic, amphipathic α-helical peptides was used to probe how the depths and shapes of hydrophobic and polar faces and the conformational stability affect antimicrobial activity and selectivity with respect to eukaryotic cells. Cytotoxic activity, in general, correlated strongly with the hydrophobic sector depth and required a majority of aliphatic residue side chains having more than two carbon atoms. It also correlated significantly with the size of polar sector residues, which determines the penetration depth of the peptide via the so-called snorkel effect. According to the authors, both an oblique gradient of long to short aliphatic residues along the hydrophobic face and a stabilized helical structure increased activity against host cells but not against bacteria. The mode of interaction changes radically for a peptide with a stable, preformed helical conformation compared with others that form a structure only on membrane binding. The close correlation between effects observed in biological and model systems suggests that the carpet model correctly represents the type of peptides that are bacteria selective, whereas the behavior of those that lyse host cells is more complex.

Other alternative polymers aiming to mimic the properties of natural peptides include acrylamide and phenylene ethynylene backbone polymers [68, 81–83] that are able to establish hydrogen bonding and therefore produce conformational changes or polymers fabricated by modification of polynorbornenes in which the monomers composing the polymer chain can facially amphiphilic [84].

3.2.4 Other Antimicrobial Functional Groups

In addition to the above-mentioned functional groups, other functionalities used for their antimicrobial performance in polymers have been reported [7, 85]. One illustrative example is the use of sulfonium groups. Sulfonium functional groups resemble somehow quaternary ammonium group since they exhibit the same charge but some promising studies have showed less toxicity than ammonium [86].

Zwitterionic polymers have also been explored as antimicrobial bear charged monomer units having simultaneously a positive and a negative charge and thus exhibiting a net neutral charge. Some of these studies are conducted by Low et al. [87] that studied a number of these polymers and some of them have shown antimicrobial activity toward *S. aureus* and *E. coli*. Other studies using zwitterionic

polymers were made by Jiang et al. [88]. Many of these polymer systems have been equally tested for their low binding of bacteria to surfaces and their interesting antifouling properties.

Finally, other examples of chemical groups introduced into polymer structures to provide antibacterial activity are: nitric oxide (NO) that has also been used as antimicrobial group in some polymers [89] or silver [90] and silica [91] compounds that have also been extensively used in combination with polymers as antimicrobial groups.

Finally, another class of functional polymers also currently explored for their antimicrobial performance; they are guanidines and biguanides containing polymers. Polyguanidines and polybiguanides are attracting extensive attention as antimicrobial compounds, mainly due to their excellent solubility in aqueous solution, high biocidal efficiency against a wide variety of microorganisms and non-toxicity. The synthesis usually is carried out by two different alternatives. On the one hand, these polymers can be obtained by reaction of a diamine with chlorcyan, cyanamid, or dicyanamid (polybiguanides). On the other hand, they can be prepared by polycondensation of a guanidinium salt with a diamine [92–94, 99].

3.3 Synthetic Strategies to Prepare Antimicrobial Polymers

There are two ways in order to have antimicrobial functional groups in polymers.

(a) The first one is to polymerize monomers containing these antimicrobial functional groups.
(b) The second one is modifying the final polymer structure by post-polymerization reaction. In this last approach, it is possible to modify the structure maintaining the original molecular weight.

As has been depicted above, there exists a wide variety of monomers with antimicrobial activity that can be incorporated into polymeric structures using different polymerization techniques. In principle, any polymerization approach can be employed to produce antimicrobial polymers. In this context, anionic and cationic polymerizations as well as radical polymerization techniques have been widely employed to prepare these polymers. While it is true that different alternatives available nowadays to construct polymers, most syntheses to fabricate antimicrobial polymers are performed by free radical polymerization [15]. Conventional synthesis techniques are used especially for homopolymers or random copolymers.

The synthesis of well-defined polymer structures require, however, advanced polymerization techniques. These techniques include living/controlled free radical polymerization. Atom transfer radical polymerization (ATRP) and reversible addition fragmentation chain transfer polymerization (RAFT) are mostly used in these approaches to obtain amphiphilic block copolymers and several polymers with specific topologies [95, 96].

In addition, for some monomers ring-opening polymerization has been used, for instance in the preparation of antimicrobial peptides from α-amino acid-*N*-carboxyanhydrides (NCA) [97].

Finally, Sampson and coworkers [98] explored the effect of charge positioning with reference to the other charge groups by using polymeric mimics based on hydrocarbon backbones generated by ring-opening metathesis polymerization (ROMP). Through controlled alternating ROMP, systematic variations of the arrangement and spacing between the cationic pendants were achieved to further probe the structural effects on antimicrobial activity.

3.4 Interactions Between Bacteria and Polymeric Materials: Role of the Macromolecular Parameters on the Antibacterial Activity

As has been illustrated in Sect 3.2, different types of functional groups included in the polymer have been explored for their antibacterial activity. However, in addition to the chemical groups involved other macromolecular parameters such as molecular weight, hydrophilic/hydrophobic balance among others also play a key role and define the success or not of a particular active group. In this section, we will review the macromolecular parameters involved in the bacterial interaction.

3.4.1 Hydrophilic/Hydrophobic Balance

One of the most important parameters in designing antimicrobial polymers is the amphiphilicity (i.e., the hydrophilic/hydrophobic balance). The amphiphilicity affects not only the final antimicrobial but also the selectivity in the presence of mammalian cells since it is related to the manner in which the polymer interacts with the cell membrane. Typically, in order to favor the interaction between the polymer and the bacterial surfaces the hydrophilic moiety is typically positively charged. Moreover, the hydrophobic moiety is typically an alkyl group that forms the main chain of the polymeric structure [4]. As a result of this configuration, the negatively charged cell membrane will interact with the positively charge moieties, and the hydrophobic main chain will be in contact with the lipid domains of the membrane [99].

In spite of this general structure, the hydrophilic and hydrophobic interactions can be evaluated by the partition coefficient "ethanol/water" of the polymer [100]. It has been found that when the hydrophilic moiety is large, the copolymer binds better to the cell membrane. However, when the hydrophobic moiety is too large polymers have been proven to be toxic to all cell types and the selectivity is lost. Thus, the hydrophobic moiety is directly related to the toxicity of these materials [101].

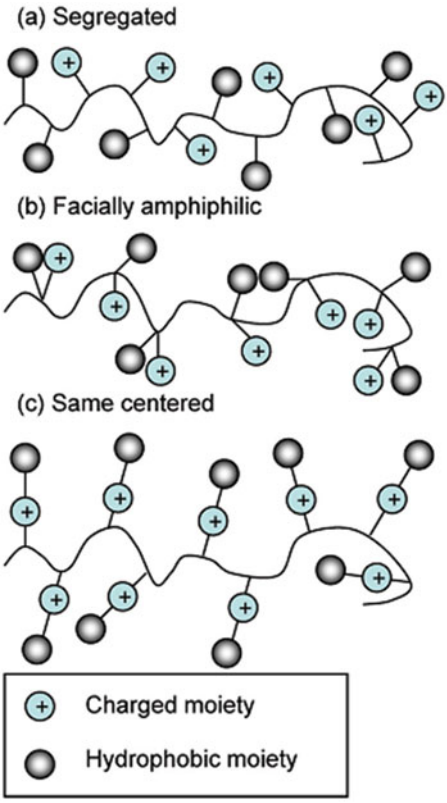
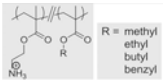
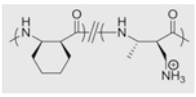
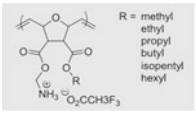
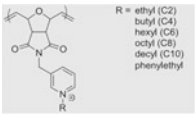
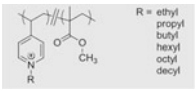


Fig. 3.2 Schematic depiction of the main strategies reported for balancing hydrophobicity and hydrophilicity. Reproduced with permission from [4]

As evidenced above, it is therefore crucial to find an appropriate balance between the hydrophilic/hydrophobic moieties. For this purpose, different strategies have been reported to vary the structure of the polymer and thus reach an appropriate balance hydrophilic/hydrophobic right (Fig. 3.2 and Table 3.1). The main alternatives as reported by Engler et al. [4] are:

(a) The first alternative involves the copolymerization of two different monomers, i.e., nonpolar monomer and charged monomer to produce a statistical copolymer. This strategy has been named the "segregated monomer" approach. As a result, the hydrophilic/hydrophobic balance can be easily modulated depending on the feed composition.
(b) The second approach resort to the use of "amphiphilic face" monomers, i.e., monomer having a nonpolar part and a cationic part to construct the polymer chain.
(c) Finally, the third approach, known as "same centered," employs monomers having an alkyl chain attached to a positive charge. It is important to note that, in (b) and (c) the hydrophilic/hydrophobic balance can be modified by using alkyl chain with variable lengths.

Table 3.1 Examples of structures of polymers with variable hydrophobic/hydrophilic balance

	General structure	Microbes tested	Reference
Segregated monomer		*E. coli*	[102]
		*E. coli*, *B. subtilis*, *S. aureus*, *E. facium*	[103]
Facially amphiphilic polymers		*E. coli*, *S. aureus*	[104]
Same-centered polymers		*E. coli*, *B. subtilis*	[105]
		*E. coli*, *B. subtilis*	[106]

Reproduced with permission from [4]

3.4.2 Molecular Weight

While the molecular weight has been demonstrated to be a key parameter on both antimicrobial and hemolytic activity, the results reported depend on the type of polymer employed and the cells explored to a large extent. The interaction between polymers and bacterial cells largely depends on the polycation charges thus suggesting that high-molecular weights give better selectivity. This is due to the increase in the forces of electrostatic attraction that simultaneously provide a better antimicrobial activity due to the increase of the hydrophobic moieties that penetrate the lipid membrane of the cell walls. However, two important drawbacks associated to high-molecular weight need to be mentioned. On the one hand, with increasing molecular weight, parameters such as solubility, diffusion and aggregation in the biological medium, and cell wall barrier become important. On the other hand, higher molecular weights are typically accompanied by not only an increase in antimicrobial activity but also an increase in hemolytic activity.

Considering the three ways of preparing monomers "segregation monomer," "same centered," and "facially amphiphilic," a direct relationship between the antimicrobial ability of polymers depending on their molecular weight was observed. However, different trends were observed depending on the type of monomer used and the macromolecular topology. For instance, Gabriel et al. [107] evidenced that facially amphiphilic (FA) monomers led to polynorbornenes with excellent antimicrobial activities and selectivities. On the contrary, polymers obtained by copolymerization

of structurally similar segregated monomers, in which cationic and nonpolar moieties reside on separate repeat units, led to polymers with less pronounced activities.

Moreover, in some cases even the same topology depicts contradictory trend depending exclusively on the monomer used. Several research groups including such Kuroda et al. [100] and Chan-Park et al. [97] evidenced different trends for different polymers. In the report of Kuroda and coworkers [100], they described the effect of molecular weight on the methacrylate polymers prepared by the segregated monomer approach. In their studies, they observed that both antimicrobial activity against Gram-negative bacteria *E. coli* and the hemolytic activity increased as molecular weight increased. On the contrary, substitution of methacrylates by the poly(norbornene) significantly changed their results. In this case, the lower molecular weight polymers were more active against all microbes tested and less hemolytic [107].

3.4.3 Polymer Topology

A crucial difference between small molecules of antimicrobial agents and macromolecular antimicrobial agents in addition to the previously mentioned molecular weight or functional groups is that in the macromolecular systems different types of architectures (topologies) can be fabricated. Examples of different topologies, including homopolymers, random copolymers, block copolymers, branched polymers, and ionic or zwitterionic telechelic polymers, are depicted in Fig. 3.3 [108].

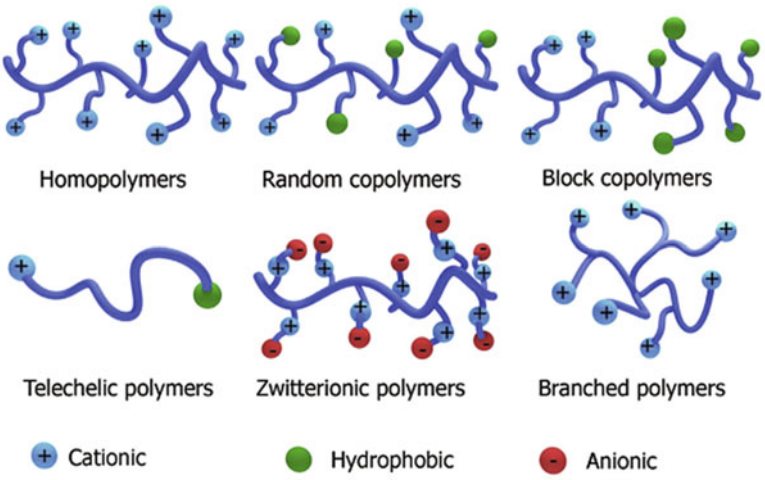


Fig. 3.3 Commonly employed polymeric architectures to prepare antimicrobial polymers. Reproduced with permission from [108]

3.4.3.1 Homopolymers/Copolymers/Telechelic Polymers

There are several types of homopolymers/copolymers used as antimicrobial polymers depending on the placement of the polar groups (same-centered, facially amphiphilic, and segregated). The correct hydrophilic/hydrophobic balance results in adequate antimicrobial activity and good selectivity between bacterial and mammalian cells, i.e., low hemolytic [4].

In addition to homo-/copolymers, telechelic polymers have been taken great interest to design molecules with antimicrobial activity. Telechelic polymers, generally prepared from multifunctional initiators typically by living polymerization techniques, have a reactive functionality in both their ends. An illustrative example of the use of these types of polymers with antimicrobial activity was reported by Waschinski et al. [45] who used polyoxazoline that contain amino functionalities in their structure.

3.4.3.2 Block Copolymers Versus Random Copolymers

The arrangement of the polar and nonpolar groups within the macromolecules also influences their antimicrobial activity. Two different main situations have been explored. On the one hand, random copolymers are typically obtained by copolymerization of a hydrophobic monomer with other hydrophilic (typically cationic). On the other hand, block copolymers are formed by covalently bonding segments of two distinct polymers.

While it is true that random copolymers exhibit good antimicrobial activity, they often exhibit high hemolytic activity. The toxicity of cationic copolymers may result in adverse effects to the host. In this regard, Yang et al. [109] have reported the synthesis of random copolymers incorporating antimicrobial methacrylic acid and 2-aminoethylmethacrylate hydrophobic methacrylate. These polymers can be acid activated, but, under normal physiological pH (neutral) have little hemophilic activity; whereas at acid pH they are active toward bacteria. Likewise, they have also reported random copolymers with improved antimicrobial selectivities [110].

In the case of block copolymers, they exhibit a large tendency to form nanoobjects in solution upon self-assembly. By controlling the molecular weight of the blocks and the use of specific monomers precisely, it controls the amphiphilic balance. Their use as antimicrobials is rare. This may be due to the low values of critical micelle concentration (CMC) of these amphiphilic block copolymers.

Reports regarding their antimicrobial activity of block copolymers in solution have indicated a manner similar to random copolymers containing the same type of monomer [111] activity. However, there has been a decrease in hemolytic activity. Therefore, it has not seen a marked difference between these two types of copolymers except for their hemolytic activity.

3.4.3.3 Dendrimers and Brush Polymers

Dendrimers and brush polymers with well-defined architectures have equally explored as antimicrobial macromolecules. Both structures exhibit antimicrobial activity as reported by Ortega et al. [112]. They reported a family of amine- and ammonium-terminated hyperbranched polycarbosilanes (PCS) and dendrimers has been synthesized. The functionalization of a polycarbosilane matrix was carried out with peripheral allyl groups by two strategies in the case of PCS: (1) hydrosilylation of allyl amines with PCS containing terminal Si–H bonds, or (2) hydrosilylation of PCS–allyl with an aminosilane. Dendrimers with terminal amine groups were synthesized by hydrosilylation of allyl dimethylamine and quaternized systems with MeI. The antibacterial properties of the ammonium-terminated hyperbranched polycarbosilanes and dendrimers demonstrated that they act as potent biocides against Gram-positive and Gram-negative bacterial strains [112].

Linear and branched structures have been used to increase the valency of short, active peptides. For instance, Kallenbach et al. [113] tested several series of multivalent AMPs and compared with the natural AMP, indolicidin. The macromolecular architectures reported include multivalent displays with different sequences, repeats, and scaffolds, including dendrimers, brush-like structures, and polymeric displays constructed by linking various peptides to polymaleic anhydride (PMA). As a result, they evidenced that branched tetramer of dipeptides (RW)4D demonstrates the highest level of effectiveness. More interestingly, they later found that the dendrimer (RW)4D preferentially kills Gram-negative bacteria relative to Gram-positive bacteria. This response differs from many natural AMPs, not only Arg- and Trp-rich peptides. (RW)4D thus confers different specificity by a putative membranolytic mechanism [114]. Moreover, they also show that the dendrimer inhibits bacterial growth in both planktonic and biofilm states [115].

In a recent study, Yang et al. [116] fabricated linear, 2-arm branched, and 4-arm star-like peptides and evaluated their antimicrobial and hemolytic activities (Fig. 3.4). Branching has been demonstrated to enhance antimicrobial activity and reduce undesired hemolysis, leading to better selectivity toward microbes over mammalian cells.

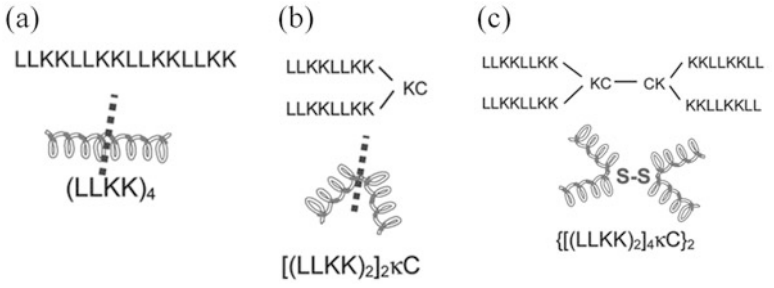


Fig. 3.4 Schematic and mass spectra of (**a**) linear $(LLKK)_4$, (**b**) 2-arm branched $[(LLKK)_2]_2\kappa C$, and (**c**) 4-arm starlike ($[(LLKK)_2]_2\kappa C\}$) α-helical peptides [116]

A large variety of parameters can be varied in the fabrication of antimicrobial dendrimers. In addition to the variable, density of functional groups provided by the dendrimer generation antimicrobial dendrimers can also be amphiphilic in nature [4]. In this case, the backbone contains hydrophobic segments or cavities whereas the polar groups at the periphery are typically amine or quaternary ammonium functionalization of its periphery [39, 41, 112] or from amine-containing branches with poly(ethylene glycol) groups [117, 118].

Amphiphilic dendrimers exhibit, in addition, several specific factors that affect antimicrobial efficacy. In an excellent report, Cooper et al. [39] reported that antimicrobial activity of quaternary ammonium-terminated poly(propyleneimine) dendrimers (PPIs) against *E. coli* did not increase monotonically with dendrimer generations. On the contrary, the antimicrobial activity varied according to G5>G4>G1>G2>G3 proceeding from strongest to weakest. This trend was explained by Cooper et al. by the contribution of two factors, i.e., the ability to penetrate bacterial cell membranes and charge density.

On the one hand, in the lower generations the molecular weight of the dendrimer is rather low, which according to the authors induced antimicrobial activity by diffusing across the cellular membrane affecting intracellular pathways. On the other hand, for higher generation dendrimers an increasing high charge density is available at the periphery of the dendrimer that may enhance the interaction with the cell membrane inducing the lysing of the membrane and inhibiting bacterial growth.

In addition to the generation and the molecular weight, the variation of the chemical functionality/charge on the periphery of the dendrimer has also been demonstrated to affect the antimicrobial activity. For instance, Cai et al. [118] described the modification of poly(amidoamine) (PAMAM) with polyethylene glycol (PEG) chains. In their study, they showed that 43 % PEGylation of a G5 amino-terminated PAMAM dendrimer abolished its antimicrobial activity against Gram-positive *S. aureus*. However, the antibacterial activity appears to be selective against Gram-positive bacteria. In effect, the same degree of PEGylation did not significantly alter its antimicrobial activity against Gram-negative *P. aeruginosa*.

Another alternative was explored by Kannan et al. [119] studied the bactericidal activity of hydroxyl and carboxylic acid-terminated PAMAM dendrimer was evaluated against Gram-negative *E. coli* and compared with amine-terminated PAMAM dendrimers. The G4-PAMAM dendrimers effectively inhibited growth of *E. coli* with the antimicrobial activity decreasing in the order of G_4-NH_2, G_4-OH, and $G_{3.5}$-COOH [119] (Fig. 3.5). The G_4-PAMAM-NH_2 dendrimer is known to be potent antibacterial agent, however, it was found to be highly cytotoxic to above 10 μg/mL to human cervical epithelial (End1/E6E7) cells and immune cells (BV-2) while the G_4-OH dendrimer was noncytotoxic up to 1 mg/mL concentrations to both cell lines. The authors proposed different action mechanisms depending on the functional groups introduced in the dendrimer. The possible mechanisms involve the G_4-PAMAM-NH_2 acting as polycation binding to the polyanionic lipopolysaccharide, the G_4-PAMAM-OH binding via hydrogen bonds to the hydrophilic O-antigens and the $G_{3.5}$-PAMAM-COOH acting as a polyanion chelating the divalent

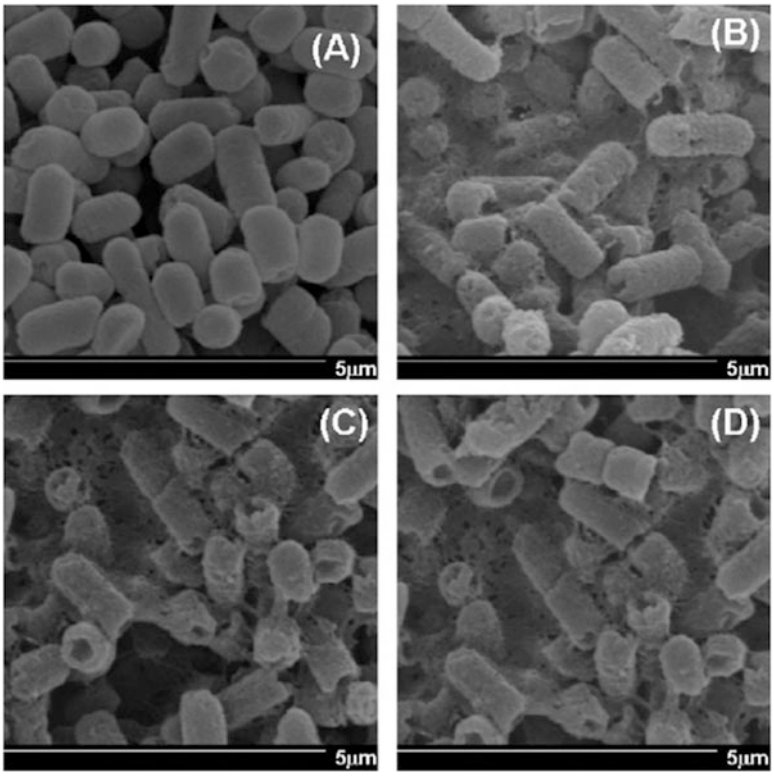


Fig. 3.5 SEM images of *E. coli*. (**a**) Untreated *E. coli*, (**b**) 8 h treatment of $G_{3.5}$-PAMAM-COOH, (**c**) 8 h treatment of G_4-PAMAM-OH, and (**d**) 8 h treatment of G_4-PAMAM-NH_2. Scale bars indicate 5 µm. The treatment with dendrimers shows the damage to the bacterial cell wall. Reproduced with permission from [39]

ions in outer cell membrane. One of the major findings concerns the bactericidal effect of G_4-PAMAM-OH dendrimer and its ability to treat *E. coli* infections in vivo in pregnant guinea pigs [119].

The polyvalency of branched dendrimers and brush-like structures provide a high antimicrobial efficacy. In the case of using peptides, by branching α-helical peptides, improved both selectivity and antimicrobial activity can be obtained. However, major drawback efforts need to be made to reduce production costs and ensure batch-to-batch reproducibility before wide range of clinical applications can be realized [4].

3.4.4 Monomer Derivatization with Alkyl Chains: Spacer Length and Alkyl Chain Effect

It is reasonable that the antimicrobial activity is dependent on the spacer length due to change in both conformations and charge density of the polymer, which obviously affects the mode of interaction with the membrane. In this regard, Nonaka et al. [120] has found that increasing the length of the spacer also enhances the antimicrobial activity.

Cooper et al. [39] reported that antimicrobial activity of quaternary ammonium-terminated poly(propyleneimine) dendrimers (PPIs) against *E. coli* is clearly influenced by the alkyl chain length of quaternary ammonium compounds. In their study, alkyl chain length of quaternary ammonium compounds was systematically varied from C_8 to C_{16} and counterion effect was investigated. When studying antimicrobial activity as a function of alkyl chain length, a parabolic trend was observed, with C_{10} having the optimal antimicrobial activity. In addition, dendrimers with bromide counterions had a higher antimicrobial activity compared to those with chloride counterions [39].

3.4.5 Other Macromolecular Parameters Involved in the Antibacterial Activity

Additional parameters that have an effect on the antimicrobial activity of the polymers are:

(a) *Cationic group:* As has been depicted, cationic groups have been largely employed to favour the interactions with the bacterial membrane. Although the effects of varying the type of charged group on antimicrobial activity has been extensively studied, there are few examples to investigate their effects on the hemolytic activity and cell toxicity. In this sense, several cationic groups have been explored [4]. For instance, in natural antimicrobial polypeptides, this charging unit is either a primary amine or a guanidine group. Other types of cationic groups have been explored including quaternary ammonium salts and phosphonium [3].

There are several examples which have used different types of amines: primary, secondary, tertiary, and quaternary ammonium [121]. In general, these studies found that primary amines exhibit antimicrobial activity with low hemolytic activity.

(b) *Charge density and their localization:* The charges present in the monomers that compose the polymer play a crucial role in the antimicrobial activity. This effect has been evidenced by Al-Badri et al. [122] and reported that when the charge density in a moderately hydrophobic polymer increased, the antimicrobial activity remained constant while decreasing their hemolytic activity. However, an increase of the amount of charge within the polymer structure resulted in an increase of the antimicrobial activity but they were hemolytic.

The localization of the charged groups within the polymer chain has also an effect on the antimicrobial and hemolytic activity. Sampson et al. [98], using both random and alternating polymers, found that polymer structures in which the charges are regularly spaced with distances between 6 and 8 carbons was optimal for both antimicrobial activity and selectivity.

Additionally, the location of cationic polymer group has a pronounced effect on both antimicrobial and hemolytic activities. Chen et al. [123, 124] prepared cationic polymers from quaternary ammonium containing poly(*N*,*N*-dimethylaminoethyl methacrylate) with natural rosin as the pendant groups (PDMAEMA-g-rosin).

They prepared first rosin-grafted polymer, in which the quaternary ammonium group was located at the periphery of the entire polymer [124]. In this polymer, the cationic groups were located on the termini of the pendant moiety, acting like small needles. Thus, this cationic polymer could be easily absorbed onto the bacterial cell surface through electrostatic interactions and subsequently diffuse through the cell wall and kill bacteria. On the contrary, when the positive charges are sandwiched between the PDMAEMA backbone and rosin moiety there could exist a significant steric hindrance effect from its pendent rosin moiety to impede the interaction of the polycation with the bacterial cell wall (Fig. 3.6).

(c) *Polar neutral groups:* The polar neutral groups have been used to reduce the hemolytic activity of antimicrobial polymers, e.g., polyethylene glycol as reported by some researchers [125]. They established that the incorporation of hydrophilic groups in biocompatible cationic polymers improve the biocompatibility of the molecules. However, a proper balance must be maintained to preserve the antimicrobial activity.

(d) *Counterion effect:* Counterions influence cationic polymers antimicrobial activity in terms of their solubility and ion-pair formation. The effect of counterions in antimicrobial polymers has been rarely studied. One of these studies, carried out by Kanazawa et al. [126] concluded that a weak ion pairing and hydrophilic anions may enhance the antimicrobial activity of cationic macromolecules.

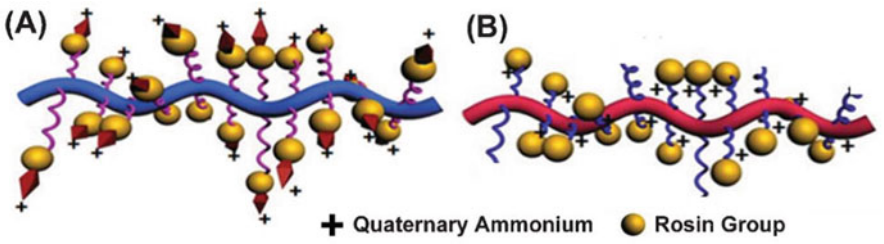


Fig. 3.6 A comparison between two amphipathic structures having cationic charges at different locations with respect to the rosin moiety and polymer backbone: (**a**) polymer with cationic charges located at the periphery [124]; (**b**) a polymer cationic charges embedded inside [123]. Reproduced with permission from [123]

3.5 Evaluation of the Antimicrobial Activity: In Vitro Testing

In view of the growing interest in the development of novel antimicrobial substances, a simultaneous interest has been focused in the elaboration of methodologies to screen and evaluate their antimicrobial activity [127].

Antibacterial properties of antimicrobial materials and products are usually tested in in vitro test systems. Various different methods, such as the ISO 22196/JIS Z 2801 or ASTM E 2149, ASTM E 2180 are often used for testing the antimicrobial properties of materials and products. However, the conditions and setup parameters differ significantly between the test methods. There are many factors influencing the efficiency of the antimicrobial additive in a given polymer. Among the most prominent key factors are the bacteria strains used for testing, the number of bacteria for inoculation of the test samples, volume of the bacterial inoculum, incubation time, temperature, and humidity. Each test method uses its own specific setup conditions, possibly leading to disparate results. Therefore, it is essential to choose the right assay to test antimicrobial materials under meaningful conditions for the respective material or product. The widely used agar diffusion assay, minimum inhibitory concentration assays (MIC), or the adherence tests are restricted to leachable additives [35].

As reported recently by Balouiri et al. [128], the most relevant methods are:

(a) Diffusion methods

Among the diffusion methods the agar-disk diffusion is a routine testing to measure antimicrobial susceptibility generally employed in microbiology laboratories. This method has several advantages in comparison to other methods including its simplicity, the ability to test enormous numbers of microorganisms, low cost as well as the ease to interpret results observed [129, 130].

This method involves the inoculation of agar plates with a standardized inoculum of the evaluated microorganism. Consequently, filter paper discs (around 6 mm in diameter) are impregnated with the test compound at particular concentrations and positioned on top of the agar surface. During the incubation (using suitable conditions), the antimicrobial agent diffuses into the agar and prevents both the germination and growth of the test microorganism. By measuring the diameters of inhibition growth zones, the activity can be estimated. The diameters of the inhibition zones have standardized to several bacterial pathogens including *streptococci*, *Haemophilus parainfluenzae*, *Haemophilus influenzae*, *Neisseria meningitidis*, and *Neisseria gonorrhoeae*. For this purpose, specific culture media, various incubation conditions, and interpretive criteria for inhibition zones have been employed [129]. However, this method does not result appropriate to define the minimum inhibitory concentration (MIC) since it is difficult to determine the quantity of the antimicrobial agent that has diffused into the agar medium. It is worth mentioning that the MIC value is defined as the lowest concentration of the assayed antimicrobial agent that inhibits the visible growth of the microorganism tested usually expressed in mg/mL or mg/L.

In order to determine the MIC, the antimicrobial gradient method that combines the principle of dilution methods with that of diffusion methods is more appropriate. In this methodology, a strip incorporating an increasing concentration gradient of the antimicrobial agent from one end to the other is placed on the agar surface, in which the microorganism to be tested has been previously inoculated.

Other diffusion methods include agar well diffusion method [131, 132], agar plug diffusion method [133, 134], cross streak method [135], and the poisoned food method [136–138].

(b) Thin-layer chromatography (TLC)—direct bioauthography
While several variants using TLC have been described, the direct bioauthography is the most widely employed method among these methods. In this approach, the TLC plate is either dipped into a microbial solution or sprayed with a microbial suspension, and the bioautogram is incubated at 25 °C for 48 h under humid conditions [139]. In order to follow the microbial growth, tetrazolium salts, e.g., *p*-Iodonitrotetrazolium violet are employed [140]. These salts undergo a conversion to intensely colored formazan by the dehydrogenases of living cells [141, 142]. For that purpose, these salts are sprayed onto the bioautogram that is again reincubated either at 25 °C for 24 h [143] or at 37 °C for 3–4 h [144] depending on the experimental protocol followed.

(c) Dilution methods
The dilution approaches are the most suitable for the analysis of the MIC values. These approaches enable the estimation of the concentration of the tested antimicrobial agent in the agar (agar dilution) as well as in the broth medium (macrodilution or microdilution). In order to quantitatively measure the in vitro antimicrobial activity against bacteria and fungi both approaches, broth or agar dilution method can be employed.

In spite of the many different guidelines approved to carry out these tests, the most generally accepted standards are those provided by the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST).

Following these standards, for instance, in the broth dilution method, the method comprises the preparation of twofold dilutions of the antimicrobial agent in a liquid growth medium dispensed either in tubes (minimum volume of 2 mL—macrodilution) or with smaller volumes using 96-well microtitration plate (microdilution). Each tube or well is then inoculated with the microbial solution prepared after dilution of standardized microbial suspension usually adjusted to 0.5 in the McFarland scale. Upon mixing, the tubes/plates are incubated under suitable conditions depending upon the test microorganism.

(d) Time-kill tests
Time-kill tests are generally employed to estimate bactericidal or fungicidal effect of a particular antimicrobial. It is particularly powerful in obtaining information about the dynamics of the interactions between the microbial strain evaluated and antimicrobial biocide employed. Depending on the information required, this test displays either a time-dependent or a concentration-dependent

antimicrobial effect [145]. In the case of bacteria, this test is now standardized in the M26-A document of CLSI [146]. By using this protocol, the experiment is carried out in broth culture medium by using three tubes containing a bacterial suspension of 5×10^5 CFU/mL. The first and the second tubes contain the antimicrobial molecule/polymer at concentrations of 0.25 and 1 MIC. The third tube is employed as growth control. The incubation is carried out under suitable conditions at different intervals (0–24 h) [145, 147], and then the percentage of dead cells is calculated in comparison with the growth control.

In Table 3.2 are briefly summarize the experimental conditions recommended by the CLSI to estimate the antimicrobial susceptibility.

The above-mentioned tests are usually employed to leaching materials as well as antimicrobials soluble in the culture media. However, for nonleaching antimicrobial systems these methods cannot be employed [127]. In this case, alternative methods have been proposed such the ISO 22196/JIS Z 2801 [152] in order to evaluate the antibacterial activity on plastic surfaces or the ASTME2180 [153] to determine the activity of Incorporated Antimicrobial Agent(s) in Polymeric or Hydrophobic Materials. These tests are based on the inoculation and recovery of bacteria from test specimens after a well-defined time. While these methodologies are widely extended, they have a major drawback concerning the geometries they require. More precisely, these tests are carried out using flat geometries with sizes from ~3 × 3 cm up to 5 × 5 cm. However, there are many commercial products that could benefit from the use of antimicrobial polymers with other geometries.

An alternative to these methodologies for the evaluation of the antimicrobial efficiency in other geometries is the Certika assay [154]. In addition to the geometry, this method permits the evaluation of the antimicrobial effectiveness of both leaching and nonleaching additives. This method is based on the reproduction and release of daughter cells over a period of 18 h after inoculation [127]. Independently of the antimicrobial mechanism, the effect for bacterial growth on the surface is evaluated by the release of vital daughter cells, which are, in turn, those that will be at the origin of further infections. The growth daughter bacteria can be monitored over time and the antimicrobial activity will be determined by the time needed to reach a defined optical density (OD). The latter is directly related to the number of released cells and antimicrobially active materials will be compared with untreated controls. If the surface exhibit antimicrobial properties, a delayed or even inhibited growth will be observed.

3.6 Conclusions

This chapter summarized the most significant aspects related to the types of functional groups that can be employed as antimicrobial agents as well as their incorporation to form larger macromolecules. Natural antimicrobial peptides and polymers exhibit better antimicrobial properties in comparison with low-molecular weight antibiotics.

Table 3.2 Culture media, microbial inoculum size, and incubation conditions for antimicrobial susceptibility testing methods as recommended by CLSI

Methods	Microorganism	Growth medium	Final inoculum size	Incubation temperature (°C)	Incubation time (h)	References
Disk-diffusion method	Bacteria	MHA	(0.5 McFarland) $(1–2)\times10^8$ CFU/mL	35 ± 2	16–18	M02-A [129]
	Yeast	MHA + GMB[a]	(0.5 McFarland) $(1–5)\times10^6$ CFU/mL	35 ± 2	20–24	M44-A [130]
	Molds	Non-supplemented MHA	$(0.4–5)\times10^6$ CFU/mL	–	–	M51-A [148]
Broth microdilution	Bacteria	MHB	5×10^5CFU/mL	35 ± 2	20	M07-A [149]
	Yeast	RPMI 1640[b]	$(0.5–2.5)\times10^3$CFU/mL	35	24–48	M27-A [150]
	Molds	RPMI 1640[b]	$(0.4–5)\times10^4$ CFU/mL	35	48 for most fungi	M38-A [151]
Broth macrodilution	Bacteria	MHB	5×10^5 CFU/mL	35 ± 2	20	M07-A [149]
	Yeast	RPMI 1640[b]	$(0.5–2.5)\times10^3$ CFU/mL	35	46–50	M27-A [150]
	Molds	RPMI 1640[b]	$(0.4–5)\times10^4$ CFU/mL	35	48 for most fungi	M38-A [151]
Agar dilution	Bacteria	MHA	10^4 CFU/spot	35 ± 2	16–20	M07-A [149]
Time-kill test	Bacteria	MHB	5×10^5 CFU/mL	35 ± 2	0, 1, 18 and 24	M26-A [146]

Reproduced with permission from [128]

MHA Mueller Hinton Agar, *MHB* Mueller Hinton Broth

[a]GMB: the medium was supplemented with 2% glucose and 0.5 mg/mL methylene blue

[b]RPMI 1640: Roswell Park Memorial Institute medium (with glutamine, without bicarbonate, and with phenol red as a pH indicator) was 1640, buffered to pH 7.0 with MOPS (morpholine propane sulfonic acid) at 0.165 M

Macromolecules inhibit and/or kill pathogens with a significantly lower likelihood of inducing drug resistance in the long term.

More importantly, the distribution of the active moieties, the molecular weight, or the topology that largely determine the activity of the macromolecule can be easily modulated.

However, as depicted by Engler et al. [4] several aspects require consideration for further developments including synthesis strategies, safety and efficacy evaluations, and formulation issues.

For instance, concerning the synthetic strategies from the examples analyzed in this chapter, it can be concluded that highly branched structures and/or nanostructures have an increasing cationic charge valency thus resulting both in an enhanced antimicrobial activity and selectivity.

Finally, recent progresses have been made in the development of synthetic-targeted antimicrobials. These systems may confer the ability to seek out and kill specific strains of microbes. This is a highly promising development in the emerging field of synthetic macromolecular antibiotics [155].

A large variety of applications can be envisaged for performant antimicrobial polymers that included medical device coating, intravenous formulations as well as in ointments and creams just to mention a few of them.

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Chapter 4
Nano-Micro Polymeric Structures with Antimicrobial Activity in Solution

Abstract Pioneer strategies to combat infectious diseases focused on the improvement of pharmacokinetics of the antibiotics by prolonging their blood circulation. These initial approaches permitted the antibiotic to reach difficult-to-target sites of infection and, as a consequence, to reduce dose frequency of antibiotics and more interestingly to reduce undesired rapid clearance of therapeutic agents. However, this strategy can only be accomplished in combination of the advancement of the appropriate techniques both in chemical synthesis and the understanding of macromolecular chemistry.

This chapter describes the alternatives to fabricate nanometer scale polymeric structures with antimicrobial properties. In particular, we will describe the different alternatives developed to produce efficient antimicrobial polymer nanostructures in solution.

Organic (based on polymers) or hybrid inorganic/organic nanostructures have peculiar properties that distinguish them from materials structured at the micro scale. In particular, their large surface area to volume ratio may enhance the interaction of the nanostructured material with a given microbe as a result of a larger number of functional sites. The most studied antimicrobial nanostructures in solution are nanoparticles and within nanoparticles those made of silver have been extensively explored.

Moreover, antimicrobial polymers and, in particular, the nanostructures resulting from the self-assembly processes in solution has been recently demonstrated to be of interest for different applications including animal and human health care. Of particular interest are those cases in which the polymers form self-assembled nanostructures with a large concentration of antimicrobial moieties. Moreover, these self-assembled structures are able to incorporate other additional antimicrobials such as silver nanoparticles.

Keywords Self-assembly • Block copolymers • Hybrid nano-assemblies • Polymeric nanocapsules • Nanoparticles • Core/shell nanoparticles

4.1 Introduction

Pioneer strategies to combat infectious diseases focused on the improvement of pharmacokinetics of the antibiotics by prolonging their blood circulation. This strategy would permit the antibiotic to reach difficult-to-target sites of infection [1, 2]

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and, as a consequence, to reduce dose frequency of antibiotics [3, 4] and more interestingly to reduce undesired rapid clearance of therapeutic agents [5]. However, this strategy can only be accomplished in combination of the advancement of the appropriate techniques both in chemical synthesis and the understanding of macromolecular chemistry [6].

As has been depicted in Chap. 3, amphiphilic macromolecules have been demonstrated to be excellent candidates for antimicrobial purposes. In this context, Engler et al. [7] established the criteria that must be encompassed to have effective antimicrobial polymeric materials. As a result, efficient antimicrobials include a sufficient contact with the microbes, cationic charge to promote adhesion to the microbial cell envelope and, finally, a hydrophobic moiety that will be integrated into the cellular membrane.

Organic (based on polymers) or hybrid inorganic/organic nanostructures have peculiar properties that distinguish them from materials structured at the micro scale. In particular, their large surface area to volume ratio may enhance the interaction of the nanostructured material with a given microbe as a result of a larger number of functional sites. The most studied antimicrobial nanostructures in solution are nanoparticles and within nanoparticles those made of silver have been extensively explored.

In this context, antimicrobial polymers and in particular the nanostructures resulting from the self-assembly processes in solution has been recently demonstrated to be of interest for different applications including animal and human health care [8]. Of particular interest are those cases in which the polymers form self-assembled nanostructures due to their concentration in antimicrobial polymers. Moreover, these self-assembled structures are able to incorporate other additional antimicrobials such as silver nanoparticles.

In this chapter, we will describe the different alternatives developed to produce efficient antimicrobial polymer nanostructures in solution.

4.2 Amphiphilic Antimicrobial Structures in Solution: Key Variables to Take into Account

As has been already introduced in the previous chapter, several important aspects require attention in the design of amphiphilic antimicrobial polymers. Herein, we will briefly describe the main parameters to be considered in the design of self-assembled nanostructures for the treatment of infectious diseases,

(a) Linear versus branched topologies
Amphiphilic nonlinear architectures have been equally explored. Dendrimers based on poly(amidoamine) (PAMAM) [9, 10] and poly(propyleneimine) (PPI) [11, 12] have been reported to have enhanced antimicrobial activities in comparison with linear macromolecules. Most probably, high charge density presented on the periphery of the dendrimer was a dominating factor in lysing the membrane and inhibiting bacterial growth.

(b) Hydrophilic/hydrophobic balance
The interaction of the antimicrobial with bacteria is determined by the polarity of the macromolecules it is crucial to find an appropriate balance between the hydrophilic/hydrophobic moieties. For this purpose, different strategies have been reported to vary the structure of the polymer and thus reach an appropriate hydrophilic/hydrophobic balance. The main alternatives are: the "segregated monomer," "amphiphilic face" monomers, i.e., monomer having a nonpolar part and a cationic part to construct the polymer chain and "same centered," employs monomers having an alkyl chain attached to a positive charge [7].

(c) Distribution of the hydrophilic groups
The charge group position within the backbone also has an effect on both antimicrobial and hemolytic activities. For instance, according to Song et al. [13] an ordered microstructure is required for optimal antibacterial activity even in the context of a polymer in which the backbone may have an irregular conformation. In particular, the authors evidenced that the hydrophobic spacer distance along the backbone between the antibacterial groups should greater than 4 Å and at least 8–10 Å.

4.3 Antimicrobial Random/Alternated Copolymers in Solution

Random copolymers can be straightforwardly obtained by using two different monomers with different degrees of hydrophobicity and hydrophilicity in one polymerization step. By simply varying the amount of the components in the initial feed, amphiphilicity of the synthesized copolymers can be easily varied. As a result, random copolymers are most commonly used polymers in the design of antimicrobial polymers [7]. In addition to the direct polymerization of the two or more monomers, another strategy for the fabrication of amphiphilic random copolymers involves the post-modification of either a homopolymer or a copolymer. Typically, the antimicrobial agent is immobilized in the side chain groups. Antimicrobial commonly functionalized copolymers include: polymethacrylamides, poly(β-lactams), polymethacrylates, polynorbornenes, and polycarbonates among others [14].

An interesting example was reported by Ilker et al. [15, 16] that described the preparation of amphiphilic cationic polynorbornene derivatives, soluble in water, from modular norbornene monomers, with variable molecular weights (M_n = 1600–137.500 g/mol) while maintaining narrow polydispersities (PDI = 1.1–1.3). According to their reports, the presence, and balance, of a hydrophobic group and a cationic group within the polymer structure were critical to achieve high activities. While small modifications to the hydrophobic character of the cationic amphiphilic polymer were shown to dramatically change the antibacterial and hemolytic activities, they succeeded in the preparation of polynorbornenes exhibiting good antibacterial activities and high selectivity for bacteria versus red blood cells. The overall

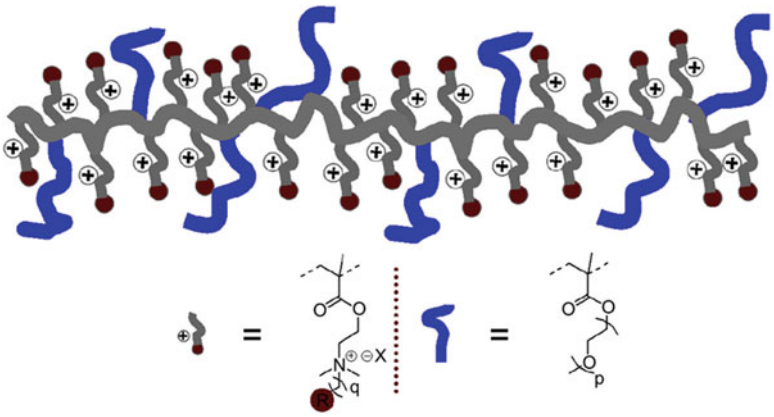

Fig. 4.1 Structure of the PEGylated methacrylate-based antimicrobial polymer containing quaternary ammonium groups developed by Venkataraman et al. Reproduced with permission from [17]

efficacy toward both Gram-negative and Gram-positive bacteria was strongly dependent on the length of alkyl substituents on the repeat units. The activity of each homopolymer with similar molecular weights ($M_n \sim 10{,}000$ g/mol) was probed against *E. coli*, *B. subtilis* and human red blood cells.

The advances in the polymerization techniques in particular in controlled radical polymerization allow preparing macromolecules with well-defined structures and a variety of functionalities. Taking advantage of the reversible addition fragmentation chain transfer (RAFT) polymerization, Yang et al. [17] have synthesized well-defined PEGylated polymers with tertiary amines using 2-(dimethylamino)ethyl methacrylate (DMAEMA) and oligo(ethylene glycol) methyl ether methacrylate (Fig. 4.1). The minimum inhibitory concentration (MIC) analyzed against Gram-positive bacteria *B. subtilis* was found to be dependent both on the nature of functional group and the hydrophobicity of the polymer. Moreover, the hemolytic properties of polymers tested against mouse red blood cells demonstrated little hemolysis in those polymers with a short alkyl or hydroxyl group while a strong antimicrobial activity is retained.

Probably, one of the most illustrative examples of the antimicrobial performance of alternating and random copolymers has been reported by Song et al. [13]. They synthesized four series of polymers (Fig. 4.2), i.e., alternating copolymers, random copolymers, and two series of homopolymers from two monomers cyclobutene and cyclooctene. As a result, the cationic and hydrophobic groups were distributed along the backbone with variable charge spaced (Fig. 4.3). They tested the polymers against six different bacterial species (both Gram-positive and Gram-negative) and for host cytotoxicities (red blood cell lysis). The authors evidenced that the most effective of the polymers studied were those regularly spaced, featuring a 6–8 carbon stretch along the backbone between side chains that present positively charged groups.

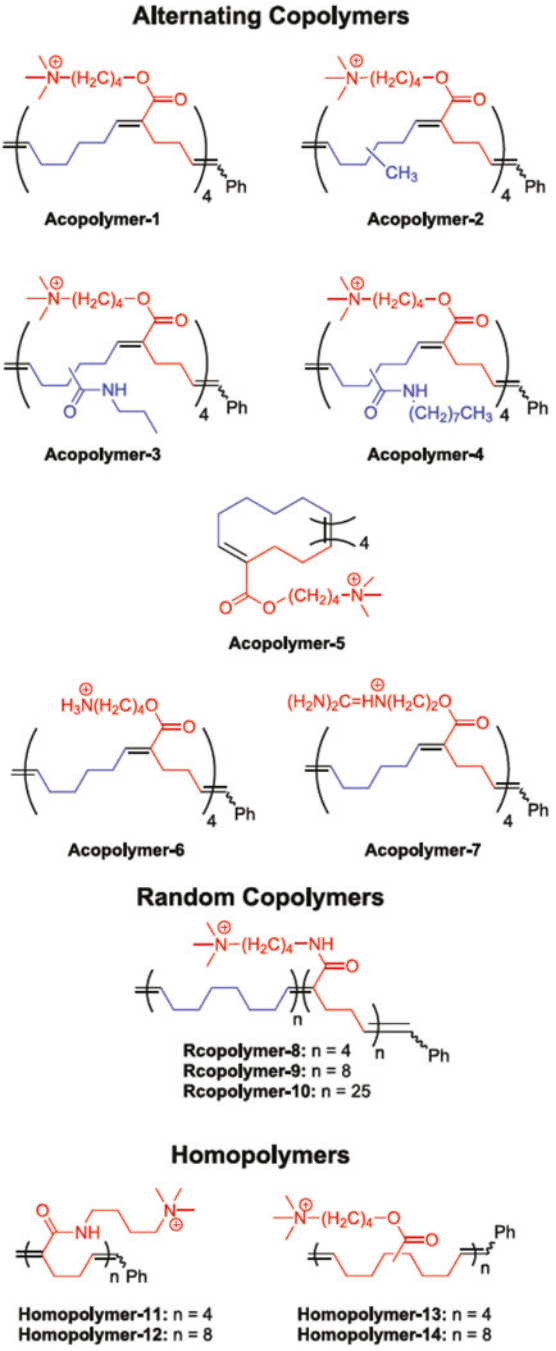

Fig. 4.2 Structures of amphiphilic polymers. The reported *n* is based on experimentally determined number-average molecular weights. Reproduced with permission from [13]

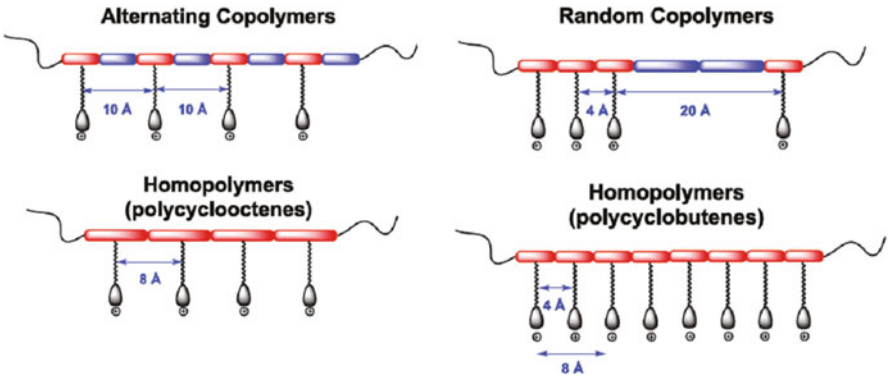

Fig. 4.3 Structure–activity relationship for amphiphilic polymers. Polymers containing longer spacing exhibit better antibacterial activities. Reproduced with permission from [13]

4.4 Self-Assembled Block Copolymer-Based Antimicrobial Nanostructures

As has been extensively described, amphiphilic block copolymers are able to self-assemble into micellar assemblies in aqueous media as a result of intermolecular forces, such as hydrophobic interactions, hydrogen bonding, stereocomplexation, weak Van der Waals forces, and electrostatic interactions [18–20]. One of the interesting features of nano-assembled structures is that the antibacterial efficacy may be increased when the antibacterial polymer chains assemble into micelles or vesicles due to increased local concentration of positive charges.

The effect of polymer sequence and self-assembly on antimicrobial activity against *E. coli* and selectivity has been investigated, for instance, by Oda et al. [21]. As depicted in Fig. 4.4, they examined the antibacterial and hemolytic activities in a series of amphiphilic block and random copolymers of poly(vinyl ether) derivatives prepared by base-assisting living cationic polymerization. More precisely, they compared block copolymer and random copolymers systems from poly(vinyl ether) derivatives containing primary amines and isobutyl hydrophobic repeat units with similar composition. Block and random amphiphilic copolymers with similar monomer compositions showed the same level of activity against *E. coli*. However, they observed that the hemolytic activity of the random copolymers was ~2000 times higher than that of the block copolymers. Thus, a priori the amphiphilic copolymer structure is a key determinant of activity. Furthermore, the block copolymers induced selective dye leakage from lipid vesicles consisting of *E. coli*-type lipids, but not mammalian lipids. Random copolymers disrupted both types of vesicles.

An important observation in this study was that both copolymers displayed bactericidal and hemolytic activities at concentrations 1 or 2 orders of magnitude lower than their critical (intermolecular) aggregation concentrations (CACs), as determined

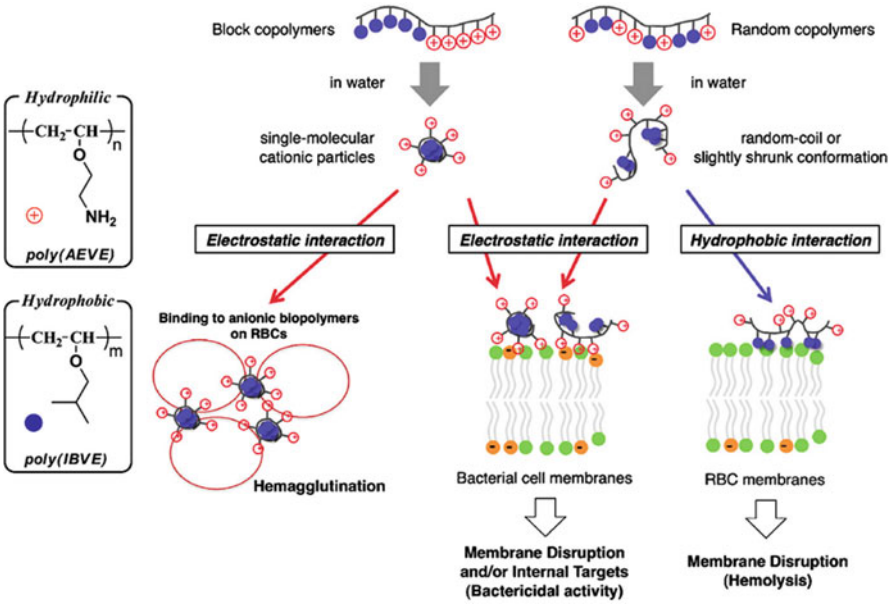

Fig. 4.4 Schematic presentation of proposed antibacterial and hemolytic activities of block and random poly(vinyl ether)s. Reprinted with permission from [21]

by light scattering measurements. This suggests that polymer aggregation or macromolecular assembly is not a requisite for the antibacterial activity and selectivity against bacteria over human red blood cells (RBCs). According to the authors, most probably the different single-chain conformations between the block and random copolymers play an important role in the antibacterial action and underlying antibacterial mechanisms.

This observation, i.e., the selectivity toward bacterial cells has also been reported by other groups. For instance, comparison between poly(carbonate)-based random copolymers prepared by Qiao et al. [22] and block copolymers reported by Nederberg et al. [23] evidenced that the formation of well-defined nanostructure enhances selectivity for microbes over red blood cells. Most probably, this behavior comes from the increasing multivalency and reduction of the hydrophobic components exposure.

Nederberg et al. [23] reported the fabrication biodegradable cationic poly(carbonate) micelles resulting from the self-assembly of cationic poly(carbonate)-*b*-poly(carbonate) triblock copolymers (Fig. 4.5). The polymers self-assembled into nanostructured micelles with average diameters of 43–198 nm and positive zeta potentials ranging from 47 to 65 mV. Both micelle diameter and zeta potential are directly related to both the length of cationic and hydrophobic poly(carbonate) blocks and the total molecular weight. These nano-assembled structures exhibit excellent antimicrobial properties against Gram-positive bacteria and fungi and had equally strong microbicidal activity against clinically threatening MRSA at a concentration

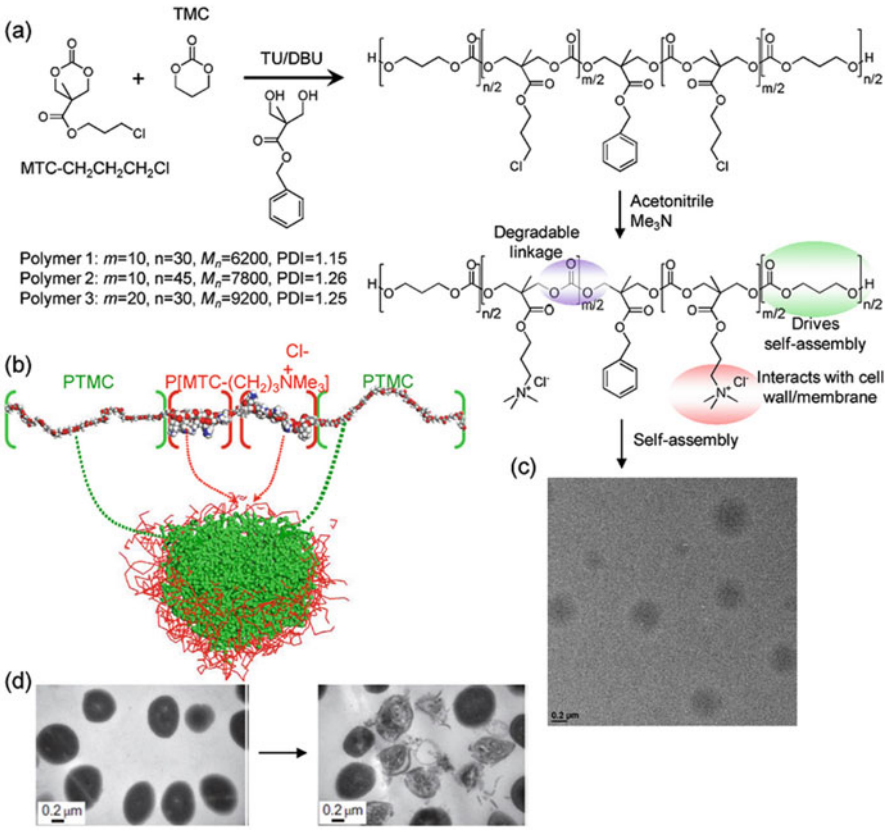

Fig. 4.5 Synthesis and micelle formation of cationic amphiphilic polycarbonates. (**a**) Chemical structure of the cationic amphiphilic polycarbonates, (**b**) these molecules self-assemble to form micelles, (**c**) TEM microgram of micelles, (**d**) MRSA before and after treatment with polymer micelles. Reprinted with permission from [23]

that did not induce toxicity to liver and kidney functions in a mouse model. Moreover, these nano-objects did not significantly affect electrolyte balance in the blood after 48 h post-injection or 14 days post-injection.

While selectivity of the nanostructured polymers toward antibacterial cells observed by Oda et al. [21] was confirmed in this study, the microbicidal function of these nanostructures occurred at polymer concentrations above their critical micellar concentrations. Therefore the nanostructure formation to enhance multivalency of the polymers plays a key role in selectively interacting with microbial membranes.

Interestingly, homologous random copolymers formed highly dynamic micelles. These structures exhibited stronger microbicidal potency against Gram-negative bacteria than the block copolymer structures. This behavior was explained by the authors by the easier access to hydrophobic components of the bacterial membrane [22].

Another interesting example was reported by Yang et al. [24]. They designed a short amphiphilic peptide which contains a hydrophilic block based on a cell penetrating Trans-Activator of Transcription (TAT) peptide and six arginine aminoacids and hydrophobic block of cholesterol. A hydrophobic moiety of cholesterol (C) was connected to the hydrophilic shell via three glycine spacers to drive self-assembly of the peptide. As a result, this amphiphilic structure is able to form cationic micellar nanoparticles with average diameter of 177 nm and positive zeta potential. This self-assembled structure showed stronger antibacterial activity in comparison to the pure polypeptide against yeasts and fungi and compared to amphotericin B, a commonly used antimicrobial drug for fungi-induced brain infections, the nanoparticles were less hemolytic.

All the above-mentioned examples involved the auto-organization of block copolymers to produce spherical shaped nanostructures. However, self-assembly of block copolymers permits the formation of nano-objects with variable shape. This approach has been employed by Yao et al. [25] reported a procedure to fabricate antibacterial core/shell polymer nano-objects with sheet-like, cylindrical, and spherical shapes depending on the block copolymer composition. As depicted in Fig. 4.6, they prepared nano-objects with chemically cross-linked polysiloxane cores and densely grafted polyammonium shells by dispersing cross-linked microphase separated materials of diblock copolymers, poly(2-(dimethylamino)ethyl methacrylate)-*block*-poly (3-(triethoxysilyl)propyl methacrylate) (PDMAEMA-*b*-PTEPM). The antibacterial activities of these quaternized nano-objects have been preliminarily assessed against bacteria *E. coli*. Compared with that of QPDMAEMA homopolymer, the antibacterial properties of all shaped nano-objects were greatly enhanced due to higher quaternary ammonium density. However, the shapes of nano-objects did not show is this preliminary study great difference in antibacterial performance.

4.5 Hybrid Organic/Inorganic Nano-Assemblies in Solution

An alternative to the use of exclusively antimicrobial polymers involves the incorporation of nanoparticles that may act synergistically and, thus, enhance the antimicrobial activity. Metal nanoparticles and in particular silver nanoparticles have shown great antimicrobial activity against a broad range of Gram-negative and Gram-positive bacteria [14, 26, 27]. However, a major drawback of using metal nanoparticles is related to their agglomeration problem that dramatically reduces their efficiency [28]. Agglomeration can be prevented by encapsulation on polymer micelles/vesicles [8, 29–32], or using nonlinear polymer architectures such as microgels [33] or hyperbranched polymers [34] thus leading hybrid nanostructured materials.

For instance, Lu et al. [29] described the preparation of water dispersible silver-decorated polymer vesicles and micelles based on an amphiphilic block-statistical copolymer with *tert*-butyl groups protected PEO-*b*-P(DMA-*stat*-tBA) or partially

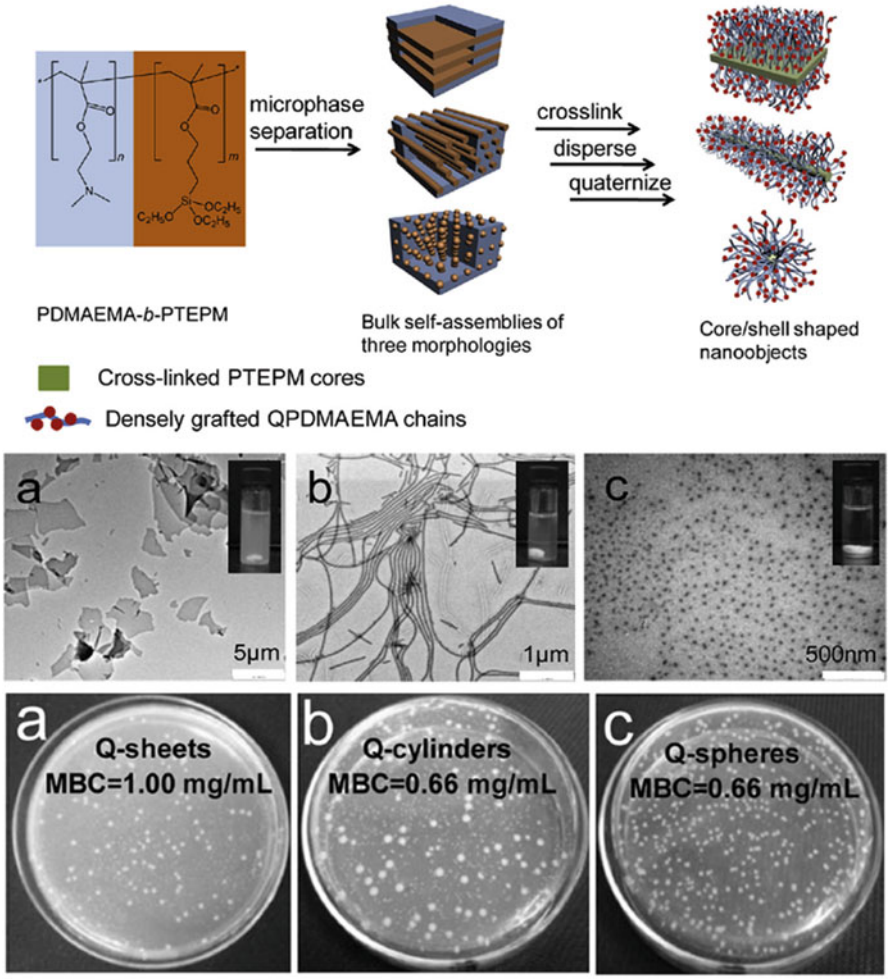

Fig. 4.6 *Above*: schematic representation of synthetic procedure for shaped antibacterial nano-objects (*red* dots along hairs of particles are ammonium groups). *Center*: TEM images of quaternized shaped nano-objects dispersed in water: (**a**) sheets, (**b**) cylinders, and (**c**) spheres. *Below*: representative photographs of bacteria *E. coli* incubated with different samples for 24 h. MBC stands for minimum bactericidal concentration of quaternized materials. Reproduced with permission from [25]

hydrolyzed PEO-*b*-P(DMA-*stat*-tBA-*stat*-AA). PEO stands for poly(ethylene oxide), DMA for 2-(dimethylamino) ethyl methacrylate, *t*BA for *t*-butyl acrylate, and AA for acrylic acid.

Different self-assembled nanostructures (micelles with variable sizes and vesicles) were obtained simply by changing the pH of the solution as a result of the pKa

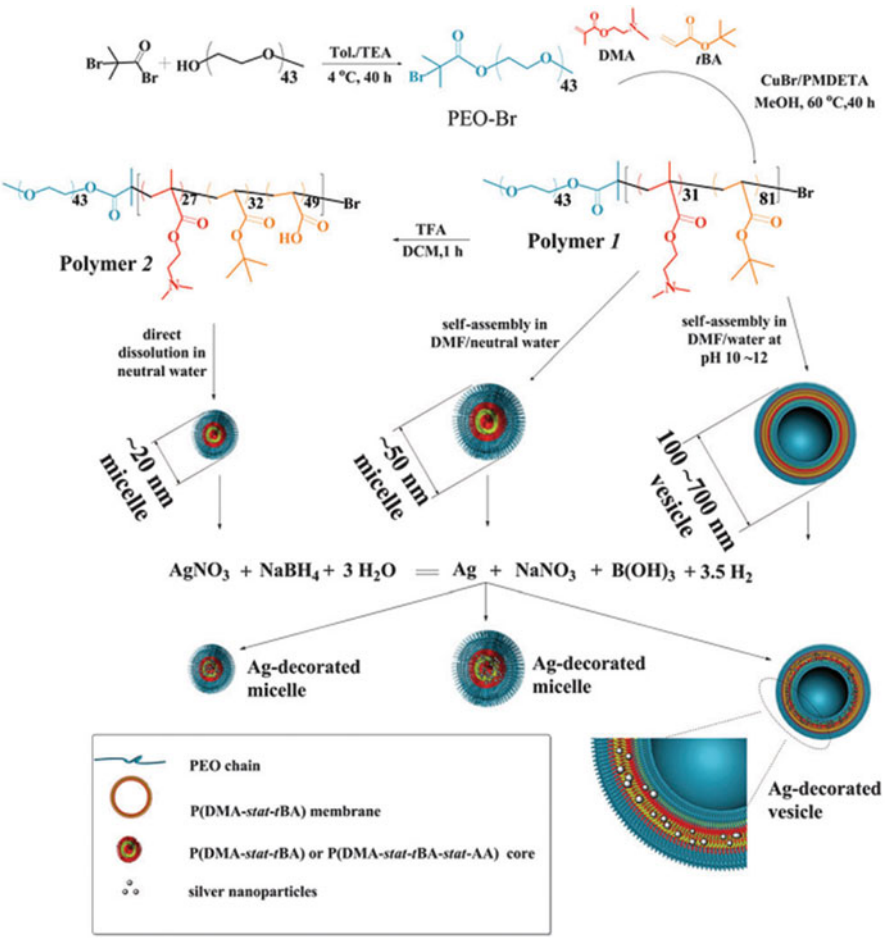

Fig. 4.7 Approach for the preparation of silver-decorated polymer vesicles and micelles developed by Lu et al. [29]. **Polymer *1***, PEO_{43}-*b*-P(DMA_{31}-stat-tBA_{81})-*block*-statistical copolymer prepared by ATRP, form either polymer vesicles by self-assembly in basic water–DMF or polymer micelles in neutral water–DMF. **Polymer *2***, PEO_{43}-*b*-P(DMA_{27}-stat-tBA_{32}-stat-AA_{49})-*block*-statistical copolymer obtained upon partial hydrolysis forms small polymer micelles. By encapsulation of the silver precursors inside the micelles core or the vesicle membrane, silver nanoparticles are formed in situ. Reproduced with permission from [29]

of the PDMA chains. As depicted in Fig. 4.7, in situ reduction of the silver ions allowed the preparation of nanoparticles inside of either the vesicle membrane or the micelles core. More interestingly, both micelles and vesicles with encapsulated silver nanoparticles showed high efficacy against *E. coli*.

4.6 Polymeric Nanocapsules

Nano-reservoirs have been explored to provide an alternative to transport and deliver the antibacterial agents. Baier et al. [35] developed an interesting concept fabricating nanocapsules able to release the antibacterial agents could be triggered by the presence of the bacteria themselves. They proposed the preparation of hyaluronic acid nanocapsules (HA-NCs) and hydroxyethyl starch nanocapsules (HESNCs) (with and without the antimicrobial agent polyhexanide) synthesized via interfacial polyaddition reaction using the inverse miniemulsion technique.

The hyaluronic acid (HA)-based nanocapsules containing the antimicrobial agent polyhexanide can be specifically cleaved in the presence of hyaluronidase, a factor of pathogenicity and invasion for bacteria like *S. aureus* and *E. coli*. As a result, the presence of bacteria increased the delivery of the antimicrobial agent thus producing an efficient killing of the pathogenic bacteria by the antimicrobial agent.

4.7 Polymeric Nanoparticles

Another strategy explored to produce soluble antimicrobial nanostructures resort to the preparation of polymer nanoparticles [36]. One of the most extended systems involves the use of Chitosan. Chitosan is a linear polysaccharide consisting of (1,4)-linked 2-amino-deoxy-β-D-glucan units derived from alkali deacetylation of chitin [37]. As a result, chitosan is a polycation whose charge density depends on the degree of deacetylation and pH. More interestingly, the positive charges contained within the chitosan offers antibacterial properties against a wide variety of microorganisms [38, 39]. Chitosan is a very versatile antimicrobial with demonstrated activity against different bacteria [40, 41]. Qi et al. [41] demonstrated that chitosan nanoparticles inhibit the growth of various bacteria such as *E. coli*, *S. choleraesuis*, *S. typhimurium,* or *S. aureus.* They reported MIC values below 0.25 μg/mL and the MBC values of nanoparticles up to 1 μg/mL. However, the activity largely depends on the pH. As a consequence, chitosan nanoparticles are ineffective at pH<6, probably because of the absence of protonated amino groups.

N-halamine-derivatized cross-linked polymethacrylamide nanoparticles (NPs) by copolymerization of the monomer methacrylamide (MAA) and the cross-linker monomer *N*,*N*-methylenebis(acrylamide) (MBAA) were fabricated by Natan et al. [42]. These nanoparticles were loaded in a subsequent step with oxidative chlorine using sodium hypochlorite (NaOCl). In addition to the antibacterial properties of the particles, the chlorinated NPs exhibit remarkable stability. They were resistant both to repetitive bacterial loading cycles as compared with the common disinfectant NaOCl (bleach) and also to organic reagents. The authors elucidated the mechanisms and demonstrated that the antibacterial mechanism involves the generation of reactive oxygen species (ROS) that occurs only upon exposure to organic media and not in water. Therefore, they evidenced a specific interaction of the chlorinated NPs

with *S. aureus*. Interestingly, this bacterial encircling does not require targeting biomolecules (e.g., an antibody or a ligand).

Recently, Cai et al. [43] reported the preparation of amine *N*-halamine copolymerized polystyrene (ANHCPS) nanoparticles via the surfactant-free emulsion copolymerization for antibacterial applications. By tuning reaction conditions such as monomer molar ratio, temperature, and copolymerization period, the morphology and size of the ANHCPS could be varied. Moreover, antibacterial evaluation test showed that the ANHCPS exhibited the capability of killing bacteria (Fig. 4.8).

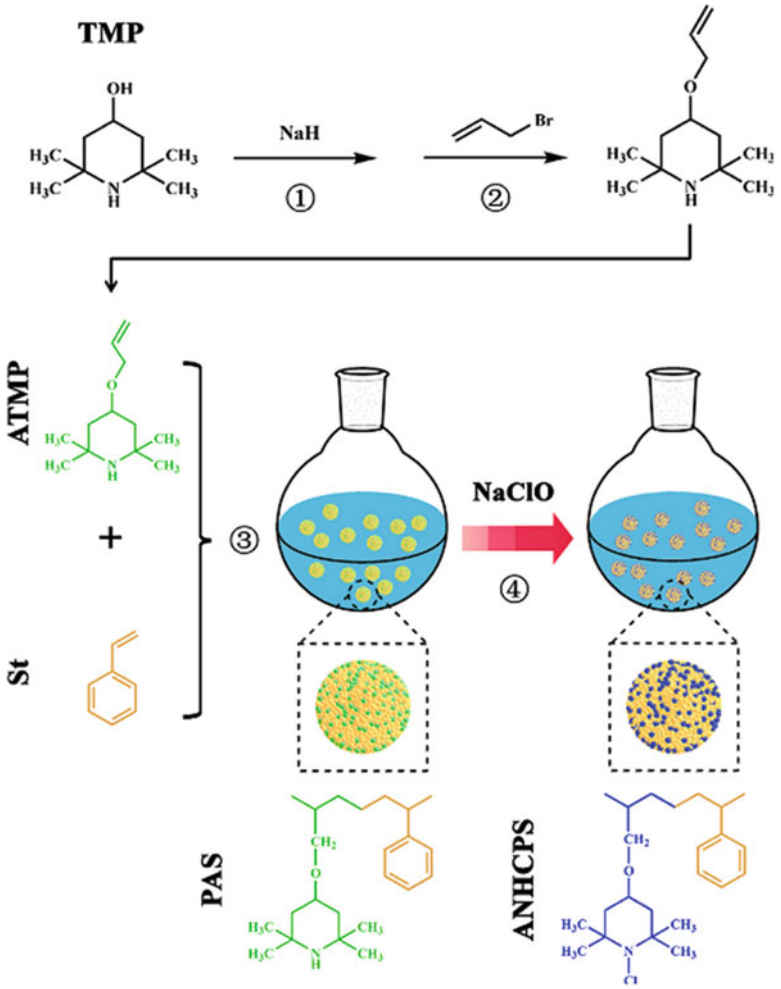

Fig. 4.8 Schematic illustration of the synthesis of the amine *N*-halamine copolymerized polystyrene (ANHCPS) nanoparticles. Reproduced with permission from [43]

4.8 Core/Shell Nanoparticles

Core/shell nanoparticles are formed by an inorganic nanoparticle that forms the core and a polymer coating forming the shell. Different approaches have been reported in order to coat a polymer shell onto a nanoparticle substrate. These methodologies include the incorporation of an initiator embedded in the nanoparticle base polymerization or by using grafting-onto or grafting from methodologies [36]. The formation of a polymer layer has associated two important advantages. On the one hand, the polymer layer covering the particle surface prevents the agglomeration of the nanoparticles. On the other hand, the synthetic strategies permit the incorporation of antimicrobial functionalities.

Song et al. [44] fabricated silica–poly(TBAM-*co*-EGDMA) core/shell nanospheres and evaluated their activity toward bacteria (Fig. 4.9). To achieve this goal the authors treated the inorganic colloids with chlorodimethylvinylsilane (CDVS) to improve the chemical affinity of the silica particles with the organic monomer. The surface-modified nanoparticles were obtained by centrifugal precipitation. Upon addition of azobisisobutyronitrile (AIBN) (employed as radical initiator) the mixture was evacuated and the liquid monomer mixture was introduced and

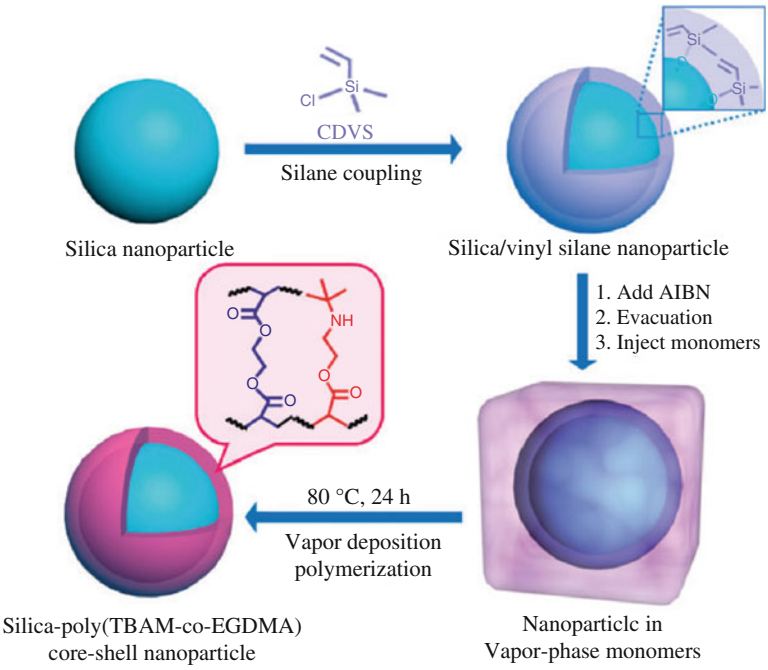

Fig. 4.9 Illustration of the strategy followed for the fabrication of silica–poly-(TBAM-*co*-EGDMA) core/shell nanoparticles. Figure reprinted from [44]

polymerized. More interestingly, the synthesized core/shell nanoparticles were well dispersed in aqueous solution and showed excellent antibacterial activities against planktonic *E. coli* and *S. aureus*.

Biocidal silver, ZnO, and TiO_2 core/shell nanoparticles have also been recently described [45–48]. These systems combine the intrinsic antimicrobial properties of the nanoparticles encapsulated with an antimicrobial polymer layer that enables their appropriate dispersion and improves the antimicrobial efficiency. Kong et al. [47] prepared core/shell nanoparticles by surface-initiated photopolymerization using titania as an initiator. As polymer shell, the authors employed vinyl monomer mixtures of nontoxic secondary amine-containing biocidal 2-(tert-butylamino)ethyl methacrylate and antifouling ethylene glycol dimethacrylate. When comparing the pristine TiO_2 nanoparticles with the modified core/shell nanoparticles, they observed an increase of the photocatalytic antibacterial activity. The particles exhibit antibacterial activity both in dark conditions and under UV light. Whereas, in the dark condition, the TiO_2/biocidal polymer nanoparticles exhibited high antimicrobial efficiency (95.7 %) against Gram-positive *S. aureus*, during UV irradiation, the TiO_2/biocidal polymer showed improved inhibition of bacterial growth against Gram-negative *E. coli* and Gram-positive *S. aureus* in comparison to the pristine TiO_2 nanoparticles.

Xu et al. [49] studied the conditions and mechanism of antibacterial activity of hydrophilic polymer-coated silver nanoparticles (AgNPs) against *E. coli* in various treatment conditions of pH and temperature. The coating employed is an amphiphilic polymer that introduced carboxyl groups on the surface to make it water soluble. They evidenced that the antibacterial activity occurs through the formation of reactive oxygen species (ROS). They established that the conditions of higher antimicrobial effect where those that generated more ROS and the antibacterial efficiency was dependent on the presence of oxygen. As a result, the antibacterial activity was suppressed in the presence of an antioxidant.

4.9 Fabrication of Microspheres for Antibacterial Purposes

Microspheres with antimicrobial activity have been equally explored. Microspheres have several advantages including a large specific surface area, ease of handling, ease of packing (in pack column applications) and recovery, and finally ease of dispersion.

Microspheres have been straightforwardly prepared with relatively narrow dispersion following two different methodologies, i.e., suspension or emulsion polymerization.

Chen et al. [50] reported the preparation of polymer microspheres with permanent antimicrobial surfaces. The synthetic route involves three different steps as depicted in Fig. 4.10. The first step involves the synthesis of cross-linked poly(4-vinylbenzyl chloride) (PVBC) microspheres via suspension polymerization. Then, the microspheres were modified by covalently grafting poly[2-(dimethylamino)

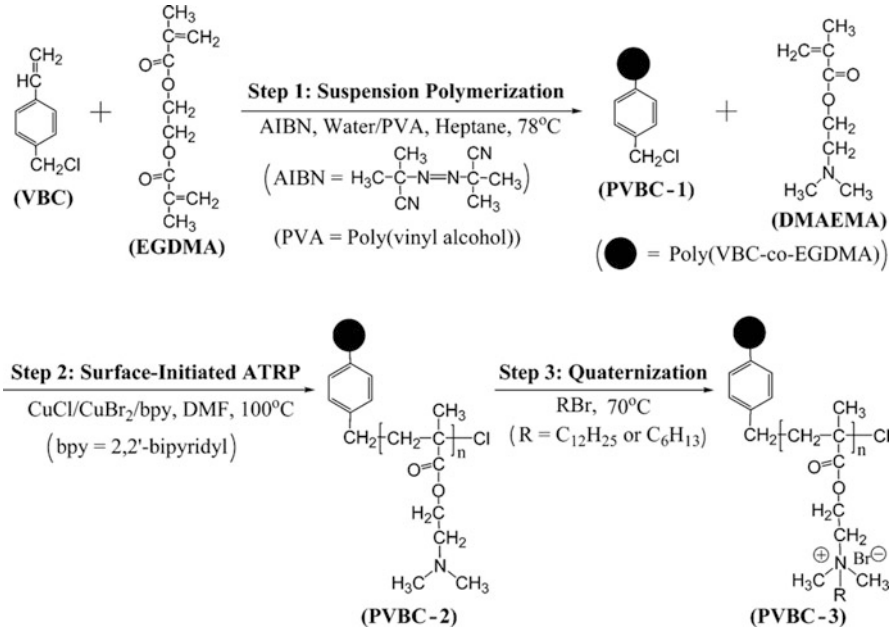

Fig. 4.10 Schematic diagram illustrating the process for preparing microspheres with permanent bactericidal surfaces. Reproduced with permission from [50]

ethyl methacrylate] (PDMAEMA) brushes. For this purpose, the authors employed the surface-initiated grafting from approach using controlled radical polymerization techniques. Finally, the authors quaternized PDMAEMA brushes by using alkyl bromides (1-bromododecane or 1-bromohexane). The authors evidenced the bactericidal effect of the QAS-functionalized microspheres on *E. coli* and *S. aureus* as well as the permanence of the bactericidal activity demonstrated through the repeated applications of the surface-modified PVBC microspheres without any significant loss of their surface activity or functionality.

Another example of polymer microspheres with permanent antibacterial surface from surface-initiated atom transfer radical polymerization of 4-vinylpyridine and quaternization has been reported by Zhenping et al. [51].

Biodegradable microspheres have also been proposed as an alternative to the previous examples to control the delivery of antibiotic drugs. Ravindra et al. [52] reported an illustrative examples in which ciprofloxacin (CF) was loaded biodegradable of microspheres composed of poly(lactide-*co*-caprolactone)-PF127 (a poloxamer block copolymer of ethylene oxide/propylene oxide). The microspheres were prepared by using solvent evaporation technique. The cumulative release characteristics of the microspheres for CF, the antibiotic drug, were investigated in pH 7.4 media and evidenced that it is possible to release CF in controlled manner up to 72 h.

4.10 Responsive Nanoparticles/Assemblies

Stimuli-responsive assemblies have been largely employed to release drugs in response to environmental changes. This concept has been, for instance, employed to deliver anticancer drugs for cancer therapy purposes. This section will summarize several illustrative examples in which stimuli-responsive systems have been employed in order to deliver antimicrobial agents or to improve the antibacterial efficiency.

(a) pH-responsive polymers
Zheng et al. [53] reported a graft-from strategy to prepare pH-responsive mesoporous silica nanoparticles (MSN) through the *N*-carboxyanhydride (NCA) ring-opening polymerization of γ-benzyl-L-glutamate *N*-carboxyanhydride (BLG-NCA) on the surface of MSN.

In their approach, the release of the anticancer drug doxorubicin hydrochloride from MSN-PLGA is pH dependent. Whereas the drug loading was achieved at pH 8.0, drug release occurs at different pH (5.5, 6.8 and 7.4).

Another interesting example was reported by Radovic-Moreno et al. [54] that develop drug-encapsulated, pH-responsive, surface charge-switching poly(D,L-lactic-*co*-glycolic acid)-*b*-poly(L-histidine)-*b*-poly-(ethylene glycol) (PLGA-PLH-PEG) nanoparticles (NP) for treating bacterial infections. These NP exhibit surface charge switching achieved by selective protonation of the imidazole groups of PLH at low pH. As a result, the NP are able to shield non-target interactions at neutral pH values but bind avidly to bacteria in acidity, delivering drugs and mitigating in part the loss of drug activity with declining pH (Fig. 4.11).

(b) Temperature-responsive assemblies
Quaternized methacrylamide (MA) poly(*N*-isopropyl acrylamide) (PNIPA) thermo-responsive copolymers were developed in literature [55, 56]. Copolymers with NIPAAm and a low MAHA content showed temperature-responsive behavior in an aqueous environment [56]. The lower critical solution temperatures (LCSTs) of these polymers varied between 32 and 44 °C. The LCSTs of quaternized copolymers were higher than those of neutral copolymers because they were more hydrophilic. The obtained homopolymers and copolymers were tested for antibacterial activities against *S. aureus* and *E. coli*. The quaternized water-soluble copolymers showed antibacterial activities against *S. aureus*. The quaternization resulted in the synthesis of both antibacterial and temperature-responsive copolymers.

Dizman et al. [55] also reported temperature-responsive polymers with antibacterial response. A new methacrylamide monomer (MAMP) containing a pyridine moiety was synthesized by reacting methacrylic anhydride and 3-(aminomethyl) pyridine. The monomer was homopolymerized in 1,4-dioxane and copolymerized with *N*-isopropyl acrylamide in DMF at two different compositions using AIBN as an initiator. The pyridine groups of the homopolymer and copolymers were reacted with various bromoalkanes containing 12, 14, and 16 carbon alkyl chains to obtain the polymers with pendant pyridinium groups.

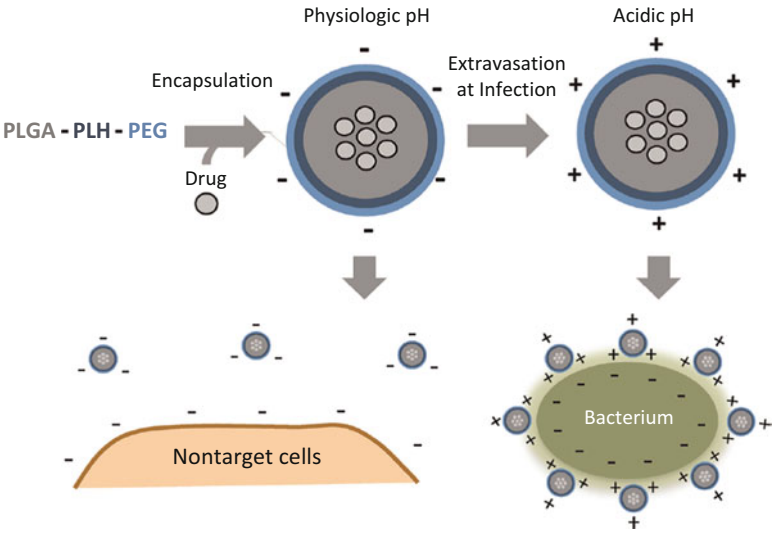

Fig. 4.11 Schematic representation of the designed nanoparticle (NP)-mediated drug targeting to bacterial cell walls. Drugs are encapsulated into NPs using a double emulsion/solvent evaporation process. The NPs avoid uptake or binding to nontarget cells or blood components at physiologic pH 7.4 due to a slight negative charge and surface PEGylation. Inflammation at a site of infection causes increased local vascular permeability, promoting NP extravasation. The weakly acidic conditions at sites of certain infections activate the surface charge-switching mechanism, resulting in NP binding to negatively charged bacteria. Finally, controlled release of the encapsulated drug leads to antibacterial effect. Reproduced with permission from [54]

The authors investigated the antibacterial activities of water-soluble copolymers against *S. aureus* and *E. coli* using the broth dilution and spread plate methods. The water-insoluble polymers were tested for the antibacterial activity against the same types of bacteria using the shaking flask method. The quaternized water-soluble copolymers showed excellent antibacterial activities against both types of bacteria, whereas the neutral polymers and quaternized water-insoluble homopolymers and copolymers were not active.

(c) Nano-objects with photoinduced antibacterial activity

Liu et al. [57] demonstrated that Chlorin e6 (Ce6) encapsulated charge-conversion polymeric nanoparticles (NPs) could be employed for efficiently targeting and killing pathogenic bacteria in a weakly acidic urinary tract infection environment. According to the authors, the NPs undergo a surface charge conversion in acidic environments that provides enhanced recognition for both Gram-positive (e.g., *S. aureus*) and Gram-negative (e.g., *E. coli*) bacteria due to the charge interaction. As a result, the NPs showed significant antibacterial efficacy in vitro while maintaining low cytotoxicity. In the same work, the effects of photodynamic therapy in urinary tract infections were investigated. They observed a significant decline in bacterial cells when using NPs after photodynamic therapy treatment.

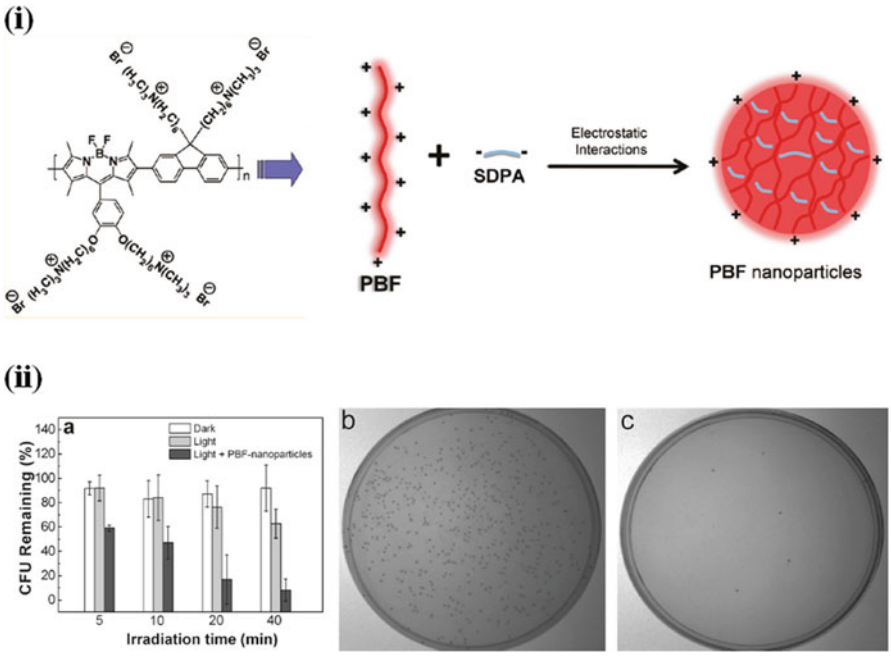

Fig. 4.12 (**i**) Schematic preparation of the polymer containing fluorene and boron-dipyrromethene repeat units in the backbones (PBF) nanoparticles. SDPA: negatively charged disodium salt 3,30-dithiodipropionic acid. (**ii**) (*a*) Biocidal activity of PBF nanoparticles toward Amp^r *E. coli* in the dark and under *white* light for varying irradiation times. Dark and light control experiments were done with the bacterial suspensions irradiated or in the dark in the absence of photosensitizers [PBF nanoparticles] = 20 μM. The light intensity was 90 mW cm^{-2}. Plate photographs for Amp^r *E. coli* incubated with PBF nanoparticles for 40 min: (*b*) in dark and (*c*) upon exposure to *white* light. Reproduced with permission from [59]

Conjugated nanoparticles have been equally employed to kill antibacterial as well as tumor cells since they can, upon photoexcitation, sensitize oxygen molecules to produce reactive oxygen species (ROS) [58]. For instance, Chong et al. [59] reported the fabrication of new water-soluble conjugated polymer containing fluorene and boron-dipyrromethene repeat units in the backbones (PBF) able to form uniform particles. These nanoparticles that absorb at 550 nm, upon photoexcitation with white light (400–800 nm and 90 mW cm^{-2}) can sensitize oxygen molecules to readily produce ROS for rapidly killing neighboring bacteria. As depicted in Fig. 4.12, irradiation times of 5 min in the presence of PBF nanoparticles led to 40 % reduction of the bacteria. More interestingly, an increase of the irradiation time to 10 and 40 min produces a reduction of bacteria of 50 and 90 %. In the absence of PBF nanoparticles, exposure to white light only 20 and 30 % bacterial reduction were obtained with irradiation for 20 and 40 min, respectively. In Fig. 4.12 are depicted the plate photographs for Amp^r *E. coli* incubated with PBF nanoparticles with the treatment of PBF nanoparticles in the dark and after white light irradiation.

Xing et al. [60] also employed conductive polymers to form complexes in order to improve light-activated antibacterial activity. In particular, they evidenced that anionic water-soluble polythiophene (PTP) and a cationic porphyrin (TPPN) can form a complex through electrostatic interactions. In this system, efficient energy transfer from PTP to TPPN occurs upon irradiation under white light (400–800 nm). The design of the systems incorporated two different features. On the one hand, the positive charges of PTP/TPPN complex promote adsorption to the negatively charged bacteria membranes of both Gram-negative *E. coli* and Gram-positive *B. subtilis* through electrostatic interactions. On the other hand, once this absorption has been produced the singlet oxygen effectively kills the bacteria. According to their findings, about 70 % reduction of bacterial viability is observed after only 5 min of irradiation with white light at a fluence rate of 90 mW cm^{-2} (27 J cm^{-2}).

(d) Multiresponsive nano-assemblies
Recently, more sophisticated approaches involving the use of multiresponsive polymer systems have been proposed. For example, Zhang et al. [61] prepared poly[2-(2-methoxyethoxy)ethyl methacrylate] ($PMeO_2MA$)\poly[2-(tert-butylaminoethyl) methacrylate] (PTA) diblock polymers with antimicrobial properties. This thermo- and pH-responsive antimicrobial diblock copolymer was directly dissolved in water to form polymer vesicles upon simply raising the temperature. Compared to individual polymer chains, the resulting polymer vesicles exhibit much better antimicrobial efficacy against both Gram-negative and Gram-positive bacteria under physiological conditions with neither quaternary ammonium moieties nor the loading of any external antibiotics as a result of their increased local concentration of cationic charge.

4.11 Conclusions

This chapter described different strategies to prepare nanostructured polymer structures that could be employed as efficient antimicrobial systems in solution. Self-assembled nano-objects, nano- and microparticles together with other complex structures such as core/shell particles have been obtained with different antimicrobial functionalities. The formation of nanostructures that concentrate a large amount of functional groups provides two important advantages. On the one hand, the nanometer size permits an increase of the contact between the polymeric nanostructure and the bacterial membrane. On the other hand, the density of active functional groups can be enhanced thus improving their efficiency.

In addition, this chapter also depicts the incorporation of polymers able to respond to a particular stimulus in antimicrobial nanostructures. According to the results in the use of these systems, this is another interesting alternative to clearly improve the antimicrobial activity. In this context, while temperature, pH, or light are among the most extended stimuli novel approaches attempt to include polymers able to respond to more than one stimulus to precisely tune the antimicrobial activity in particular environmental conditions.

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Chapter 5
Antimicrobial/Antifouling Surfaces Obtained by Surface Modification

Abstract A major issue in the use of biomaterials in natural environments and in particular in hospitals is related to the microorganism adhesion to the biomaterial surface. In this context, the focus of scientists and biomedical manufacturers turned to the development of coatings capable of resisting bacterial colonization and that can be placed on the surfaces of medical devices.

In this chapter, a variety of concepts and approaches are currently being explored in order to produce materials with anti-infective properties that could be employed for biorelated applications will be described. As will be depicted, the strategies are proposed to either reduce or prevent bacterial adhesion. They basically can be divided into two different methodologies: the first type of methodologies include those strategies that either involve chemical modification to introduce antimicrobial activity or are intrinsically antimicrobial. The second type refers to those methodologies that resort to the formation of micro/nanostructures at the biomaterial surface. This chapter will focus on the first group, i.e., the description of the different strategies to chemically modify the polymer surface to improve their antifouling properties or to provide antimicrobial activity.

However, prior to the description of the different methodologies to fabricate antimicrobial surfaces the approaches that are available in order to modify the chemical composition of a particular surface will be first analyzed.

Keywords Surface modification • Antimicrobial surface • Grafting from • Grafting onto • Biocide-releasing coatings • Bioactive materials

5.1 Introduction

A major issue in the use of biomaterials in natural environments and in particular in hospitals is related to the microorganism adhesion to the biomaterial surface. In this context, the focus of scientists and biomedical manufacturers turned to the

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development of coatings capable of resisting bacterial colonization and that can be placed on the surfaces of medical devices [1, 2].

Microorganisms and in particular bacteria adhere to almost all kind of surfaces. Upon adhesion they are able to grow and produce a matrix containing extracellular polymeric substances that may, in a further step, form a biofilm. As a result, patients might suffer from acquired infections like ventilator-associated pneumonia, catheter-associated urinary tract infection, and central line-associated blood stream infections. For instance, the annual infection rate for cardiovascular implants is even higher (7.4 %) [3]. In addition, a particular concern is that once the biofilm is formed antibiotics administered systemically are not effective against implant-associated infections. As a result, the strategy followed resort to implant removal and/or amputation.

In this context, a large variety of concepts and approaches are currently being explored in order to produce materials with anti-infective properties that could be employed for biorelated applications [4]. In Fig. 5.1 are depicted the different strategies proposed to either reduce or prevent bacterial adhesion. They basically can be

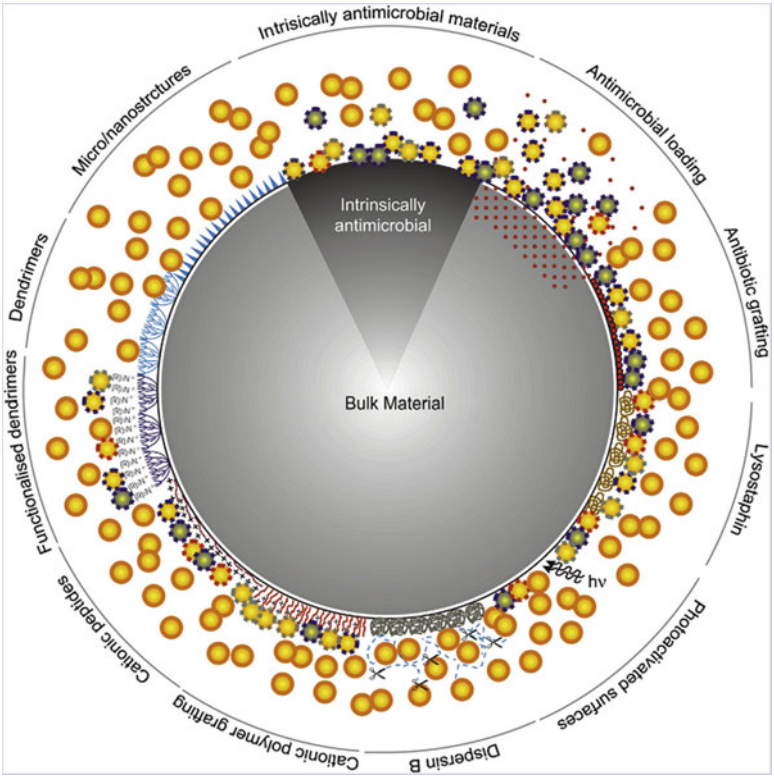


Fig. 5.1 Overview of the strategies to modify biomaterial surfaces to prevent biomaterial-associated infections. Reproduced with permission from [4]

divided into two different methodologies: the first type of methodologies include those strategies that either involve chemical modification to introduce antimicrobial activity or are intrinsically antimicrobial. The second type refers to those methodologies that resort to the formation of micro/nanostructures at the biomaterial surface. This chapter will focus on the first group, i.e., the description of the different strategies to chemically modify the polymer surface to improve their antifouling properties or to provide antimicrobial activity. Those approaches to produce antimicrobial surfaces based on their structuration will be considered in Chap. 6.

However, prior to the description of the different methodologies to fabricate antimicrobial surfaces the approaches that are available in order to modify the chemical composition of a particular surface will be first analyzed.

5.2 Polymer Surface Modification

As has been mentioned above, once the biofilms have been developed on the material surface they are extremely hard if not impossible to remove and show great resistance to a great variety of biocides. As a result, the most extended strategy to prevent infection and material deterioration is to prevent the biofilm formation. In this context, the primary adhesion of microbial cells must be avoided. As depicted in Fig. 5.2, this objective has been mainly pursued by modifying the polymeric interface using two different strategies, i.e., using repelling or killing molecules. Repelling coatings resort, for instance, to the immobilization of polyethylene glycol (PEG) segments at the surface, by anchoring highly negatively charged polymers that repel the bacterial adhesion based on electrostatic repulsion or modifying the surface with ultrahydrophobic moieties.

Alternatively, microbes adhering to the surfaces can be killed by releasing a biocide. The biocide can be either embedded in the polymer matrix or generated

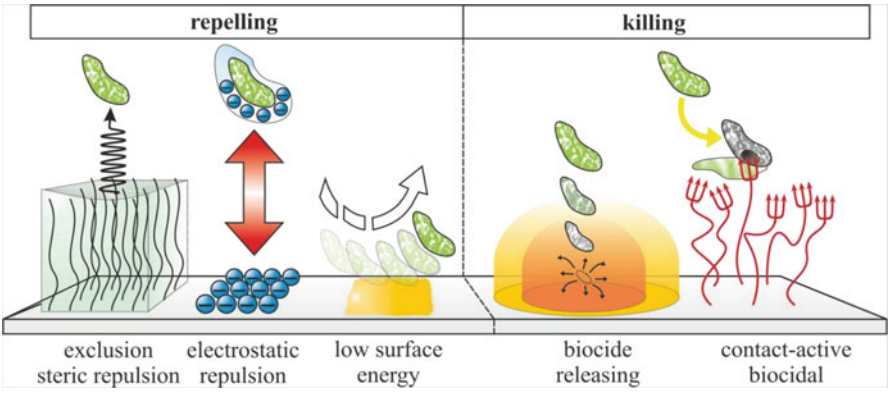


Fig. 5.2 Alternative approaches to prepare either antifouling or antimicrobial surfaces. Reproduced with permission from [5]

in situ, by formation of active species. For instance, reactive oxygen species (ROS) can attack a diverse range of targets to exert antimicrobial activity. These species are versatile in mediating host defense against a broad range of pathogens [6]. Alternatively to these strategies, surfaces can also be rendered contact-active antimicrobial upon tethering antimicrobial polymers. In this chapter, we will limit our discussion to the surface modification with antifouling and antimicrobial polymers [5].

Whereas pioneer advances on the development of materials and surfaces with antibacterial properties were based on empirical analysis, today significant advances on the causes of infection allowed us to explore different strategies to prevent bacterial adhesion. In particular, this chapter will summarize the strategies explored to modify surfaces of commonly used polymers. In order to fabricate materials with infection-resistant properties, the surface chemical composition can be varied using different alternatives including material surfaces with antimicrobials, surfactants, repellent coatings, or with selected biological molecules, such as heparin or albumin [7–12].

However, as reported by Siedenbiedel and Tiller [5] the strategies to chemically modify polymer surfaces in order to avoid bacterial adhesion and, therefore, biofilm formation can be grouped into two main alternatives (Fig. 5.2) [5, 13–19]. On the one hand, surfaces can be modified introducing repelling groups that act using different forces such as electrostatic repulsion, low surface energy, or exclusion steric repulsion. On the other hand, modified surfaces can be prepared by immobilization/release of antimicrobial compounds capable of killing bacteria upon contact with the material surface.

5.3 Techniques to Functionalize Polymer Surfaces

The strategies to functionalize polymer surfaces reported can be grouped in three main alternatives (Fig. 5.3). The first strategy involves the physical immobilization of polymer chains, i.e., by non-covalent attachment. Within this approach, layer-by-layer deposition [21] or dip coating [22] processes have been employed to prepare antimicrobial coatings. Although this strategy is very simple and can be carried out without the use of sophisticated chemical approaches, there are few limitations on their use. On the one hand, the mechanical stability of these interfaces is reduced and changes in the environmental conditions (temperature, pH, …) can produce significant changes. On the other hand, biocide leaching may lead to a rapid loss of the antimicrobial activity [23].

As an alternative to this approach, covalent immobilization of the antimicrobial moieties can be achieved by using either grafting-to or grafting-from methodologies. Grafting-to resorts to the immobilization of preformed chains to a polymer surface by a coupling reaction. This approach permits the formation of a homogeneous layer of antimicrobial polymers in which the chemical properties such as monomer composition or chain length can be easily controlled. Moreover, the

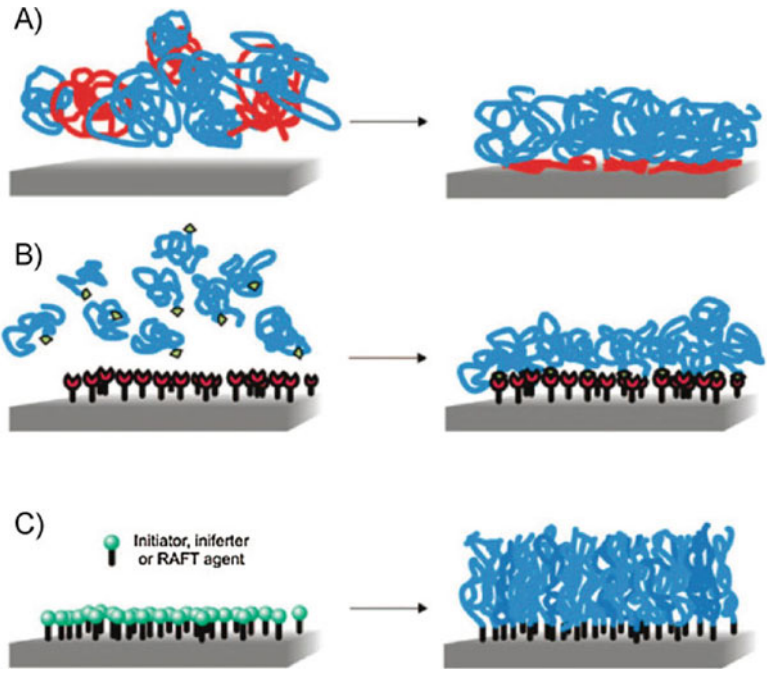


Fig. 5.3 Strategies to immobilize polymer chains (**a**) Physical adsorption by non-covalent interactions. Dominated by the preferential adsorption of the *red* blocks to the surface, e.g., LbL films, block copolymer coatings, (**b**) Grafting-to methods by creating covalent bonds with complementary groups at the surface, e.g., PEIs (poly(ethylene imine)), cationic polymers, (**c**) Grafting-from or surface-initiated polymerization via synthesis of antimicrobial coating from initiators revealed at the surface by ATRP, e.g., PVP, PDMAEMA, methacrylates. Reproduced with permission from [20]

covalent bonds established between the polymer and the surface does not allow the biocide to leach thus enabling a long-term use of the material.

Similarly to the grafting-onto, grafting-from enables produces covalently anchored functional surfaces. In this case, an initiator present at the surface can be employed to polymerize. Controlled radical polymerization such as atom transfer radical polymerization (ATRP) or reversible addition fragmentation chain transfer polymerization (RAFT) produced coatings with polymer chain having narrow polydispersity. A major advantage in comparison with the grafting-to approach, concerns the higher chain density that can be achieved using this strategy.

It is worth mentioning that most of these elaborate techniques are useful for preparations in the laboratory but not in the industry, because the required chemical finishing is often too expensive [5]. In Table 5.1 are summarized the different alternatives to obtain contact-active antimicrobial surfaces as well as the polymers employed and several illustrative examples.

Table 5.1 Examples of surface-attached biocidal polymers

	Method	Polymer	Examples
Grafting from	Immobilized initiator	QPAM	[16]
		PEtOx	[24]
Grafting to	Immobilized comonomer	QP4PVP	[25]
	End-on	AMP	[26, 27]
	Side-on	QPEI	[28]
		NB	[28]
	Parallel grafting to and modification	QP4PVP	[29]
	In situ end-on	PMOx	[30]
	In situ side-on	QPU	[31]
Coating	Layer by layer	Polylysine	[21]
		PAA	[32]
		PHGH	[33]
		Chitosan	[34]
	Particles with grafted polymer	Magnetic Fe_3O_4 with QPEI	[35]
		PA-particles with QP4PVP	[36]
	Hyperbranched polymers	QPEI	[22, 37]
	Plasma polymerization	PDAA	[38]
		Polyterpenol	[39]
	Surface-induced hydrogelation	Vancomycin	[40]
		AMP	[41]

Reproduced with permission from [5]
QP4VP quaternized poly(4-vinylpyridine), *QPAM* quaternized poly(*N*,*N*-dimethylaminoethylacrylamide), *PAA* poly(allylammonium chloride), *QPEI* quaternized polyethyleneimine, *PS* poly(styrene), *PEtOx* poly(2-ethyloxazoline), *PMOx* poly(2-methyloxazoline), *QPU* quaternized polyurethanes, *PN* norbonene-based polymers, *AMP* antimicrobial peptides, *PA* poly(acrylate), *PHGH* poly(hexamethylene guanidinium hydrochloride)

5.4 Anti-Adhesive Polymer Surfaces: Antifouling

Chemical modification of polymer surfaces has been demonstrated to be crucial in order to avoid bacterial contamination. For this purpose, highly hydrophobic and hydrophilic groups have been anchored on polymer surfaces. Table 5.2 includes few illustrative examples in which modified polymer surfaces have shown low bacterial adhesion properties [4].

Hydrophilic synthetic polymers can repel or reduce the microorganisms adhesion by steric hindrance [9, 53–57]. In this category also referred as "passive approach" or "bacteria-resistive" [58] we can include the formation of coatings of highly hydrated polymer chains, such as poly(ethylene glycol) (PEG) on a surface exhibits a large exclusion volume effect, which inhibits both protein and bacterial

Table 5.2 Examples of anti-adhesive coatings

Polymer coating	Monomer charge	In vitro-tested efficacy		Refs
		Gram-negative	Gram-positive	
Fluorosiloxane coatings	Superhydrophobic		SA	[42]
Poly(ethylene oxide) (PEO)	Hydrophilic, no charge	EC, PA	SA, SE, SS	[43, 44]
Poly(epsilon-caprolactone) (PCL)/PEG copolymer	Hydrophilic, no charge	BS		[45]
Phosphorylcholine (PC)-based polymers	Zwitterionic	EC, PA	SA, SM	[46, 47]
2-Methacryloyloxyethyl phosphorylcholine (MPC) polymer	Zwitterionic	PA	SA, SE	[48]
Zwitterionic poly(sulfobetaine methacrylate) (pSBMA)	Zwitterionic	PA	SE	[49–51]
Peptide-functionalized poly(L-lysine)-grafted-poly(ethylene glycol) (PLL-g-PEG/PEG-RGD)	Positively charged		SA	[52]

Adapted from [4]
EC *Escherichia coli*, PA *Pseudomonas aeruginosa*, SA *Staphylococcus aureus*, SE *Staphylococcus epidermidis*, SM *Streptococcus mutans*, SS *Streptococcus salivarius*

adhesion [59]. Equally, coatings based on heparin (highly hydrophilic polymer) also prevented the adhesion of bacterial cells [9, 10, 12].

Other alternative involves the functionalization of the surface with zwitterionic polymers and derivatives that have been employed for their antifouling properties. Zwitterionic polymers have an equivalent number of homogeneously distributed anionic and cationic groups on their polymer chains [60]. In contrast to the use of PEG, zwitterionic polymers have a broader chemical diversity and greater freedom for molecular design.

As reported by Mi and Jiang [60] important aspects related to the chemical diversity mentioned above include:

(a) Types of ionic groups (anionic and cationic) to be incorporated into the polymer structure. On the one hand, anionic groups include carboxylates [61], sulfonates [62, 63], or phosphates [64]. On the other hand, quaternary ammonium [63, 65], phosphonium [66], pyridinium [67], or imidazolium [68] have been typically employed as cationic groups.
(b) Distribution and arrangement of the charged groups. In this context, two main aspects can be varied. First, the proximity between positive and negative charges within the same monomeric unit [69]. Secondly, the total separation of oppositely charged ionic groups onto different polymer side chains (the latter case is also known as "mixed charge" polymers); [70]
(c) More sophisticated designs include the modification of typically employed zwitterionic polymers to form new polymers able to switch between zwitterionic

and non-zwitterionic forms [71–74]. Equally, these modified systems could be designed to carry a charged biologically active molecule as a part of the zwitterionic constituent [75].

It is important to mention that even if zwitterionic polymers have been mainly employed as antifouling molecules, the possibility of adjusting functional aspects, such as the ionic nature of zwitterionic materials, polymer charge density, pH sensitivity, or counterion association, have open new paths for their use as antimicrobial compounds [60].

5.5 Antibacterial Coatings

In contrast to the "passive" strategies to develop antifouling surfaces, the so-called active approaches also known as "bacteria killing" have been focused on the anchoring of molecules able to kill bacteria upon contact.

5.5.1 Biocide-Releasing Antibacterial Coatings

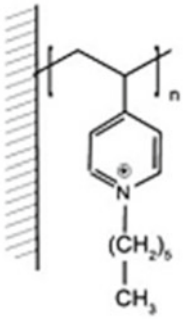
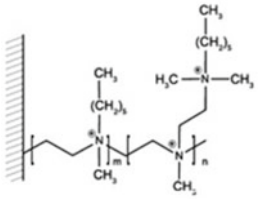
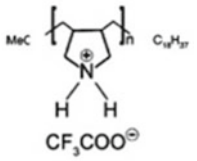
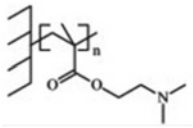
Most of the systems explored involve the incorporation of antimicrobial agents that can be gradually released into the solution for a large periods of time and simultaneously kill the bacteria present in the media [76–78].

Within this category many different antimicrobial agents have been explored with more or less success. These include quaternary ammonium compounds, iodine, silver ions, nitric oxide, or even antibiotics [4, 79]. As an example of microbicidal coating, Klibanov et al. [80] prepared both inorganic glass and polyethylene interfaces modified with of *N*-hexyl, *N*-methyl-PEI (polyethylene imine) [35, 81–83]. This strategy involves the non-covalent interactions between the PEI and the substrates. In this system, polycations leached from the surface act as antimicrobials against *S. aureus* [22]. More interestingly, replacing the short hexyl chains by longer docecyl chains resulted in a material with improved the integrity while retaining their antimicrobial activity for longer periods of time [84, 85]. However, as has been mentioned above, in some cases specially structured robust coatings and effective in resisting biofilm formation are required.

5.5.2 Intrinsically Bioactive Materials: Contact-Active Biocidals

The most extended class of polymers employed as antimicrobials are cationic polymers that are effectively adsorbed at the bacterial cell surface directed by the net negative charge of microbial cells. As depicted in Table 5.3, many different

Table 5.3 Antimicrobial coatings obtained by surface modification with cationic polymers

Name and typical structure of cationic polymeric coatings	Surface	Grafting strategy	Reference
P4VP polymeric coating 	Glass plastic	Covalent modification	[58, 61]
		Covalent modification	
PEI-based polymeric coating 	Glass textile	Covalent modification	[19, 26, 59]
		Dip coating	
Polymers with incorporated quaternary ammonium ODDMACPDDMAC 	Cellulose glass	Covalent modification	[31, 165]
		Dip coating	
PDMAIMA 	Glass inorganic surfaces plastic (polypropylene)	ATRP (grafting from) RAFT (grafting from) ATRP + covalent (grafting onto)	[70, 71, 74]

Reproduced with permission from [23]

examples have been reported in the literature of surface modification with cationic polymers involving covalent and non-covalent interactions [23]. One of pioneer works was reported by Klibanov et al. [25] that covalently linked poly(4-vinyl-*N*-alkylpyridinium bromide) to amino-modified glass slides via acylation with acryloyl chloride followed by copolymerization with 4-vinylpyridine, and finally *N*-alkylation with different alkyl bromides.

In addition to cationic polymers, another highly effective functional group in killing bacteria is based on cyclic *N*-halamine polymeric compounds [86]. In *N*-halamine, one or more halogen atoms are covalently bond to nitrogen atoms in a cyclic structure. According to current models, *N*-halamines exhibit antimicrobial properties as a consequence of the direct transfer of active halogen from the halamine groups to the cell wall of the microorganisms by direct contact followed by oxidation or by dissociation into water followed by diffusion over the microorganisms. The released halogen groups interact with the bacterial receptor thus inactivating the cell. In comparison with cationic polymers, *N*-halamines act faster but require to be regenerated. The latter occurs by exposure to dilute halogen solutions. *N*-halamines, are in addition inexpensive, nontoxic, and noncorrosive.

An illustrative example of the potential of using *N*-halamines was reported by Sun et al. [87] that described the surface modification of a polyurethane using an *N*-halamine precursor (5,5-dimethylhydantoin (DMH)). According to the authors, the *N*-halamine-based PU potent antimicrobial effects against a large variety of microorganisms: *Staphylococcus aureus* (Gram-positive bacterium), *Escherichia coli* (Gram-negative bacterium), *methicillin-resistant Staphylococcus aureus* (MRSA, drug-resistant Gram-positive bacterium), *vancomycin-resistant Enterococcus faecium* (VRE, drug-resistant Gram-positive bacterium), and *Candida albicans* (fungus). Moreover, these modifications are stable and prevented both bacterial and fungal biofilm formation during months. More interestingly, when the antimicrobial efficiency is lost due to their extensive use, it could be regenerated again by chlorination treatment as depicted in Fig. 5.4.

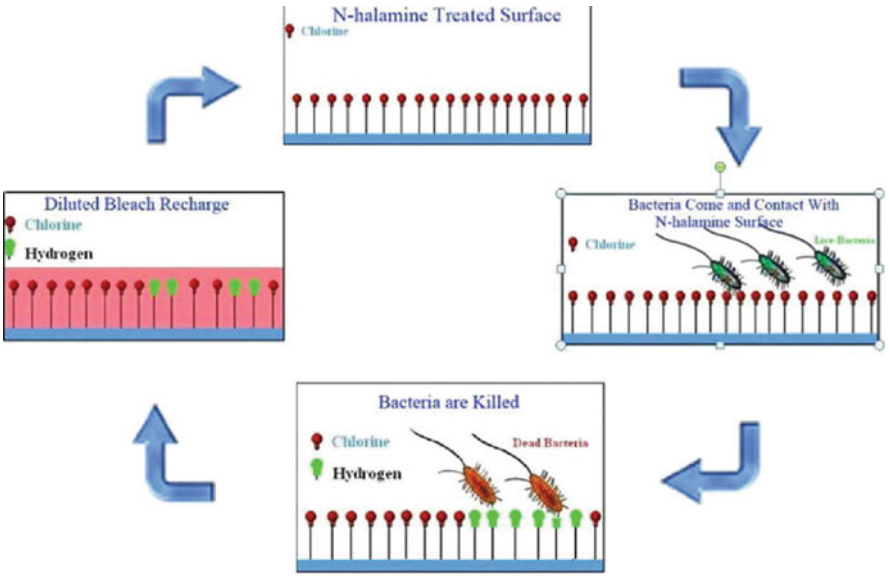


Fig. 5.4 *N*-halamine-based polyurethane surfaces are able to kill both bacteria and prevent biofilm formation. Moreover, their antimicrobial activity can be regenerated after treatment with dilute bleaching solutions. Reproduced with permission from [87]

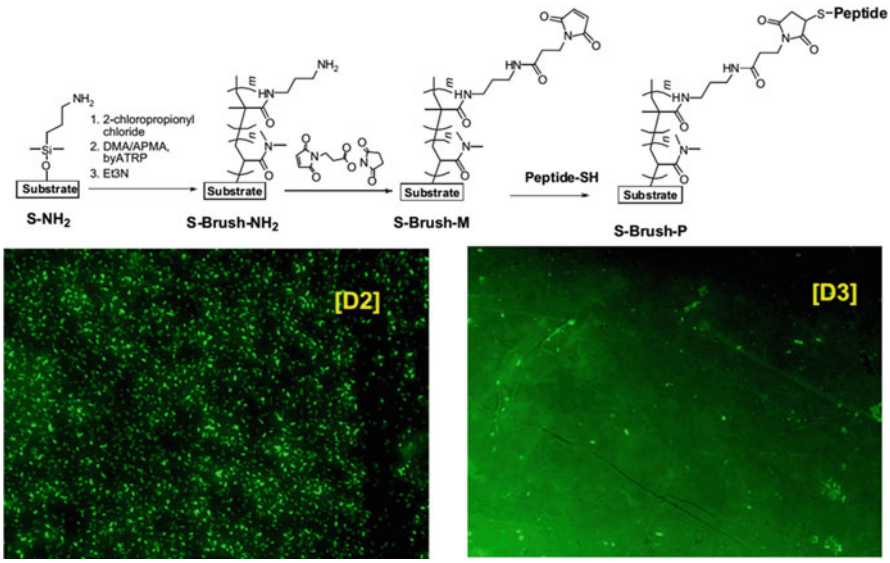


Fig. 5.5 *Above*: synthetic route for copolymer brushes and peptide conjugation. The strategy involves four steps: (*1*) surface functionalization with an initiator, (*2*) surface-initiated ATRP of *N*,*N*-dimethylacrylamide and *N*-(3-aminopropyl) methacrylamide hydrochloride, (*3*) synthesis of maleimide group immobilized Ti surface, and finally (*4*) coupling with the appropriate peptide. *Below*: (**D2**) Fluorescence image of bacteria on titanium surface, (**D3**) Fluorescence image of bacteria on peptide (Tet-26) immobilized copolymer brush on titanium surface. Reproduced with permission from [89]

Antibacterial coatings prepared by covalent immobilization of antimicrobials have been equally reported using antimicrobial peptides (AMPs). For instance, Bagheri et al. [88] reported examples of different biomaterials employed as surface supports (such as gold surfaces, resin beads, cellulose membranes, polymer brushes, and block copolymers) employed to covalently anchor cationic antimicrobial peptides. AMPs were also employed by Gao et al. [89] to modify titanium surfaces. As depicted in Fig. 5.5, this group prepared infection-resistant coatings on implants based on covalently grafted hydrophilic polymer brushes conjugated with an optimized series of tethered antimicrobial peptides. These immobilized AMPs showed broad spectrum activity against different pathogenic bacteria and yeast when immobilized on a surface.

While it is true that most of the strategies employed are directed either to prevent bacterial infections by reducing the adhesion of bacteria to the surface or to kill them when in contact with the surface recent progresses in the understanding on the molecular mechanisms of the biofilm have open the path to new alternatives to reduce the biofilm formation [4, 90, 91]. As depicted in Table 5.4, recent investigations evidenced that a large variety of substances possesses antibiofilm activities. These substances can be introduced in the grafted or can be released from the biomaterial surface [115]. Campoccia et al. [4] recently reviewed the different

Table 5.4 Examples of molecules immobilized on polymer surfaces to prevent biofilm formation

Antibiofilm molecule	Action mechanism	Ref
Hamamelitannin	Reduced biofilm metabolic activity	[92, 93]
Proteinase K	Degradation of the extracellular proteic substances of bacterial biofilms	[91]
D-aminoacids (e.g., D-leucine, D-methionine, D-tyrosine, and D-tryptophan)	They trigger biofilm disassembly and may represent a widespread bacterial signal for biofilm disassembly	[94, 95]
Norspermidine	It interacts directly and specifically with exopolysaccharide causing biofilm disassembly	[96]
Trypsin	Degradation of the extracellular proteic substances of bacterial biofilms	[97]
rhDNase I	Degradation of the extracellular-DNA (eDNA) component of bacterial biofilms	[90, 98]
Dispersin B	Degradation of the exopolysaccharidic component of bacterial biofilms	[99]
Antimicrobial peptides (AMPs)	Permeabilization of the cytoplasmic membranes. Active against quiescent bacteria	[100–102]
N-acetylcysteine (NAC)	Disruption of clinically relevant and drug-resistant bacterial biofilms. NAC inhibits exopolysaccharide expression and is also bactericidal	[91, 103]
EDTA	At low concentration bacteriostatic for planktonic cells, at higher concentrations inhibiting biofilm	[104]
Hydroxypropyltrimethyl ammonium chloride chitosan, HACC	Inhibition of polysaccharide intercellular adhesin (PIA) expression through downregulation of icaAD and upregulation of icaR in SA and SE	[105]
RNA III inhibiting peptide (RIP)	Quorum sensing-targeting	[106, 107]
Furanones	Quorum sensing-targeting	[108–110]
3-oxo-C12-(2-aminophenol)	Quorum sensing-targeting	[111]
4-Nitro-pyridine-*N*-oxide (4-NPO)	Quorum sensing-targeting	[111]
Horseradish juice extract	Quorum sensing-targeting	[110]
Norspermidine and some biomimetic guanidine and biguanide compounds	Release the protein component of EPS from the bacterial cell wall	[112]
Lysozyme	Destruction of staphylococcal cell wall. Active against quiescent bacteria	[113, 114]

Reproduced with permission from [4]

action mechanisms or currently explored active substances and distinguished four main types:

(a) Bactericidal molecules capable of killing even metabolically quiescent bacterial cells within biofilms (e.g., lysostaphin, certain AMPs)
(b) Enzymes capable of selectively degrading extracellular polymeric substances of the biofilm (e.g., Dispersin B [99], rhDNase I [90, 98])

(c) Molecules downregulating the expression of biofilm extracellular polymeric substances (e.g., *N*-acetylcysteine [91, 103]) or anyway reducing biofilm metabolism (e.g., hamamelitannin [92, 93])
(d) Molecules acting with the Quorum sensing system and inducing biofilm dispersion (e.g., furanones) [106–111]

5.6 Dual-Function Antibacterial Surfaces for Biomedical Applications

The strategies depicted above involving either the fabrication of bactericidal surfaces or bacteria-resistant surfaces have supposed important steps toward effective antimicrobial surfaces. However, limited success has been achieved since most of the systems are effective during a short-medium periods of time. In order to improve the performance of antimicrobial surfaces, many efforts have been focused on the combination different functionalities [116]. In this section, we will analyze the alternatives developed that combine two strategies acting simultaneously in one system.

5.6.1 Repelling and Releasing Surfaces

This strategy involves the use of an inherent low adhesive material incorporating active molecules. An example of this strategy involves the use of poly(vinyl alcohol) (PVA), PEG-bearing copolymers or poly(acrylic acid) derivatives hydrogel coatings that exhibit reduced microbial adhesion (around two orders of magnitude lower than uncoated control). Moreover, these hydrogels are charged with antibiotics or other biocides, so that these coatings are capable of simultaneously repelling and releasing. A rather complex design but illustrative of this approach was described by Ho et al. [117] who prepared an antimicrobial coating provided by silver ion release with a contact-killing and microbe-repelling surface. As depicted in Fig. 5.6, they fabricated a coating based on a hydrophilic polymer network of poly(2-hydroxyethylacrylate) with PEI cross-linking points. Moreover, PEI are able to form complexes with the silver ions from aqueous solution and, for upon reduction silver nanoparticles. Finally, PEGylation of these co-networks resulted in materials that efficiently kill *S. aureus* cells and still repel them after exhaustion of the silver.

5.6.2 Contact-Killing and Repelling

Laloyaux et al. [118] reported the preparation of temperature-responsive polymer brushes switching from bactericidal to cell-repellent. The system reported consists of have presented a surface that consists of surface-attached antimicrobial peptide

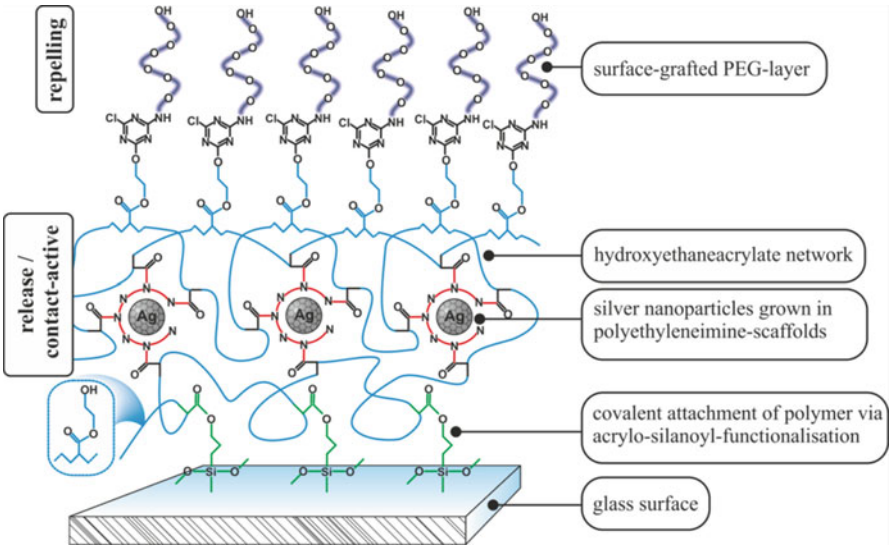


Fig. 5.6 Concept of repel and release of a designed network. Reproduced with permission from [117]

(Magainin) grafted with oligo(ethylene glycol) methacrylates (OEGMA). At room temperature, the OEGMA chains are stretched and the Magainin groups are available at the interface and effectively kill microbial cells on contact. However, upon heating above 35 °C the OEGMA collapses, the surface is mainly covered by PEG moieties at the surface. In this situation, the attached and nonattached Gram-positive bacterial cells are repelled efficiently. It is interesting to mention that, by lowering the temperature, the killing properties are reactivated. In principle, this allows to kill or repel microbial cells by reversible heating/cooling temperature cycles (see Fig. 5.7).

Another interesting examples of this strategy has been reported by Ji et al. [119]. Their approach combines heparin and chitosan embedded in a multilayer film constructed layer by layer. Chitosan (antibacterial agent) and heparin (anti-adhesive agent) were alternatively deposited onto aminolyzed poly(ethylene terephthalate) (PET) films. In their study, they correlated the hydrophobicity or hydrophilicity with the microbial adhesion. Chitosan, a pH-responsive natural polymer, exhibits significant structural changes by changing the environmental pH. Thus, at higher pH values the chitosan chains adopted loopier-type structures and tend to be adsorbed as thicker layers. On the contrary, a decrease in the pH values resulted in a reduced adsorption of chitosan to the surface. The amount of adsorbed chitosan and the hydrophilicity had a direct relation with the anti-adhesive properties of the film. The films assembled at lower pH are more hydrophilic, and this more hydrophilic surface prevented the adhesion of *E. coli* (Fig. 5.8).

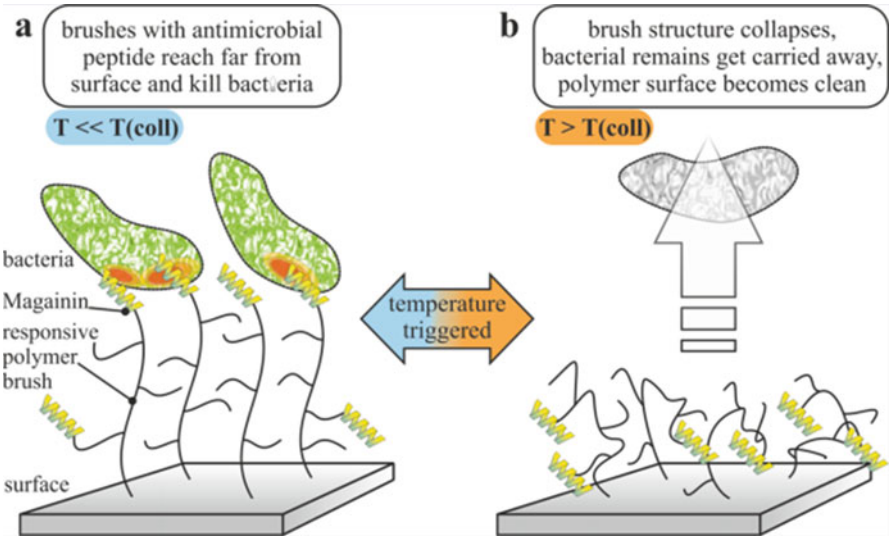


Fig. 5.7 Double contact-killing and repelling surfaces. Magainin grafted via thermoresponsive oligo(ethylene glycol) methacrylates (OEGMA) are able to (**a**) kill bacterial cells below and (**b**) repel them above the transition temperature. Reproduced with permission from [118]

5.6.3 Releasing and Contact-Killing

Biser et al. [24] developed a coating based on cellulose with an antimicrobial *N*,*N*-dimethyl-dodecylammonium (DDA) group grafted via poly(2-ethyl-1,3-oxazoline) ($PEtO_x$). The system worked as follows. First, the immobilized antimicrobial was able to kill approaching microbial cells on contact. The dead microbial cells deliver cellulose to the environment. Second, the liberated cellulose is capable of degrading the cellulose coating and reactivated the antibacterial activity again (Fig. 5.9). As major advantages over previous strategies, the authors mentioned that the cellulose-based coating reported can act as a contact-active system, is biologically compatible, degradable, and additionally might release biocides in case of a biological contamination only.

5.7 Responsive Antibacterial Surfaces

Modification of the surface with stimuli-responsive polymers has also been evaluated to make surfaces "antibacterial" [120]. As has been already mentioned, in general, previous designs of antibacterial surfaces resort to the delivery of antibiotics, antibacterial agents, or inorganic nanoparticles. Some of these strategies resulted in

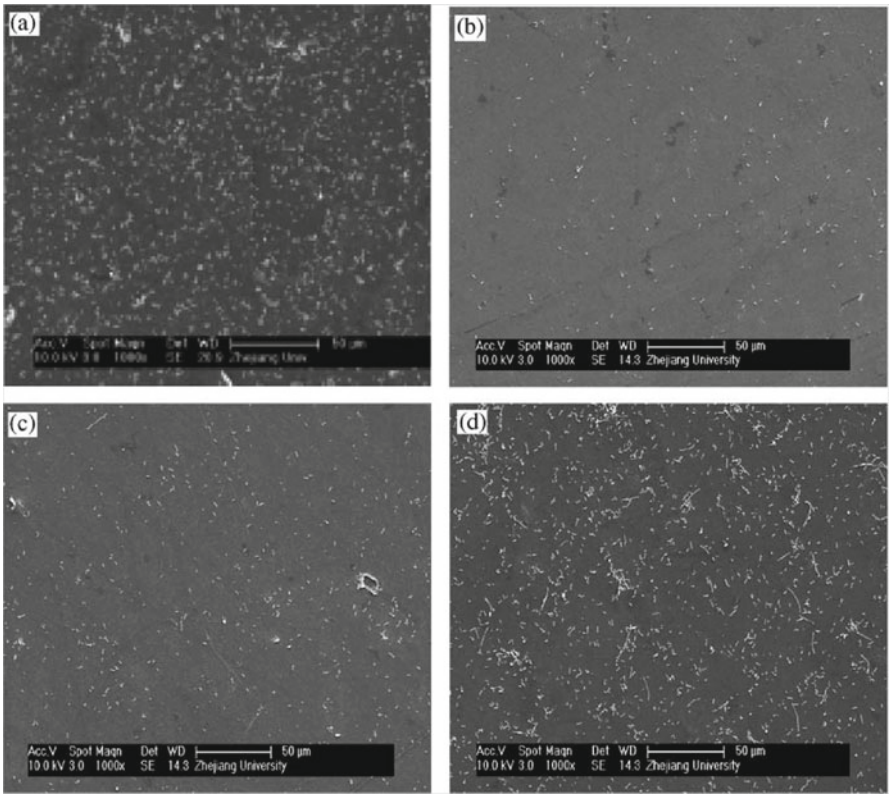


Fig. 5.8 Scanning electron micrographs of (**a**) pristine PET, (**b**) the (heparin/chitosan)$_6$ multilayer film assembled at pH = 2.9, (**c**) the (heparin/chitosan)$_6$ multilayer film assembled at pH = 3.8, and (**d**) the (heparin/chitosan)$_6$ multilayer film assembled at pH = 6.0 after exposure to 5×10^7cells = mL *E. coli* for 4 h. Reproduced with permission from [119]

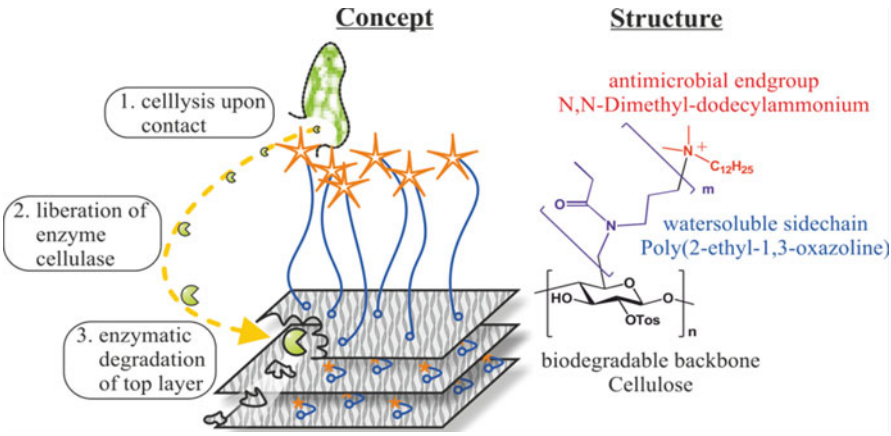


Fig. 5.9 Concept of contact-killing and releasing using a cellulose-based coating with an attached biocidal polymer. The cellulase deliver upon microbial killing can degrade the coating. Reproduced with permission from [24]

the increase of bacterial resistance, toxicity, or even the development of inflammatory responses. As a consequence, different studies evidenced the interest of designing novel antimicrobial coatings that respond only when infection occurs thus limiting the negative side effects. In general, these systems involve first the encapsulation of antimicrobial agents inside of the responsive thin coating. In a second step, using an external stimulus (temperature, pH, etc.) the antimicrobial agent is released [120]. As will be depicted, in other cases, the antimicrobial is covalently linked and they are exposed or hidden depending on the environmental conditions.

5.7.1 *Thermoresponsive Surfaces*

For instance, thermosensitive antimicrobial surfaces induce an increase or a decrease of the bacterial adhesion depending on the environmental temperature. Thermosensitive antimicrobial coatings reported by Laloyaux et al. [118] were able to switch from bactericidal for ambient storage conditions to passive in vivo. They prepared thermoresponsive coating formed by polymer brushes of copolymers based on 2-(2-methoxyethoxy)ethyl methacrylate (MEO_2MA) and oligo(ethylene glycol) methacrylate (OEGMA). Moreover, an antimicrobial peptide, Magainin-I active against Gram-positive and Gram-negative bacteria [121, 122] was grafted on the hydroxyl groups of the brush. As depicted in Fig. 5.10, the structure of the

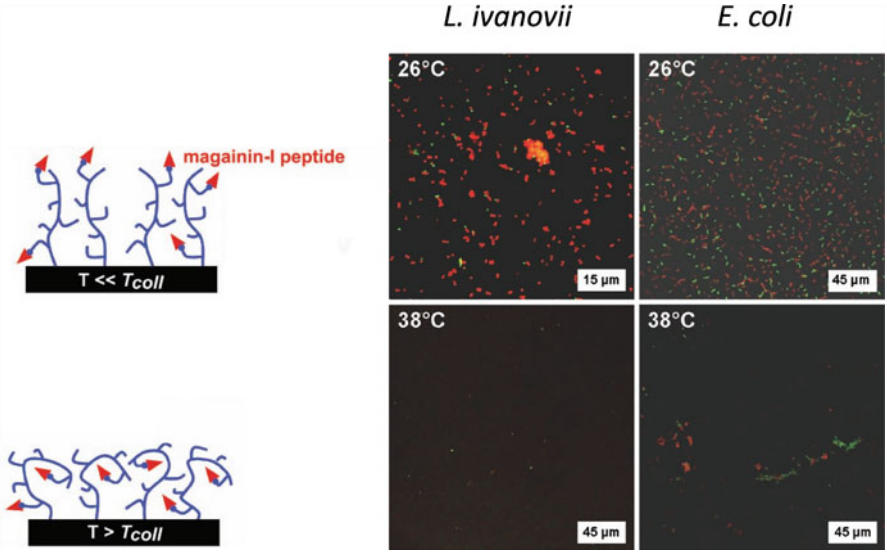


Fig. 5.10 (*Left*) Schematic drawing of the brush conformations below and slightly above LCST (T_{coll}). (*Right*) (MAG-Cys)-functionalized P(MEO_2MA_{50}-$HOEGMA_{20}$-$HEMA_{30}$) brush incubated in the presence of *L. ivanovii* or *E. coli* and subsequently stained with the LIVE/DEAD viability kit; samples incubated at 26 °C (*top*) and 38 °C (*down*). Reproduced with permission from [118]

temperature-responsive copolymer brushes based on oligo(ethylene glycol) methacrylates can be modified depending on the temperatures producing significant changes in the adhesion against various bacteria. The brushes switch from bactericidal to cell-repellent below and slightly above 35 °C, respectively, due to the progressive vertical collapse of the brush.

Pangilinan et al. [123] developed carbon nanotube (CNT)/PNIPA brush films exhibiting thermodependent antimicrobial action. They prepared the temperature-responsive carbon nanotube (CNT)/poly(*N*-isopropylacrylamide) (PNIPAM) hybrid brush films by combining the layer-by-layer and surface-initiated polymerization (LbL-SIP) techniques and evaluated the antimicrobial activity against *Exiguobacterium* sp. AT1b and Exiguobacterium sibiricum strains. The authors observed that CNT films showed antimicrobial action independently of the external temperature. On the contrary, CNT–PNIPAM films have antibacterial properties below 32 °C, which is below the lower critical solution temperature (LCST), but allows biofilm formation above the LCST.

5.7.2 pH-Responsive Surfaces

pH has been equally employed in the fabrication of smart antibacterial surfaces with on-demand switchable behaviors. For instance, Wei et al. [124] reported the fabrication of silicon nanowire arrays modified with a pH-responsive polymer, poly(methacrylic acid). This polymer has two main tasks. First, serves as a dynamic reservoir for the controllable loading and release of a natural antimicrobial lysozyme. Moreover, it works as self-cleaning platform for the release of dead bacteria and the reloading of new lysozyme thus enabling a repeatable use. Interestingly, using this strategy, the functionality of the surface can be simply switched via stepwise modification of the environmental pH and can be effectively maintained after several kill/release cycles.

5.7.3 Bioresponsive Surfaces

Bioresponsive materials refer to those interfaces that exhibit changes in response to enzymes or other constituents of the biological fluid or environment [125]. An extensively employed methodology to prepare antimicrobial surfaces takes advantage of biodegradable polymers charged with the appropriate active molecule. Some illustrative examples of biodegradable polymers employed in the fabrication of bioresponsive surfaces are depicted in Table 5.5.

Another strategy to prepare bioresponsive surfaces concerns the design of enzyme-responsive surfaces [120], where enzymes act on specific bonds that are activated in order to deliver the antimicrobial [140, 141]. In an illustrative report, Baier et al. [140] take advantage of this strategy to release an antimicrobial agent

Table 5.5 Biodegradable polymers and active molecules employed in the elaboration of antimicrobial bioresponsive surfaces

Biodegradable polymer	Active molecules	Reference
Polyphosphazenes	Ciprofloxacin and Norfloxacin	[126]
DL-dilactide polymer	Ciprofloxacin and Pefloxacin	[127]
Diisopropylcarbodiimide/poly (e-caprolactone)diol	Loaded with Nalidixic acid and Nalidixic acid derivatives	[128]
Poly(lactide-*co*-caprolactone)	Ciprofloxican-loaded biodegradable microsphere	[129]
1,6-Hexane diisocyanate/polycaprolactonediol polymers	Films of Ciprofloxican loaded	[130]
Chitosan have been shown to inhibit fungal and bacterial growth	Biodegradable composite films	[131–138]
Poly(lactic-*co*-glycolic acid) (PLGA)	Collagen	[139]

based on the action of an enzyme. In particular, they employed hyaluronic acid-based polymers that are known to be cleaved by enzymes called hyaluronidases. They designed and fabricated hyaluronic acid nanocapsules containing the antimicrobial polymer polyhexanide. The capsules were cleaved by enzymes and allow for polyhexanide release.

Using a similar approach, Tanihara et al. [141] reported the fabrication of a thrombin-sensitive peptide linker. Based on the fact that the presence of *S. aureus* in a wound is accompanied by increased thrombin-like activity and taking advantage of the fact that thrombin cleaves fibrinogen, these authors prepared fibrinogen-based thrombin-sensitive peptides. These peptides served as bridges between a hydrogel and a particular antibiotic. As a result of the cleaving of the thrombin-sensitive peptide, the antibiotic could be released to the environment.

Another strategy to prepare bioresponsive surfaces has been reported by Cavallaro et al. [120]. They proposed the fabrication of surfaces that contain partially exposed enzymes or coatings that leach-specific enzymes capable of protecting the surfaces from biological contamination or having antimicrobial effect [142–144]. This approach was employed by Wu et al. [142] that functionalized surfaces with exposed enzyme granules. The latter were able to protect them from various contaminations.

Finally, Satishkumar et al. [144] evaluated the in vitro antimicrobial activity of hernia repair meshes coated by the antimicrobial enzyme lysostaphin at different initial concentrations. In this study, the authors evidenced that leaching of lysostaphin significantly decreased the *S. aureus* infection within rat models. The antimicrobial activity of the lysostaphin-coated meshes suggests that such enzyme-leaching surfaces could be efficient at actively resisting initial bacterial adhesion and preventing subsequent colonization of hernia repair meshes.

5.7.4 *Other Responsive Interfaces*

Other stimuli have been equally employed to activate surfaces rendering them antimicrobial. These include the use of light onto photoactive surfaces, counterion-assisted modulation to facilitate the bacterial release or the fabrication of salt-sensitive surfaces.

Photoactive surfaces change their properties by variation of light wavelength, polarization, or light intensity. In this context, photodynamic antimicrobial chemotherapy (PACT) offers an alternative for the inactivation of pathogenic microorganisms based on the "photodynamic effect." In this approach, a photosensitizer, preferentially associated with a microorganism, is activated with nonthermal visible light of appropriate wavelength(s) to generate toxic species that inactivate the microorganism [145]. Upon absorption of a photon, such agents are able to release reactive oxygen species (ROS). Typically, reactive oxygen species can be generated in two forms: superoxide anions or hydroxyl radicals (type I) or singlet oxygen (type II) [146, 147]. The reactive radicals released from such coatings target bacteria in a non-site-specific manner. Unlike site-specific antimicrobial agents, i.e., antibiotics, it is difficult for bacteria to develop resistance to non-site-specific antimicrobials [147].

Photochemistry has revealed that both inorganic photocatalysts and organic photosensitizers could generate some reactive oxygen species (ROSs) on certain polymeric surfaces under light exposure, and these ROS can provide antimicrobial and decontaminating functions. Thus, researchers have been trying to incorporate the photoactive agents into various polymeric substrates to prepare self-decontaminating materials for medical applications, protective clothing, etc. [148].

Organic photosensitizers [145] employed as antimicrobials include phenothiazinium-based photobactericidal materials such as methylene blue (MB) or toluidine blue O (TBO), ruthenium complexes, rose Bengal, or phthalocyanines. These have been successfully employed for the inactivation of various Gram (+) and Gram (−) bacteria [149], such as Escherichia coli [150, 151], Staphylococcus aureus [151, 152], Streptococcus mutans [153], Porphyromonas gingivalis [154], and Pseudomonas aeruginosa [152, 155], have been documented in the literature.

Other alternative explored involves the use of UV irradiation on TiO_2-based coatings that are able to destroy cancer cells, bacteria, viruses, and algae [156]. For instance, Tallosy et al. [157] prepared photocatalysts (nanosilver-modified TiO_2 and ZnO photocatalysts)/polymer nanohybrid films by spray coating on the surface of glass plates. The photoreactive surfaces were activated with visible light emitting LED at $l = 405$ nm. The antibacterial effect of the nanohybrid films was evidenced by measuring the decrease of the S. aureus amount on the surface as a function of illumination time. The authors evidenced that the photocatalyst/polymer nanohybrid films could inactivate 99.9 % of the investigated bacteria on different thin films after 2 h of illumination with visible light source. In a recent example, Charpentier et al. [158] synthesized nano-titania/polyurethane ($nTiO_2$/polyurethane) composite coatings, where $nTiO_2$ was chemically attached to the backbone of the polyurethane

polymer matrix. The functionalized $nTiO_2$-polyurethane composite coatings showed excellent antibacterial activity against Gram-negative bacteria Escherichia coli; 99 % of E. coli were killed within less than 1 h under solar irradiation.

TiO_2 have been employed in the elaboration of other composites using PP [159], nylon [160], PS [161], or PMMA [162] as polymer matrices.

Counterion-activated nanoactuators permit to reversibly kill/release bacteria. Huang et al. [163] reported an strategy to release attached bacteria from surface-grafted bactericidal poly((trimethylamino)ethyl methacrylate chloride) (pTMAEMA) brushes. They prepared pTMAEMA brushes by surface-initiated atom transfer radical polymerization, and the surfaces were washed with electrolyte solutions containing anions with different lipophilic characteristic, charge density, polarity, and adsorbility to quaternary ammonium groups in polymers. Because of the special ion-pairing interactions, the interfacial properties, including wettability and ζ-potential, can be manipulated in a controlled manner. As a result, the counterion-assisted modulation of pTMAEMA brushes facilitates the bacterial release and regeneration of antimicrobial polymer films.

Finally, as demonstrated by Yang et al. [164] also the salt concentration can play a key role on the antifouling properties. They fabricated zwitterionic poly(3-(1-(4-vinylbenzyl)-1H-imidazol-3-ium-3-yl)propane-1-sulfonate) (polyVBIPS) brushes as ion-responsive smart surfaces via the surface-initiated atom transfer radical polymerization. They examined the salt-response and evaluated the variation on the surface hydration and as a consequence on both friction, and antifouling properties. In particular, they compared both in water and in salt solutions with different salt concentrations and counterion types. According to the authors, the polyVBIPS brushes exhibited reversible surface wettability switching between in water and saturated NaCl solution. As a result, polyVBIPS brushes in water induced larger protein absorption, higher surface friction, and lower surface hydration than those in salt solutions. Interestingly, at appropriate ionic conditions, polyVBIPs brushes were able to switch to superlow fouling surfaces (<0.3 ng/cm^2 protein adsorption) and superlow friction surfaces ($u \sim 10^{-3}$).

5.8 Conclusions

This chapter depicts the multiple strategies reported to reduce or to completely avoid bacterial contamination onto polymeric surfaces. As has been shown, surface modification is crucial in order to achieve this goal. In this context, different strategies can be employed.

Grafting approaches or the deposition of coatings onto the surfaces have been extensively employed to reduce the bacterial adhesion. More recent strategies resort to responsive materials. Temperature, pH, UV-light, or even salt has been demonstrated to be interesting stimuli that can produce the bacterial detachment in a precise manner.

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Chapter 6
Nano/Microstructured Antibacterial Surfaces

Abstract Today, it is well accepted that micro- and nanoscale surface topographical features can play a key role in controlling bacterial attachment. For instance, surface roughness has been directly related to the reduction of the initial surface contamination that can thus improve the reduction of biofilm formation. Thus, in addition to the chemical surface modification depicted in the previous chapter, in this chapter alternative attractive strategies to reduce bacterial adhesion would be simply acting on the features of the biomaterial surface will be described.

Moreover, the particularly astonishing advances in nanotechnologies permit today the controlled fabrication of surfaces with higher resolutions down to the nanometer scale. This area has currently become an area of intense research. The interest in the preparation of nanometer size features on material surfaces relies on the fact that they have been demonstrated to alter the 3D conformation of adsorbed proteins. As a result, it is expected that this behavior could potentially have an effect also on host adhesins which are the base of biofilm formation at biomaterial surfaces. This chapter provides an overview over the different strategies employed to fabricate micro/nanostructures and the effects observed when in contact with microorganisms. Equally, examples in which an additional surface functionalization supposes a significant improvement of the antibacterial/antifouling properties of the micro/nanostructured surfaces are depicted.

Keywords Hierarchical structuration • Nanostructured surfaces • Surface roughness • Micro-patterning • Surface instabilities • Bioinspired surfaces

6.1 Introduction

In addition to the chemical surface modification depicted in the previous chapter, another attractive strategy to reduce bacterial adhesion would be simply acting on the features of the biomaterial surface [1]. Today, it is well accepted that micro- and nanoscale surface topographical features can play a key role in controlling bacterial attachment. For instance, surface roughness has been directly related to the reduction of the initial surface contamination that can thus improve the reduction of biofilm formation [2, 3].

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It is important to note that pioneer studies were, at least apparently contradictory. Some studies carried out by the groups of Vanhaecke and Flint did not evidence a direct relationship between surface roughness (in terms of Ra) and the bacterial adhesion to surfaces [4, 5]. On the other hand, other authors reported that an increase of the surface roughness has associated a high retention of bacteria [6–8]. However, as reported by Whitehead et al. [2] this, a priori, contradictory observations can be justified by the range of roughness of the substrata employed in these studies. In particular, they precisely define the ranges in which the surfaces features have an influence on the bacterial adhesion. In their report, they evidenced that surface features whose dimensions greatly exceed those of the microorganisms will have little effect on retention [9]. Equally, features of dimensions largely below the microorganism size also have little effect [7, 8, 10]. On the contrary, enhanced bacterial retention on different surface features was observed in those cases in which the surface exhibit features in the size of the microorganisms. According to the authors, this could be due to an increase in bacterial attachment sites (for a given surface area), leading to stronger bacterial attachment and enhanced protection from cleaning shear forces [11]. Similar conclusions were reported by Verran et al. [12] demonstrating that *Staphylococcus sciuri* cells were most strongly held within the features of dimensions comparable to the cells.

The bacterial adhesion on surfaces with variable roughness has also been studied from a theoretical point of view. For instance, Decuzzi and Ferrari [13] presented a theoretical model for predicting the strength of cellular adhesion to originally inert surfaces as a function of the substrate topography. According to the authors, three different regimes can be identified as a function of the surface energy of the substrate (γ). For small γ, any increase in roughness results in a detrimental to adhesion; for large γ, an optimal roughness exists that maximizes adhesion; and for intermediate γ, surface roughness has a minor effect on adhesion. Thus, inspired by mathematical model, taking into account nanotopography and ad hoc surface functionalization it may be possible to achieve the selective adhesion of eukaryotic cells necessary for tissue integration, while contrasting bacterial adhesion.

Finally, it is worth to mention that in addition to the length scale, the distribution of the surface patterns has also been explored. The above-mentioned systems involved surfaces in which the roughness is based on a randomly distributed valleys and hills. However, as will be depicted later, an engineered surface topography based on skin of sharks disrupts the formation of bacterial biofilms without the use of bactericidal agents [14]. Thus, both pattern size and the morphology cannot be studied separately but required to be considered simultaneously [15].

Moreover, the astonishing advances in nanotechnologies permit today the controlled fabrication of surfaces with higher resolutions down to the nanometer scale. This area has currently become an area of intense research [16, 17]. The interest in the preparation of nanometer size features on material surfaces relies on the fact that they have been demonstrated to alter the 3D conformation of adsorbed proteins. As a result, it is expected that this behavior could potentially have an effect also on host adhesins which are the base of biofilm formation at biomaterial surfaces.

Based on this behavior, the remarkable properties of nanostructured materials are expected to be of interest in the field of bacterial adhesion and proliferation as well [1].

In particular, the role of the surface nanotopography and architecture in the bacterial attachment and biofilm formation are still under investigation [14, 16–18]. On the one hand, nanostructured interfaces are expected, based on the size of the microorganism, to decrease their affinity for the substrate and thus reduce the colonization. This general expected behavior, however, is not universally applicable and a depth analysis of the interface employed is required. In some cases, bacteria can successfully colonize surfaces with an average surface roughness (Ra) of the order of only a few nanometers or sub-nanometers [17]. On the other hand, in the attempt to find valid alternatives to classic antibiotics and in view of current limitations in the efficacy of antimicrobial-coated or -loaded biomaterials, Ivanova et al. [19] described the possibility to produce antifouling surfaces by acting on the nanotopology. Nanotopology reduces the area available for bacterial attachment, but the analysis of the role of the nanostructure appears to go far beyond simply limiting bacterial adhesion. In their report, by taking advantage of the nanopillar arrays present on the cicada wing surface the observed that these arrays can induce bacterial cell death.

In summary, the bacterial adhesion issue requires a global analysis in which the size of the surface patterns (micro/nanostructure), their distribution (random, ordered) as well as the shape of the motifs can dramatically affect the final surface behavior.

This chapter provides an overview over the different strategies employed to fabricate micro/nanostructures and the effects observed when in contact with microorganisms. Equally, examples in which an additional surface functionalization supposes a significant improvement of the antibacterial/antifouling properties of the micro/nanostructured surfaces are depicted.

6.2 Fabricating Micro- and Nanometer Size Patterns on Polymer Surfaces

During the last decade, significant efforts have been carried out for the fabrication of structured surfaces with greater geometrical complexity at reduced operation time and cost. These include patterns made of polymer materials possessing high aspect ratio, exhibiting several hierarchy levels, or in intricate tilted, suspended, or curved three-dimensional (3D) arrangements [20]. Herein, we will limit our discussion on the more relevant approaches that have been employed in the fabrication of micro- and nanostructured surfaces for the design of antimicrobial surfaces.

6.2.1 Innovative Lithographic Techniques

Lithography has been extensively employed to transfer a particular pattern onto a substrate by using an etching process [20]. In particular, resist lithography takes advantage of an irradiation source that is applied onto a photosensitive polymeric material responsible to transfer the pattern. Typically, the photoresist is first coated

onto a planar substrate and "soft baked" to completely remove the solvents. Then, the selected areas of the film are exposed to light and the photoresist properties change. Usually, the solubility of the film is modified, either decreasing the solubility to yield a negative-tone image or increasing the solubility of exposed areas (yielding a positive image after development).

Recent advances in lithography including the use of phase-shifting masks, illumination sources with shorter irradiation wavelength, or advanced photoresist materials permitted the fabrication of lithographic structures down to sub-100 nm dimensions [21–25].

6.2.2 Laser-Based Micro-Nanopatterning

As depicted above, surface patterning by photolithography involved first the design and fabrication of an appropriate mask. This requirement limits the flexibility, can be expensive, and also delay the fabrication process. Laser prototyping is an interesting alternative that do not require the use of masks. This patterning methodology involved the use of UV, nanosecond pulsed, excimer, and Nd: YAG lasers that precisely irradiates particular areas of the surface [20]. While the chemistry also relies on the use of resist layers and coating processes similar to lithography, this technique can be applied for the fabrication of larger areas.

Recent advances on the manufacture of picosecond and femtosecond lasers have enabled the fabrication 3D structuring with high precision in a single step using two-photon polymerizable systems. Among the latter two different approaches are currently extensively employed: stereolithography by scanning resist [26] and two-photon lithography (TPL) [27].

6.2.3 Writing Using Electron and Ion Beams

These techniques include electron beam, and ion beam uses electrons and ions to penetrate the resist material and create a well-defined path.

Electron beam lithography (EBL): uses an electron beam (typically 10–100 eV) to expose an electron-sensitive resist. The electrons irradiated onto the resist leads to free radicals and radical cations that are deactivated through fragmentation or reaction with the matrix. By using EBL, lateral resolutions to around 10 nm can be achieved. However, electrons have a small penetration depth therefore the use of this patterning technique is limited to layers with thickness below 100 nm.

Ion beam lithography (IBL): in this case high-energy ions, such as Ga^+, H^+, or He^+, are employed to create the surface pattern. In contrast to EBL, in this approach the penetration depth can be varied depending on the ion energy. As a result, increasing the beam energy from 1.0 to 3.5 MeV will accordingly increase the penetration from 20 μm up to 160 μm.

6.2.4 Molding

Molding is an alternative to photolithography to produce micro- and nanopatterns without the use of light. As depicted in Fig. 6.1, different molding processes have been developed with more or less success. Some important disadvantages of this technology includes: that cannot easily be carried out on polymeric substrates or on more sophisticated interfaces such as curved substrates, limited patterning areas, the use or harsh chemicals (resist etchants and developers, solvents, etc.) that may be incompatible with other materials or the operational cost. Nevertheless, new patterning techniques enabling micro- and nanoprocessing of plastics are currently being explored for their potential adaptation to nanofabrication. The most important are:

Nanoimprint lithography (NIL): also known as hot embossing and thermal injection molding, NIL induce the formation of surface patterns on thermoplastic polymers by conformal contact of a micro/nanostructure mold using heat to melt the polymer and pressure [28]. By applying pressure, the polymer melted is able to flow into the cavities of the structures of the mold. When the polymer occupies the cavities of the mold, the system is cooled and the mold is separated from the polymer thus leaving the structured surface.

Molding UV-sensitive materials (UV NIL): this alternative of NIL takes advantage of UV-curable resins and polymeric precursors to produce microstructured surfaces. In this case, the structured mold (transparent to UV light) is coated with the UV-curable resin. The molds are made of hydrogen silsesquioxane (HSQ) [29, 30], indium-tin oxide (ITO) [31–33], or quartz are exposed to UV irradiation that cross-linked the resin. Finally, the patterned material is demolded.

Soft lithography: soft lithography refers to those pattern-replication methodologies associated to the use of an elastomeric mold [34]. In this method, the structure of a master is transferred to an elastic polymer typically by thermal curing of a prepolymer. The most extended strategy involves the use of poly(dimethyl siloxane) (PDMS) that is coated onto the mold and heated to induce the polymer cross-linking. The elastic properties of PDMS allow it to be released from the mold without damaging the surface pattern. The structured PDMS can be the final structure and used, in turn, as mold for an additional process [35–37].

6.2.5 Pattern Formation by Surface Instabilities

Surface instabilities or in general unstable conditions are usually undesirable and are associated to lack of control of a given process. A typical example of uncontrolled processes involves film dewetting that frequently produce imperfect coatings resulting in improper functionality or bad appearance. However, understanding the dynamic behavior of a specific unstable condition may open pathways to valid patterning techniques if the outcome of the surface reconstruction due to the instability can be mastered. The interest of using surface instabilities to pattern polymer surfaces relies on the rich and complex patterns obtained as a result of spontaneous

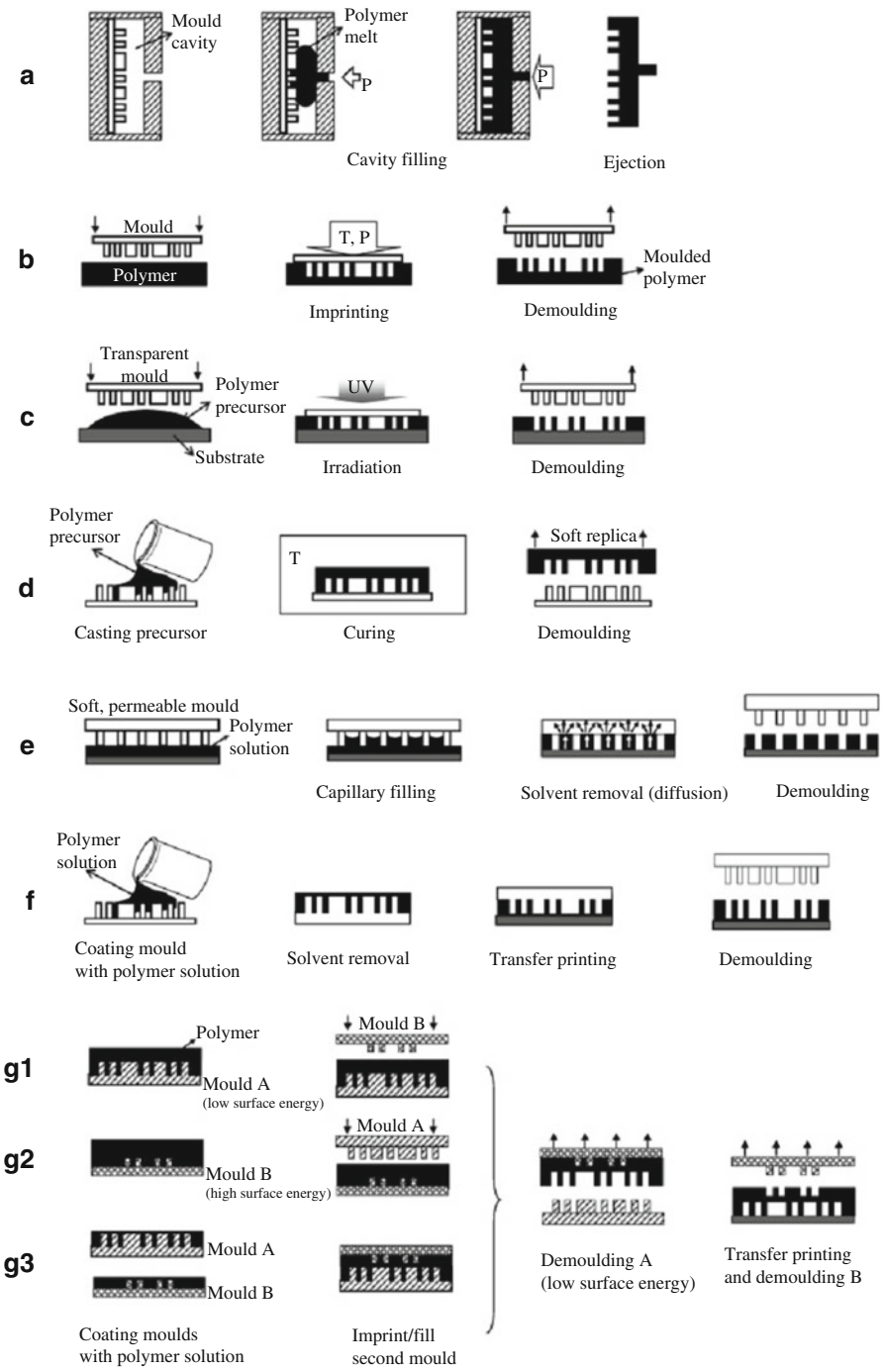

Fig. 6.1 Comparison of different molding processes. (**a**) Injection molding (NIL). (**b**) Hot embossing or nanoimprint lithography. (**c**) UV NIL. (**d**) Soft lithography. (**e**) Solvent-assisted molding. (**f**) Reversal imprinting or transfer molding. (**g**) Multilayer printing: "duomold" approach. The schematic g1, g2, and g3 represent three variants of this process. Reproduced with permission from [20]

processes. For these reasons, many research groups are currently interested in understanding and proposing alternative patterning strategies based on controlling surface instabilities or dynamic processes of polymer materials. It is possible to classify the techniques in three main families, based on the methods used to guide polymer patterning.

6.2.5.1 Spontaneous Structuration Driven by Surface/Interfacial Energy

Film thickness, temperature, or hydrophilicity of the substrate can also be employed as parameter to induce surface structures. In particular, dewetting [38] produced as a result of the film rupture on the substrate has been extensively employed to induce surface patterns. Equally, convection processes are the base of evaporative self-organization [39] processes. Finally, within this category we can include phase separation processes taking place in bulk mixtures that commonly leads to an isotropic, disordered morphology of the coexisting phases. The presence of a surface can significantly alter the phase-separation process. Phase separation of polymer blends and block copolymers [40, 41], surface segregation [42], and template guided structuration [43].

6.2.5.2 Field-Induced and Dynamic Control of Surface Structuration

The surface stability can also be altered by applying an external force. For instance, electric or magnetic fields, thermal gradients, or mechanical stresses are among the stimuli that have been employed to either modify the morphology of polymer films or induce their formation. A reasonable understanding of some of these methods has been achieved. In this category, it is possible to find the following patterning methodologies: electrohydrodynamic patterning/thermal-gradient induced surface patterning [44], elastic instability and surface wrinkling [45, 46], and reaction-diffusion surface patterns [47].

6.2.5.3 Influence of Water on Hydrophobic Polymer Surfaces

A variable physicochemical environment can also be used to modify the arrangement of polymer materials. Exposure to different solvents, vapors, water, or electrolytes may trigger the reconstruction of polymer surfaces. Three examples are: breath figures [48], ion-induced nanostructuration [49], and nanobubble-assisted nanopatterning [50].

6.3 Micro/Nanostructured Antimicrobial Surfaces in Nature

Nature has been, during the last decades, a source of inspiration for the development of novel materials with unique antimicrobial properties. In effect, many surfaces in nature exhibit exceptional antiadhesive/antimicrobial properties that, according to

several studies, is the result of both their particular surface chemical composition (hydrophobic) and hierarchical micro- and nanostructures [51–62]. In effect, a large variety of living organisms exhibit nano- and micrometer-structured surfaces with self-cleaning, anti-icing, and water-repellent properties due to a synergistic combination of hierarchically structured surfaces and low surface-free energy provided by the surface chemical functionality [63]. In general, the complex hierarchical structures depicted minimize the contact area between an abiotic surface and the physiological fluid containing bacterial cells. Nevertheless, some recent examples demonstrated that nanostructured surfaces can not only prevent bacterial adhesion but act as antimicrobials.

Hence, surface patterning and functionalization that includes approaches to develop micro/nanostructures as a means to achieve antifouling/antibacterial surfaces has been proposed as a potential solution for the long-term prevention of bacterial adhesion [64, 65].

In this section, we will first describe the use of surfaces provided by nature to prevent bacterial adhesion structured both at the micro- and nanometer scale. Secondly, different strategies reported so far for the fabricate nanostructured and hierarchically structured interfaces for the prevention of bacterial adhesion will be depicted.

6.3.1 Nanostructured Surfaces that Repel/Kill Bacteria in Nature

In principle, surface nanostructures are expected to limit the bacterial contact with the substrate and thus prevent the biofilm formation. However, recent reports evidenced the capability of nanostructured surfaces to kill bacterial cells. For instance, bacterial adhesion onto nanopillars present at the surface of cicada wings were studied by Ivanova et al. [19]. In contrast to what was anticipated these surfaces were not effective at repelling bacteria. On the contrary, a large amount of bacterial cells were able to adhere to the wing surfaces.

However, their studies revealed that *Pseudomonas aeruginosa* cells were rapidly killed upon adhesion on Cicada wing surfaces (Fig. 6.2). They evidenced rapid changes on the cell morphology when the bacteria contact the surface. When the cells come into contact with the nanostructured surfaces, the wing nanopillars penetrated the cells membrane. As a result, they observed cellular components spread over the surface and concluded that the penetrated cells were dead. As complementary experiments, they attempted to address the role of the surface chemical composition on the antibacterial properties. For that purpose, they modified the chemical composition of the surface with an ultrathin gold layer and demonstrated that is rather the physical surface structure responsible for the antibacterial behavior and not the surface chemistry.

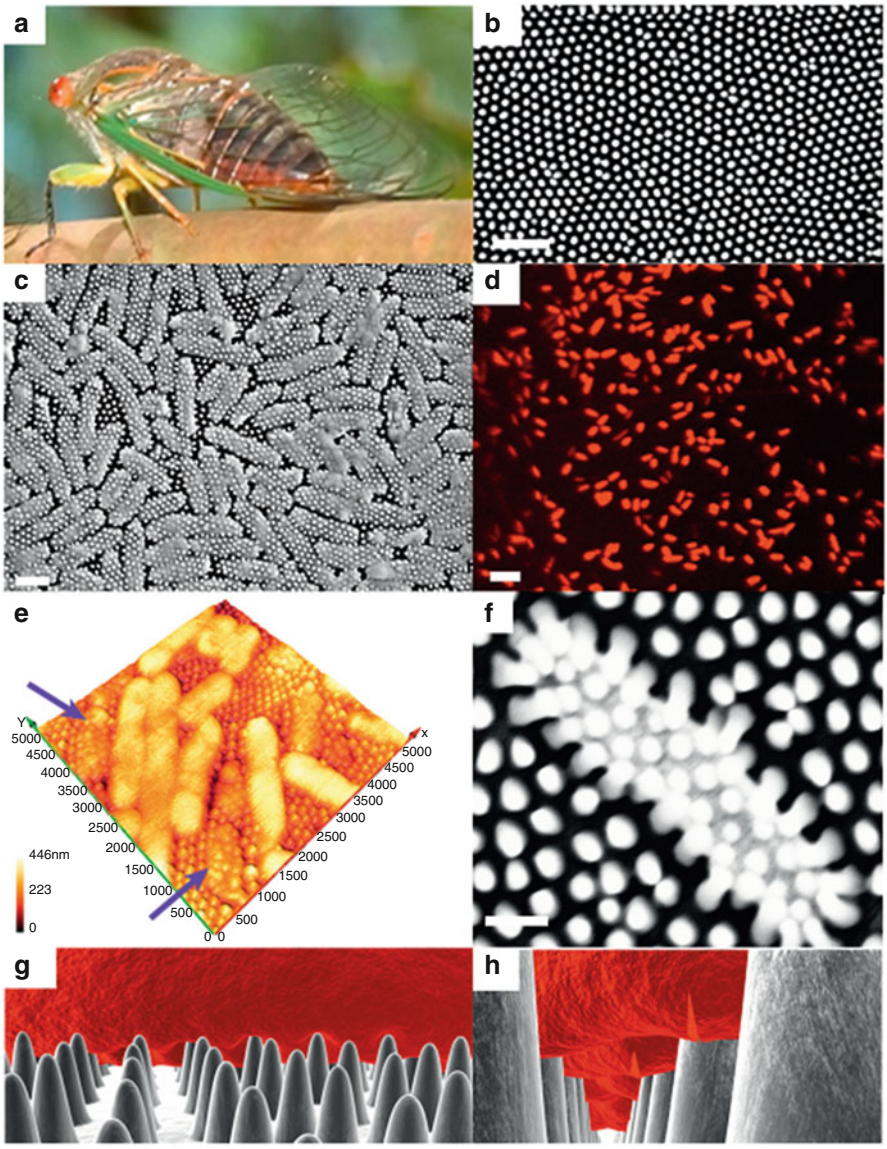

Fig. 6.2 Illustrative behavior of nanostructured surfaces in cicada wings and its antimicrobial behavior [19]. (**a**) Picture of a cicada, *Psaltoda claripennis*. (**b**) SEM micrograph of the pillar arrangement of a cicada wing. (**c**) Optical micrograph of *Pseudomonas aeruginosa* cells attached onto the wing surface, scale bar = 1 μm. (**d**) Fluorescent image of nonviable (*red color*) bacterial cells. Scale bar = 5 μm. (**e**) AFM microscopic image depicting the interaction between the surface on the bacteria and the resulting disruption of the cell membrane (*arrows*). (**f**) Bacterial cell interactions with a cicada wing surface with modified surface chemistry (gold at the interface). (**g**) and (**h**) Schematic cartoon depicting the initial bacterial attachment and the rapid rupture of the cell wall in contact with the cicada wing nanopillars [66]. Figure reproduced with permission from [19, 66]

6.3.2 *Hierarchically Structured Surfaces with Antifouling Properties*

It is today generally admitted that, dual-scale of micro- and nano-roughness of the surface morphology observed in leaves of the plant of lotus flower (*Nelumbo* sp.) with superhydrophobic properties are in addition antifouling. Water droplets onto these surfaces follow the Cassie-Baxter wetting mechanism, i.e., the water droplets are placed on top of the surface leaving below air cavities. As a result, these types of surfaces appear to prevent/limit the contact between a bacterium and the potential attachment points of the material surface. Indeed, the areas of interaction remain confined to the surface of the physical protrusions [67, 68].

Other plant leaves have also been explored for their antifouling properties include the case of *Colocasia esculenta* (taro). Both species, *Nelumbo* and *Colocasia* share common features, i.e., in both cases the surface structure consists of micrometer size surface structures with a hydrophobic epicuticle layer and a large amount of nanometer size wax crystalloids. As a result, both surfaces exhibit a large contact angle and low water roll-off angle.

However, an interesting difference between the two has been reported by Ma et al. [69]. There is a general agreement that superhydrophobic properties remain invariable when dealing with surface-air interfaces. On the contrary, it has been reported that a lotus leaf loses its superhydrophobicity in two different cases. On the one hand, when the surface is wetted by long-term immersion in water [70]. On the other hand, upon water vapor condensation on the surface [71]. While this is true for the case of *Nelumbo*, Ma et al. [69] evidenced that taro leafs exhibit excellent antifouling properties even under complete wet conditions (Fig. 6.3). In order to explain this phenomenon the authors suggested a different mechanism as a function of the environment of exposure. Under not-wet conditions, the antiadhesive property comes from the air trapped between the nanostructures. Under completely wet conditions, the antifouling property is the result of the reduced adhesion force on the area covered by dense nanometer size surface moieties. As a result, the authors suggested that appropriate nanoscale topographic structures are key to potentially reduce or even completely prevent bacterial adhesion.

6.4 Engineering Bioinspired Surfaces with Either Micro- or Nanostructured Topographic Structures

As has been depicted in Sect. 6.2, a large variety of synthetic approaches are available nowadays in order to prepare functional and structured surfaces. As a result and based on the surface structures found in nature, different strategies have explored to mimic at least to some extent, the geometries and patterns both at the micro- and nanoscale of natural surfaces.

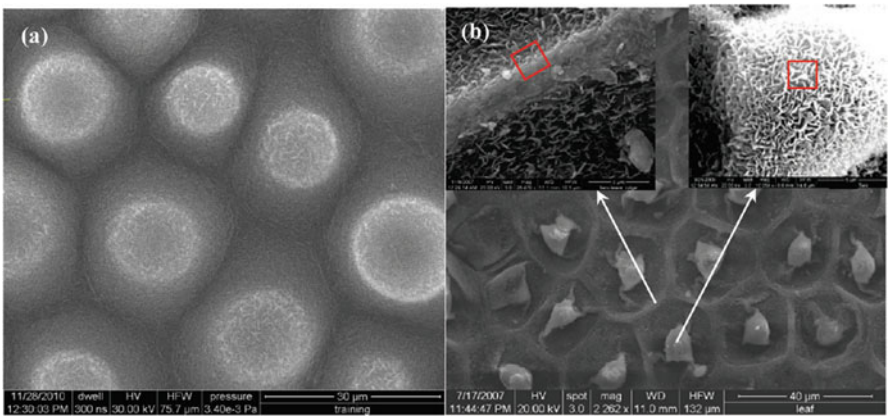

Fig. 6.3 Scanning electron micrographs of a taro leaf under wet conditions (**a**) and in a dry environment (**b**). (**b**) Images revealed the presence of two distinct regions (marked with two *red* squares) with particular nanostructures that exhibit very low adhesion. Reproduced with permission from [69]

6.4.1 *Synthetic Structured Polymer Surfaces with Micrometer Size Patterns*

An illustrative example of the role of the micrometer scale surface topography on the adhesion of *S. aureus* without using bactericidal agents has been reported by Chung et al. [14] They designed a surface microtopography that resembles the structure found on the skin of sharks (Fig. 6.4). For this purpose, they use a poly(dimethyl siloxane) (PDMS) elastomer to create patterns with dimensions of 2 μm feature width and spacing, 3 μm feature height. In their study, they selected these dimensions based on the hypothesis that hypothesized that the dimensions of the topography would be slightly too large to effectively reduce the attachment of the bacteria in the size range of ~1–2 μm but could be effective at physically disrupting the further colonization of additional bacteria and subsequent formation of biofilm. By comparison of the structured PDMS with a smooth homologue, they observed that the smooth surface exhibited early-stage biofilm colonies at 7 days and mature biofilms at 14 days, while the topographical surface did not show evidence of early biofilm colonization until day 21. Interestingly, after 14 days, the percent area coverage of *S. aureus* on the smooth surface was 54 % compared to 7 % for the structured PDMS surface.

6.4.2 *Nanoscale Surface Patterns in Polymeric Materials as Antimicrobial Materials*

The interaction of cells react with nanoscale structures remains today a controversial issue. Several studies have been focused on the understanding of how cells and bacterial respond to nanotopographical features [18]. While, cell-surface

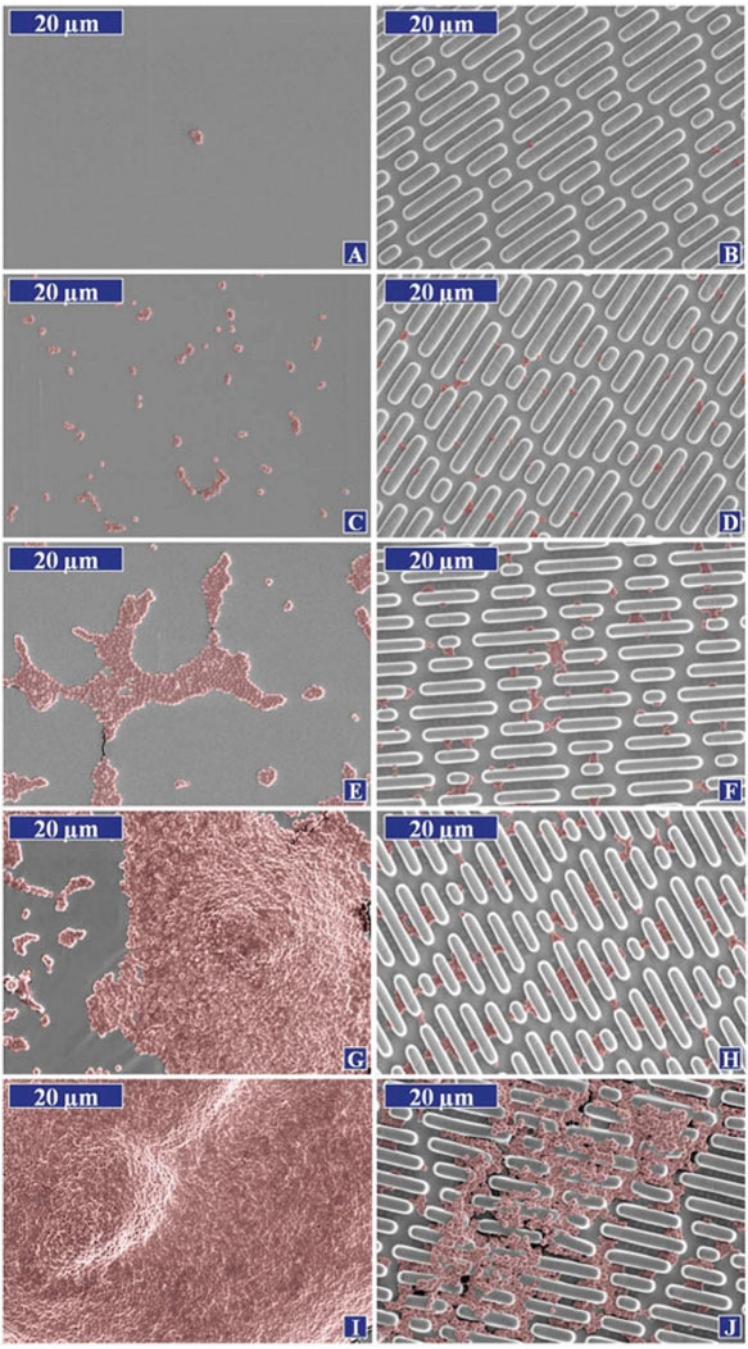

Fig. 6.4 Representative SEM images of *S. aureus* on PDMS surfaces over the course of 21 days (areas of bacteria highlighted with color to enhance contrast). On the left are smooth PDMS surfaces and the right column shows Sharklet AF™ PDMS surfaces. (**a**) and (**b**) day 0, (**c**) and (**d**) day 2, (**e**) and (**f**) day 7, (**g**) and (**h**) day 14, and (**i**) and (**j**) day 21. Reproduced with permission from [14]

interactions start to be understood as depicted in a large number of studies, examples related to bacterial adhesion on artificial nanostructured polymeric surfaces are limited. Moreover, the results reported are rather contradictory and thus require of further investigation.

For instance, nanostructured surface have, according to recent studies, a twofold role in terms of bacterial adhesion as well as on the metabolism of the bacteria. As reported by Mitik-Dineva et al. [72] using glass supports prepared with different degrees of nanometer-scale roughness, nanostructures the surface topography can exhibit a different bacterial behavior not just in terms of adhesion, but also in terms of cell metabolism, finally resulting bioactive. The three bacterial strains tested, i.e., E. coli, P. aeruginosa, and S. aureus, present significantly different patterns of attachment, all of the species exhibited a greater propensity for adhesion to the nano-rough surface. In addition, the bacteria responded to the surface modification with a remarkable change in cellular metabolic activity, as shown by the characteristic cell morphologies, production of extracellular polymeric substances, and an increase in the number of bacterial cells undergoing attachment.

Changing the glass substrate by a polymeric nanostructured supports dramatically modifies the bacterial interactions. Campoccia et al. [73] used structured and planar substrates as reference surfaces made of polyethylene terephthalate to understand the adhesion of *S. aureus*. The nanostructures prepared are cylindrical pillars (PET-N) (nanocylinders of 160 nm height and 110 nm diameter, with a spacing of 220 nm) and additionally flat ion-etched (PET-F), and tissue culture-grade polystyrene (PS) for comparative purposes. In order to assess the adherence of *S. aureus* on these surfaces, the authors explored four different media: (a) bacteria suspended in MEM medium, (b) bacteria in MEM supplemented with 10 % fetal bovine serum (FBS), (c) using test surfaces preconditioned in FBS, and (d) upon post-exposure of colonized surfaces to serum-supplemented MEM. They reported that PET-F and PET-N specimens showed high bacterial adhesion properties for all the environmental conditions tested. However, in the absence of serum both PET surfaces (nanostructured and ion-etched) exhibited greater adhesion than PS. This situation is reversed upon incorporation of 10 % serum in solution. In this case, the number of microbial cells on all surfaces was drastically reduced and PET is in these conditions less adhesive than PS. Unfortunately, in comparison with the use of nano-rough glass supports, when using polymers as substrates the specific cylindrical nanostructures created on PET did not significantly influence microbial behavior.

However, as has been mentioned for the case of lotus leaf, i.e., the superhydrophobicity is loosened when the surface is wetted by long-term immersion in water [70] or water vapor condensation on the surface [71], engineered superhydrophobic surfaces behave identically and gradually lose their antibiofouling properties when long-term testing in water are required. For instance, Zhang et al. [74] investigated smooth and roughened superhydrophobic coatings made from fumed silica (primary particle size around 50 nm), alkyltrialkoxysilane, and polysiloxane. They studied the effect of nanoscale interfacial roughness on the adhesion of Gram-negative bacterium SW8 and mixed cultures of micro-foulant for periods of up to 6 months using visual and wettability measurements. According to their results, no

microorganism was attached to the superhydrophobic structured surfaces in the first weeks of immersion. On the contrary, smooth substrates exhibited fouling within a day. Interestingly, an increase of the surface roughness leads to surfaces with higher resistance to fouling over a 6-month period. However, after periods exceeding 2 months under real ocean conditions, both films showed limited antifouling properties.

6.5 Engineered Surfaces with Micro/Nanostructured Topographic Features and Chemically Controlled Surface

Both surface chemical composition and surface topography on the macro- and micro scale strongly affect cell behavior [75] and the synergistic effect has been proposed as an interesting alternative to improve the long-term antiadhesive/antibacterial surface properties.

Aizenber et al. [76] demonstrated recently that the combination of chemical functionalization together with surface structuring appears to be a very effective methodology to prevent bacterial adhesion. Previous strategies for biofilm prevention were based exclusively on either the modification of the surface chemistry treatments or the use of microstructured surfaces. These strategies were found to only transiently affect initial attachment, finally losing their antifouling/antibacterial efficacy. Aizenber et al. reported the fabrication of slippery liquid-infused porous surfaces (SLIPS) using the methodology depicted in Fig. 6.5. The strategy involves four different steps: (1) nanostructuration of the surface, (2) surface chemical functionalization, (3) infiltration, and (4) removal of unattached lubricant. According to the authors, this strategy that combines surface structuration and chemical modification prevent 99.6 % of *P. aeruginosa* biofilm attachment over a 7-day period. This approach work equally for both static and physiologically realistic flow conditions using different bacteria: *S. aureus* (97.2 %) and *E. coli* (96 %). This result clearly improved precedent studies carried out by Campoccia et al. [73] in which PET and PS nanostructured surfaces accumulate biofilm within hours and also superhydrophobic poly(tetrafluorethylene) (Teflon) nanostructured films employed by Aizenber as model system.

Another example of antimicrobial nanostructured surfaces was recently reported by Kim et al. [65]. They prepared a nanoimprinted polymeric film using the strategy depicted in Fig. 6.6. First, the stamp was obtained in silicon wafer following a multistep procedure that comprises: (a) the employed KrF laser lithography to fabricate a large-area nanostructured surface using a Si wafer as substrate. Then, the Si wafer was coated with the bottom antireflection coatings (BARC) photoresist to reduce reflections and sidewall roughness and subsequently (b) with a 400 nm thick pattering photoresist. Upon etching (c) and by dry etching with a combination of Cl_2 and HBr gases (d) hexagonal nanopillars with a period of 300 nm were obtained. Finally,

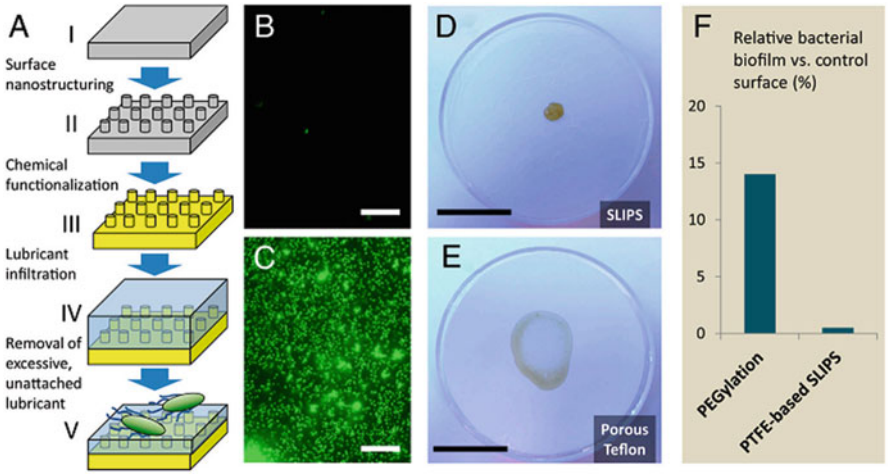

Fig. 6.5 Slippery liquid-infused porous surfaces (SLIPS) preparation and study of the bacterial attachment to the surfaces. (**a**) Stratregy developed for the preparation of slippery liquid-infused porous surface. (**b** and **c**) Fluorescence micrographs of attached bacteria following 48 h incubation of *P. aeruginosa* biofilm on SLIPS (**b**) and superhydrophobic PTFE (**c**). Scale bar ~ 30 μm. (**d**, **e**) Remains of an evaporated drop of *P. aeruginosa* biofilm-forming culture on SLIPS (**d**) and superhydrophobic PTFE (**e**). (**f**) Comparison of biofilm attachment to our SLIPS substrate after 7 days and to a PEGylated substrate after 5 h. Reproduced with permission from [76]

the shape was made parabolic (g) and the final PMMA nanostructure was obtained by thermal nanoimprinting (h).

In order to evaluate the antibacterial characteristics of the nanoimprinted PMMA films the surfaces were incubated with bacteria and mammalian cells over a week of incubation time. According to the authors observations and, as it is shown in Fig. 6.7, the initial attachment (4 h of incubation) depends on the bacteria employed. Whereas in the case of *E. coli*, the initial bacterial adhesion on the nanostructured surface was lower than that on the flat PMMA surface, when using P. aeruginosa no difference in the initial attachment could be observed. However, longer incubation times significantly changed the observations and a clear difference between the planar and nanostructured surfaces could be observed. More precisely, bacterial attachment to the flat surfaces occurs to a larger extent in comparison to nanostructured surfaces independently of the bacteria employed.

Micrometer size structures with antifouling chemistry have been equally reported. For instance, Martínez-Gómez et al. [77] fabricated membranes with micrometer size pores by using the breath figures approach (Fig. 6.8). In particular, preventing microbial adhesion onto membranes is a crucial issue that determines the durability of the membrane. For that purpose, they prepared aromatic polyimides (extensively employed for the elaboration of ultrafiltration membranes) containing PEO branches. Four polyimide-graft-polyethylene oxide (PEO) copolymers were prepared from the reaction of hexafluoroisopropylidene diphthalic anhydride (6FDA) with an aromatic diamine containing PEO-550 side groups (AD-PEO550).

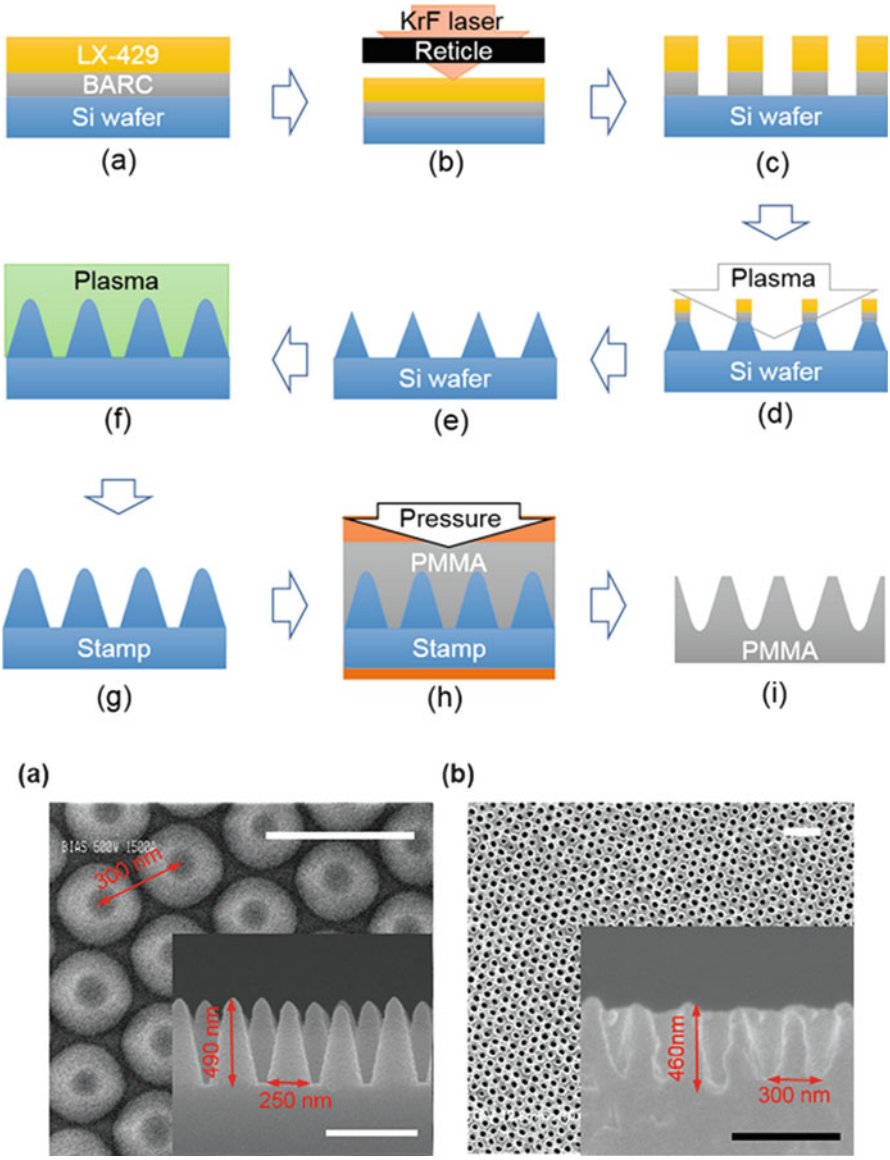

Fig. 6.6 *Above*: Fabrication process for the nanostructured surface: (**a**) photoresist coating, (**b**) exposure with a KrF laser, (**c**) developing of the photoresist, (**d**) plasma etching, (**e**) rinsing with N_2 gas, (**f**) chemical vapor deposition with high-density plasma, (**g**) rinsing with N2 gas, (**h**) direct thermal imprinting, and (**i**) demolding of the imprinted film. *Below*: SEM images of the nanostructured surface: (**a**) a top view and cross-sectional view (inset) of the silicon master surface and (**b**) the nanostructured pattern on the PMMA film and a magnified cross-sectional image thereof (inset). Scale bars = 500 nm. Reproduced with permission from [65]

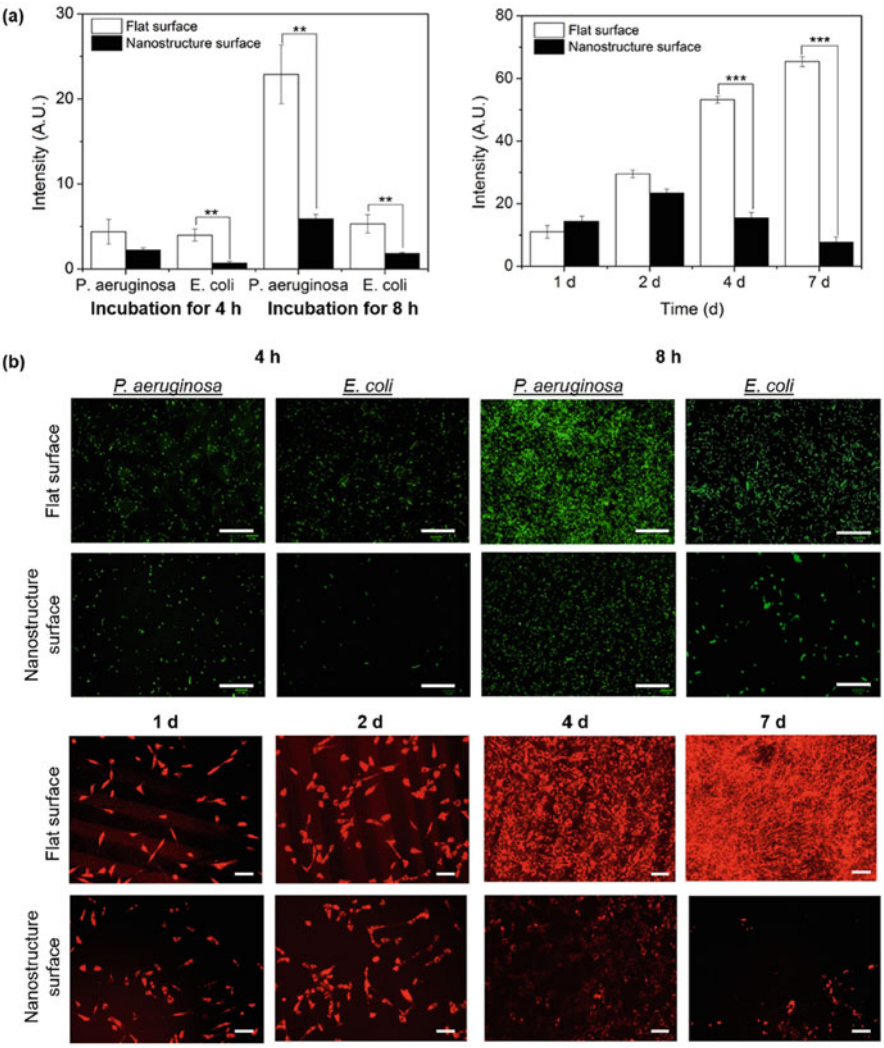

Fig. 6.7 Bacterial and mammalian cell attachment to the flat and nanostructured surfaces: (**a**) Bacterial cells (*left*) and myoblasts (*right*) were incubated with each sample over time. $^{**}P<0.01$; $^{***}P<0.001$. (**b**) Fluorescence microscopic images of the bacterial cells on the flat and nanostructured surfaces. Pseudocolors of lime green and red are used for the GFP and 700 nm NIR channels, respectively, in these images. The NIR fluorescence images for each condition were acquired with identical exposure times and normalizations. Scale bars = 50 μm. Reproduced with permission from [65]

The partial substitution of AD-PEO550 by 1,3.5-trimethyl-*m*-phenylenediamine (3MeMPD) different copolymers were obtained with increasing PEO content from P1 to P4. The breath figures technique was carried out using blends of two different polymers and permitted the fabrication of ordered surface topography, where the

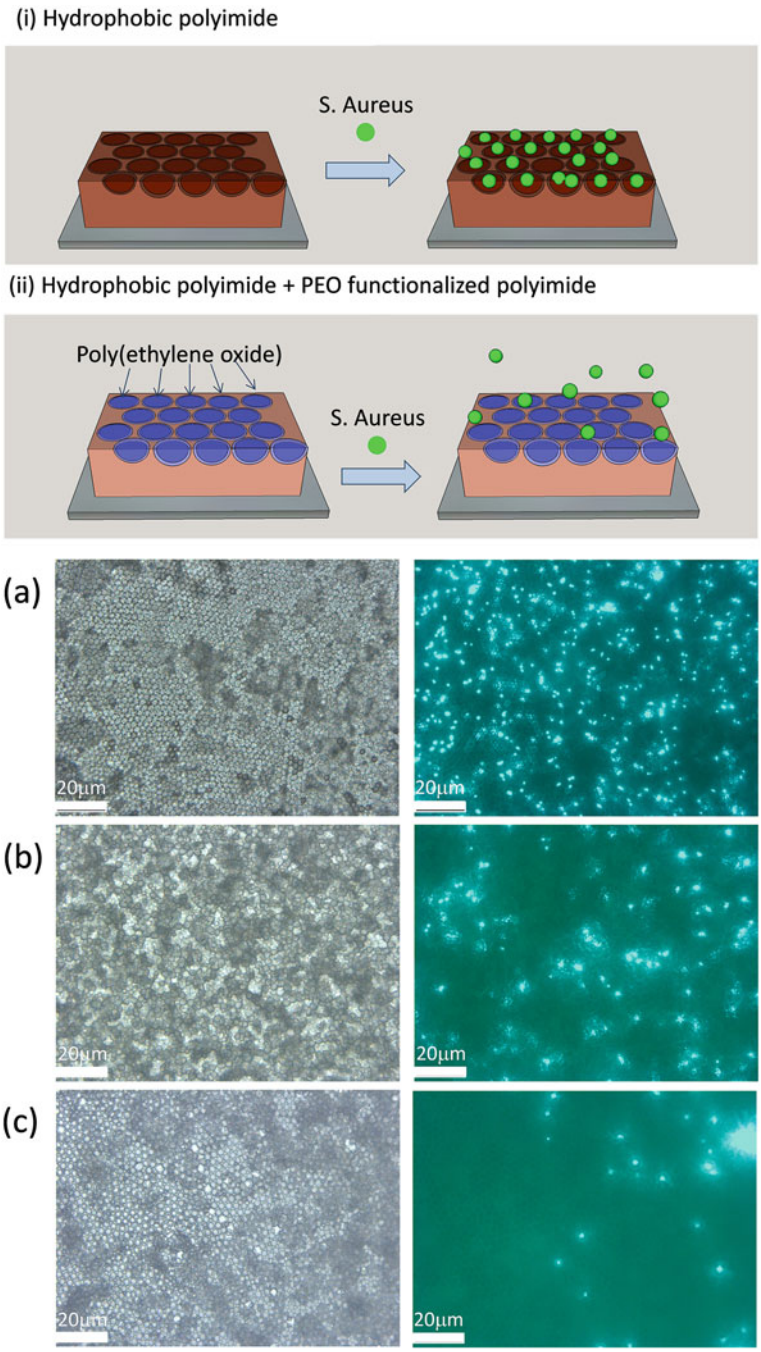

Fig. 6.8 *Above*: scheme of the hydrophobic (**i**) and hydrophilic (**ii**) polyimide films and the resulting bacterial adhesion. *Below*: Bacterial adhesion tests on honeycomb structured films prepared from (P1), and blends of P1 and P2 having different wt% ratio: (**a**) 100/0 P1/P2, (**b**) 90/10 P1/P2, (**c**) 75/25 P1/P2 (scale bar 20 μm). Reproduced with permission from [77]

PEO chains are preferentially located on the surface of the micrometer size holes. Moreover, the density of PEO chains could be finely tuned depending on the blend composition. These unique features were explored in order to reduce bacterial adhesion. They established that surface-modified polyimide membranes have a great resistance to biofouling against *S. aureus*. In particular, they observed that an increase of the PEO the content in the copolymer, and therefore inside the pore produced a significant decrease in the bacterial adhesion (Fig. 6.8a–c).

6.6 Nanostructured Composite Films

In addition to the use nanostructured surfaces composed exclusively by polymeric materials, the incorporation of inorganic nanoparticles (NPs) with antimicrobial activity has been proposed as an interesting alternative to increase the activity of polymers against bacteria. The combination of inorganic nanoparticles with organic polymers leads to nanocomposites [78] with properties that can combine synergistically the advantages of their components [15].

NPs are regularly or irregularly shaped particles with at least a dimension smaller than 100 nm that have shown very strong antibacterial activity in certain formulations of metals and metal oxides. NPs have been mainly used in two different ways: [15] they can be either used to dope bulk biomaterials or applied as a biomaterial coating. Moreover, to further improve the bactericidal properties and reduce toxicity, the surface of NPs can be modified. For instance, Jena et al. [79] coated NPs with chitosan while Lin et al. [80] reported the surface derivatization of NPs with alkylated polyethylenimines.

In addition to the strategy to incorporate nanoparticles on a polymeric material, the design of a composite requires the consideration of several other parameters such as:

(a) Concentration, size, shape, and chemical composition of NPs. For instance, a reduction of the particle size to the nanometer scale appeared to have larger antimicrobial activity when compared to micrometer size particles. For instance, Damm et al. [81] evidenced that the amount of silver in a polyamide 6/silver-microcomposite affects the final activity. Nanocomposites containing 1.9 wt% of silver kills only about 80 % of the bacteria in the same time. On the contrary, polyamide 6 filled with as low as 0.06 wt% silver nanoparticles can completely remove bacteria in 24 h.
(b) The nature of the polymer matrix may affect the release rate of the AgNP and, in turn, the activity of the nanocomposite. Three main parameters were identified by Kumar et al. [82–84] in their experiments, i.e., crystallinity of the polymer matrix [83], hydrophobicity of the matrix [85], and the filler type [82].
(c) The coating methodology to fabricate the nanocomposite can modify the final surface properties such as wettability or event surface roughness and, as a result, affect its antimicrobial activity.

(d) Also other additional factors play a key role on the final antibacterial activity. These include: type of bacteria (depending on the structure of the cell wall), metabolic and cell cycle phase (e.g., planktonic vs. sessile bacteria), environmental factors (e.g., aerobic vs. anaerobic milieu, pH), bacterial cell growth (rapid growing bacteria are more sensible), and the presence of an established biofilm (biofilm may act as a barrier reducing the exposure of inner encased cells).

The most extended nanocomposites for antibacterial purposes are developed using copper and copper oxide, silver, titanium dioxide, and zinc oxide embedded in a polymeric matrix.

The use of copper in their different forms including as copper oxide [86] or complexed [87–89] has been extended to the preparation commercial products such as paints [90] or technologically appealing materials [91, 92]. Copper-based nanocomposites release metal species [93–96] that interact in turn with the bacterial membrane. As a result, one of the key aspects is the stabilization of copper nanoparticles to control the release and enlarge the antimicrobial performance. For instance, Cioffi et al. [90] evidenced that the metal release depends on the amount of Cu nanoparticles embedded within the film.

Cellulose nanofibers have also been employed by Mary et al. [97] as support to incorporate copper (II) ions. The fabrication of these nanocomposites involves two consecutive steps. First, a periodate-induced oxidation of the cotton cellulose fibers is employed to produce dialdehyde cellulose. Secondly, the dialdehyde groups undergo a further coupling reaction through the chitosan amino groups. Finally, Cu(II) ions were immobilized by interaction with the amino groups, and the antibacterial activity was evaluated against the model bacteria *E. coli*. as shown in Fig. 6.9. This work evidenced that the amount of Cu (II) incorporated within the structure is directly related to the radius of inhibition zone. More precisely, the inhibition radius increases with the increase in the copper content within the fibers.

Food packaging materials have incorporated AgNP/polymer nanocomposites to preserve shelf life. The antimicrobial activity in these materials is obtained upon controlled release of AgNPs from the polymer matrix. The latter can be controlled and allows the material to remain active against microorganism during long periods of time.

A large variety of AgNP/polymer nanocomposites (PNCs) with high antimicrobial activity have been described employing different polymer matrices. These include polyvinylalcohol (PVA) [98], poly(methyl methacrylate) (PMMA) [99], polyamide [82–84], polyethylene oxide (PEO) [100], silicone elastomer [101], polypropylene (PP) [85], poly(acrylamide) [102], alginate [103], polyurethanes (PU) [104], cellulose [105, 106], polyvinylpyrrolidone (PVP) [107], and chitosan [108–110].

The use of TiO_2 nanoparticles have been typically focused on the fabrication of photo-catalytic disinfecting materials for meeting hygienic design requirements among others in food processing and packaging surfaces [111–114]. The generally admitted mechanism of interaction between the TiO_2 with bacteria is promoted by the photo-catalytic reaction that induces the peroxidation of the polyunsaturated

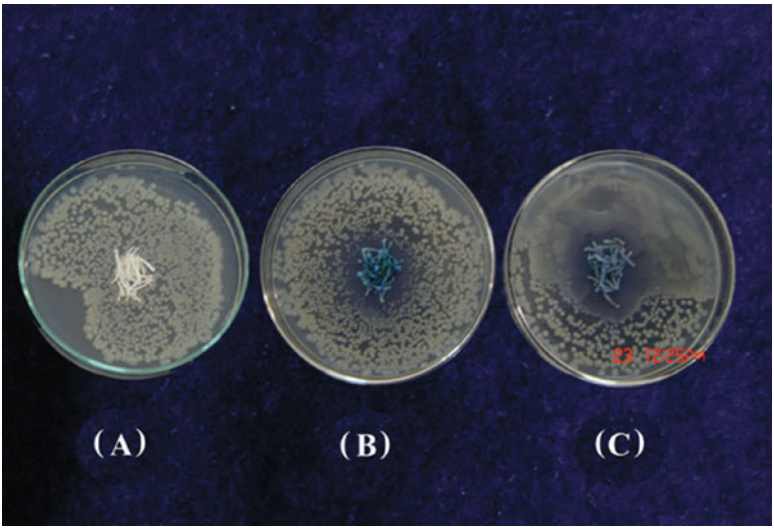

Fig. 6.9 Bacterial growth in Petri dishes supplemented with (**a**) plain fibers, (**b**) copper-bound chitosan-attached cellulose CBCAC (2), and (**c**) CBCAC (4). The number in parenthesis denotes the concentration (w/v) of Cu(II) ions in the solution used for loading of copper into fiber. Reproduced with permission from [97]

phospholipids and fatty acid of microbial cell membranes [115]. For instance, Xing et al. [116, 117] incorporated TiO_2 in PE films and observed antibacterial properties (89.3 % for *E. coli* and 95.2 % for *S. aureus* removal) upon irradiation with ultraviolet light for 1 h. Chawengkijwanich et al. [114] used also TiO_2 to form coatings on PP films. This nanocomposite exhibits antimicrobial effects toward *E. coli*. A 3 log CFU/mL reduction of *E. coli* was observed upon exposure to 20 W black-light illumination.

Finally, ZnO in the form of either nanoparticles or nanocrystals [118] have been used as additives and have been incorporated in a number of different polymers including polypropylene [119], PVC [120], PE [121, 122], or fabrics [123, 124]. In contrast to previous nanocomposites [125], ZnO exhibit antimicrobial activity both against Gram-positive and Gram-negative bacteria upon activation with visible light and exhibit antimicrobial activity. This is a clear advantage in comparison products derived from the use of the above-mentioned nanomaterials since nano-ZnO-based catalyst could be sterilized using indoor lighting.

6.7 Nanostructured Responsive Surfaces

Structured surfaces with variable morphology or able to react to environmental variations have also been explored to fabricate antibacterial surfaces. An interesting example of using films that vary as a function of the environmental pH has been reported

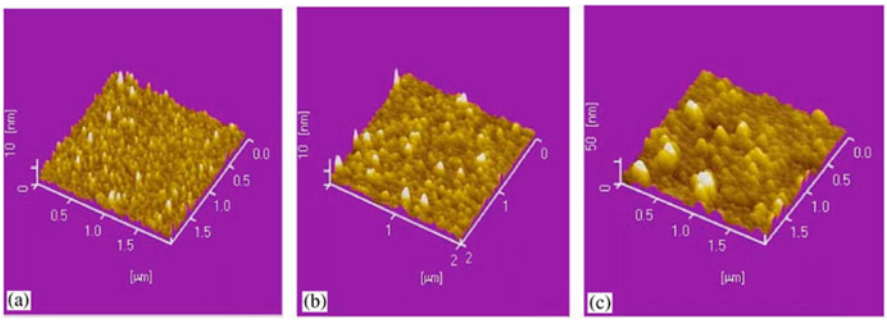

Fig. 6.10 AFM images of 12-layer chitosan-terminated heparin/chitosan multilayer films as a function of the pH employed for their assembling (**a**) pH = 2.9, (**b**) pH = 3.8, and (**c**) pH = 6.0. Reproduced with permission from [126]

by Ji et al. [126]. Their approach combines heparin and chitosan embedded in a multilayer film constructed layer by layer. Chitosan (antibacterial agent) and heparin (anti-adhesive agent) were alternatively deposited onto aminolyzed poly(ethylene terephthalate) (PET) films. These multilayer films could kill the bacteria effectively since the number of viable bacteria decreased by 7 % after 7 h in contact with the control PET films, but by 46–68 % for the multilayer-modified PET films.

In addition, the structure of the films formed appears to be dependent on the environmental pH employed. As a result, at higher pH values, the chitosan chains adopt loopier-type structures and tend to be adsorbed as thicker layers (Fig. 6.10). On the contrary, the lower the pH value, the fewer the amount of chitosan adsorbed to a surface. Interestingly, the assembly pH has a remarkable effect on the antibacterial property of the multilayer. The number of viable bacteria on the multilayer assembled at pH = 3.8, 2.9, and 6.0 decreased by 68, 58, and 46 %, respectively.

Yu et al. [127] proposed a model system that exhibit an ability to undergo noncovalent, dynamic, and reversible changes in structure that can be used to control the attachment, killing, and release of bacteria in response to changes in temperature. For that purpose, they fabricated a nanostructured surface that combines quaternary ammonium groups with antimicrobial activity with stimuli-responsive poly(*N*-isopropylacrylamide) (PNIPAAm) brushes. The authors followed the strategy depicted in Fig. 6.11i that involves three different steps: (1) nanopatterned PNIPAAm surfaces with different pattern periods and/or different polymer chain lengths by combining UV-interferometric lithography (IL); (2) surface-initiated activator regenerated by electron transfer–atom transfer radical polymerization (ARGET-ATRP) of NIPAAm from prepatterned initiator SAMs; (3) backfilling of QAS into intervals between nanopatterned PNIPAAm lines at 37 °C. Changes in the temperature-triggered hydration and conformational changes of nanopatterned PNIPAAm brushes reversibly. As a result of the particular surface distribution, these PNIPAAm chains modulate the spatial distribution of a biocidal quaternary ammonium salt (QAS) in the intervals between nanopatterned brushes. The authors studied the biocidal efficacy and release properties of these surfaces were tested against *Escherichia coli* K12. Above the lower critical solution temperature

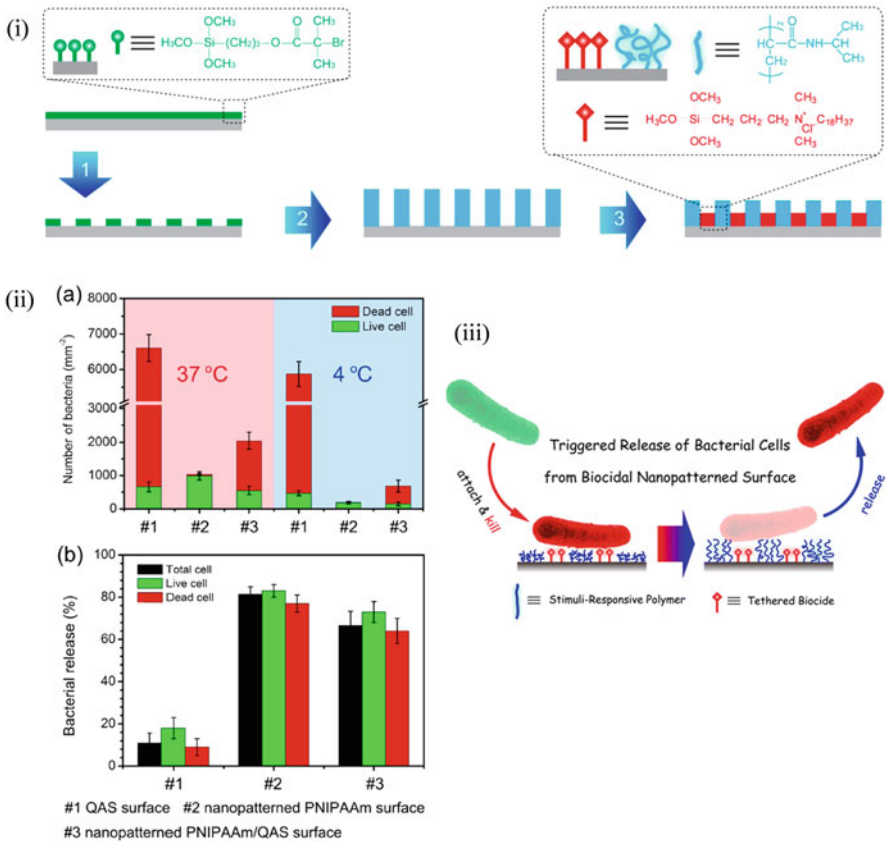

Fig. 6.11 (**i**) Schematic depiction of the procedure for the preparation of nanopatterned PNIPAAm surfaces (steps 1 and 2) and nanopatterned PNIPAAm/QAS surfaces (steps 1−3). Step 1: IL patterning of SAMs of ATRP initiators. Step 2: ARGET-ATRP of NIPAAm from prepatterned initiator SAMs. Step 3: Backfilling of QAS into intervals between nanopatterned PNIPAAm lines at 37 °C. (**ii**) (**a**) Attachment and detachment of *E. coli* on sample surfaces (#1, QAS surface; #2, nanopatterned PNIPAAm surface; #3, nanopatterned PNIPAAm/QAS surface). The surfaces were incubated in suspensions of E. coli at 37 °C for 2 h, and the average number of attached cells was determined (37 °C). Then, the surfaces were rinsed with a 0.85% NaCl solution and ultrapure water at 4 °C, and the remaining cells were counted (4 °C). The bacterial release ratio is shown in part **b**. Error bars represent the standard deviation of the mean ($n=3$). (**iii**) Above the lower critical solution temperature (LCST) of PNIPAAm, collapsed polymer chains facilitate the attachment of bacteria and expose QAS moieties that kill attached bacteria. Upon a reduction of the temperature below the LCST, swollen PNIPAAm chains promote the release of dead bacteria. Reproduced with permission from [127]

(LCST) of the PNIPAAm brushes, these collapsed polymer chains facilitated both the attachment of bacteria and the contact of QAS moieties with the bacterial membrane (Fig. 6.11ii). As a result, the membrane is damaged and the bacteria die. Moreover, upon cooling to temperatures below the LCST, PNIPAAm chains are swollen and induced the release of dead bacteria.

6.8 Conclusions

This chapter describes the role of the nano- and microstructures on polymer surfaces in the bacterial adhesion and proliferation events. Nature has inspired many of the developed systems since combination of chemical functionalities and micro/nanopatterns appears to be the most efficient strategy to control/prevent bacterial adhesion.

A large variety of synthetic systems have been developed with more or less success in which one of the above-mentioned aspects has been considered. However, only few of them have demonstrated antimicrobial activity after few days. This challenge, still unresolved in spite of the promising recent developments, for instance, using slippery liquid-infused porous surfaces (SLIPS) in which the activity is maintained during 1 week.

Finally, an increasing interest has heightened the need for developing systems that act as antimicrobials as demand instead of exhibiting a continuous antimicrobial property. In this context, stimuli-responsive polymers are excellent candidates to respond on-demand upon slight changes on the environmental properties. As a result, the system may be activated or inactivated, i.e., antimicrobial or inert depending on a particular external parameter.

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Chapter 7
Antimicrobial Fibers and Fabrics Obtained by Electro/Melt Spinning

Abstract Nanotechnology and nanoscience involve different aspects including the manipulation, control, and assembly of nanoscale components to produce materials, systems, and/or devices. In this context, the fabrication of micro/nanofibers has attracted huge interest. In particular, micro/nanofibers have different properties such as high porosity, small pore size, high surface area, and compatibility with functionalizing additives that enables their use in multiple applications. These include their use as enzyme carriers, membranes for filtration purposes, as barriers to liquid penetration, sensors, delivery purposes, and catalysts. Polymer fibers have also been explored in a large variety of medical applications such as tissue engineering or in regenerative medicine.

In this chapter, we will provide an overview of the most extended fabrication approaches and their use in medical applications, in particular to prevent microbial contamination. The fabrication of fibers treated with antimicrobials is today a standard finish for many different textile products employed in such uses as medical, institutional, and hygienic. More recently, antimicrobial fibers have been extended to other applications including women's wear, sportswear, and aesthetic clothing to impart anti-odor or biostatic properties.

Keywords Antimicrobial fibers • Micro/nanofibers • Melt/emulsion spinning • Electrospinning • Hybrid nanofibers • Responsive fibers • Biodegradable fibers

7.1 Introduction

Nanotechnology and nanoscience involve different aspects including the manipulation, control, and assembly of nanoscale components to produce materials, systems, and/or devices. In this context, the fabrication of micro/nanofibers has attracted huge interest. In particular, micro/nanofibers have different properties such as high porosity, small pore size, high surface area, and compatibility with functionalizing additives that enables their use in multiple applications. These include their use as enzyme carriers, membranes for filtration purposes [1], as barriers to liquid penetration [2], sensors [3], delivery purposes [4], and catalysts. Polymer fibers have also

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been explored in a large variety of medical applications such as tissue engineering [5] or in regenerative medicine [6].

In this chapter, we will provide an overview of the most extended fabrication approaches and their use in medical applications, in particular to prevent microbial contamination [7]. Moreover, as reported by Kenawy et al. [8], the fabrication of fibers treated with antimicrobials is today a standard finish for many different textile products employed in such uses as medical, institutional, and hygienic. More recently, antimicrobial fibers have been extended to other applications including women's wear, sportswear, and aesthetic clothing to impart anti-odor or biostatic properties [9, 10].

7.2 Approaches for Fiber Fabrication

While it is true that there exist a large number of techniques to produce fibers with diameter sizes at the micrometer scale and below, herein we will limit our discussion to the most extended methodologies employed currently. Nanofibers from polymers have been for instance prepared using approaches based on the use of a particular template. In general, these are either aluminum oxide [11] or mesoporous silica [12]. However, one of the major drawbacks of these methodologies is related to the length of the fibers obtained with remains in the best case in millimeter range. In contrast to those methodologies, different "spinning" techniques that permit the fabrication of continuous fibers with submicron diameters have been developed [13]. As will be depicted later, this procedure requires the consideration of the experimental conditions such as solution viscosity or the solution conductivity and electric-field intensity when applying an electric current to generate the fibers.

7.2.1 Melt, Solution, and Emulsion Spinning

Spinning approaches refer to those fiber fabrication techniques based on extrusion of a polymer (dissolved, melted, or in an elution) through a spinneret in a continuous mode thus allowing the production of single or, in most sophisticated setups, even multifilament materials. Within this spinning approach three alternatives have been explored for the fabrication of fibers depending on the mechanism of solidification of the extruded material [14].

Melt spinning (also found in literature as melt blowing) takes advantage of a temperature cooling to produce solid filaments. In order to allow the filament to cool, the spinneret to collector distance (TCDs) is relatively high. In melt spinning, a single filament is continuously wound onto a spool, where mechanical drawing of the solidified filament reduces the average fiber diameter. More importantly, several parameters have to be considered since they govern the mechanical properties of the resulting filaments. These include the temperature, the take-up speed, and the dray

ratio. Important advantages of this approach include their reproducibility that allows to prepare extremely long fibers or the no requirement of solvents or residues (to be removed during the fabrication process) that improve the safety during the fabrication [15].

The principle of *solution spinning* techniques relies on solvent vaporization during the drawing process of a fiber [14]. The fabrication of fibers using this methodology has been previously reported among others by Persano [15]. Solution spinning has been carried out using different alternatives such as gels spinning [16], liquid crystal spinning [17], or wet spinning [18]. Solution spinning allows in comparison with melt spinning the fabrication of fibers from thermally unstable polymers [15].

Three different variations of solution spinning have been described, i.e., wet, dry, and flash solution spinning depending on the strategy employed to remove the solvent. In wet spinning, the dissolved polymer thread passes through a coagulation bath that contains a solvent that: must be miscible with the spinning solvent and immiscible with the polymer in order to assist in fiber solidification. The dry spinning is, in comparison to wet spinning, much faster and the fiber solidification is produced by simple evaporation that could eventually be improved using gas-assisted drying around the extruded filament. The third alternative uses a difference in pressure in order to evaporate the solvent.

Finally, *emulsion spinning* has been mainly employed to produce fibers from those polymers that are either insoluble or do not melt [14]. As a result, this method is an interesting alternative to process inorganic materials [19], high melting point fluorocarbons [20], and flame-retardant formulations [21].

7.2.2 Electrospinning

Elecrospinning is today one of the most extended approaches to fabricate micro- and nanometer size fibers [22]. Electrospinning is however an old technique. It was first studied in detail by Zeleny [23] in 1914 on electrospraying and patented by Formhals in 1934 [24]. This technique uses electrostatic forces to produce fine fibers from polymer solutions or melts and the fibers thus produced have a thinner diameter (from nanometer to micrometer) and a larger surface area than those obtained from conventional spinning processes [22].

Two important advantages of using electrospinning include that the fiber formation can be carried out at room temperature and using atmosphere conditions. The setup of typical electrospinning equipment is depicted in Fig. 7.1. The standard setup consists of three major components, i.e., a high voltage power supply, a spinneret (e.g., a pipette tip) and a grounded collecting plate (usually a metal screen, plate, or rotating mandrel). Using these components, a high voltage source is applied to inject charge of a certain polarity into a polymer solution or melt, which is then accelerated toward a collector of opposite polarity [25, 26].

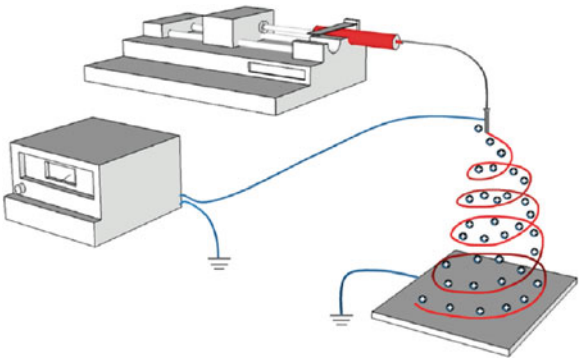

Fig. 7.1 Illustration of the electrospinning apparatus for the fabrication of fibers. Reproduced with permission from [27]

7.2.3 Melt Blowing

As has been depicted by Ellison et al. [28] during melt blowing, fibers are straightforwardly fabricated in a single step by extruding a polymer melt through an orifice die. The extruded polymer is then drawn down with a jet of hot air. This process does not require the use of solvents and has, therefore, important environmental advantages. This methodology was first developed in the 1950s at the Naval Research Laboratory with the goal of making submicron fibers to trap radioactive particles in the upper atmosphere [29]. Wente [30] first described the construction of a melt blowing die composed of a series of orifices and slots that enable the fabrication of superfine fibers. Later, the extension of this methodology at the commercial scale was carried out first by Exxon [29, 31] and later by a large number of companies including Vose, 3 M, Kimberly-Clark, Cummins, and Johns Manville that reported the use of this technology to fabricate commercial nonwoven products [29].

Today, a large number of polymers including poly(butylene terephthalate) (PBT) [32], poly(ethylene terephthalate) [33], polyethylene [30], polypropylene (PP) [33–35], poly(methyl methacrylate) [30], polyamides (e.g., nylon) [30, 36], and polystyrene (PS) [30] have been explored and successfully employed for producing blown fibers. In addition to the use of single polymers, this approach has been equally employed for the fabrication of bicomponent microfibers. For instance, Zhao et al. [33] investigated the fabrication of polypropylene (PP)/poly(ethylene terephthalate) (PET) bicomponent (bico) filaments by using the melt blowing (MB) process.

In Fig. 7.1 are summarized the most relevant technologies available for fiber manufacturing (Table 7.1).

7.3 Fibers Bearing Antimicrobial Molecules

The most extended strategy to fabricate fibers with antimicrobial properties involves the incorporation of antimicrobial molecules such as antibiotics within the fiber structure and their subsequent controlled release.

Table 7.1 Selection of fiber manufacturing processes and their advantages and disadvantages

	Fiber diameter	Advantages	Disadvantages
Melt electrospinning	<100 nm to 500 μm	Direct writing capability; solvent free; low cost; diameter is proportional to mass flow rate	Low output: device is time consuming to build. Limited number of polymers tested. Polymers require some thermal stability
Solution electrospinning	<50 nm to 10 μm	Simple to establish; low cost; suitable for many polymers; submicron diameters readily attained	Low output; direct writing is difficult; significant solvent is generated
Melt spinning	1–500 μm	High output; very consistent production; can be used in weaving technologies; industrially successful	Requires drawing onto a spool. Variable diameters at high stretching; difficult to attain submicron diameter fibers; significant cost to establish
Solution spinning	1–200 μm	Can process thermally unstable polymers; diversity in the number of configurations (e.g., flash, liquid crystal, gel spinning); industrially successful	Solvent requires removal. Complex coagulation baths needed for wet spinning. Dry spinning requires significant solvent removal systems
Melt blowing	<500 nm to 10 μm	High output: industrially successful	High cost to establish, therefore to perform research; difficult to control fiber architecture

Reproduced with permission from [14]

One of the pioneer studies using this approach was reported by Bucheńska [37]. She employed polyamide fibers (PA6) as supports to carry out a graft polymerization of acrylic acid (AA). The resultant fibers, containing carboxylic groups in their structure, were additionally modified with three different biocides, i.e., penicillin, neomycin, and gentamycin to obtain antimicrobial fibers. The activity was tested against *S. aureus*, *E. coli*, and *P. aeruginosa*, and the modified fibers showed strong biocidal effects on the Gram-positive microorganism *S. aureus* and the Gram-negative *E. coli*. The author evidenced a long-term activity since the release of antibiotics into solution proceeds for quite a long time after which there is still enough antibiotic on the fibers to provide them with antibacterial properties.

Another illustrative example of a controlled-release mechanism in the fiber with broad-spectrum antimicrobial properties based on poly(vinyl alcohol) (PVA) was described by Vigo et al. [38] (Fig. 7.2). Its strategy involves the modification of the alcohol groups (provided by the poly(vinyl alcohol)) by reaction with 5-nitrofurylacrolein in the presence of an acid catalyst. The presence of moisture leads to the slow release of the nitro compound thus producing the expected antimicrobial activity.

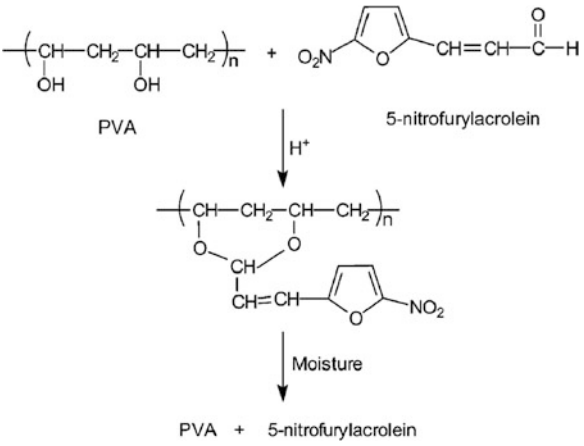

Fig. 7.2 Preparation of the broad-spectrum antimicrobial fiber based on PVA. Reproduced with permission from [8]

Another strategy has been reported recently by Ahire and Dicks [7] to prepare nanofibers containing 2,3-dihydroxybenzoic acid (DHBA). Electrospinning of DHBA into a blend of poly(D,L-lactide) (PDLLA) and PEO (24 %; 50:50) produced nanofibers of 400–450 nm in diameter. The principle behind their approach is based on the idea that free iron enhances biofilm formation, delays wound healing, and may even be responsible for persistent inflammation. They employed *Pseudomonas aeruginosa* (that readily forms biofilms in wounds, which often leads to chronic infections that are difficult to treat with antibiotics) as a model bacteria to prove that the presence of DHBA which is an iron chelator is able to reduce the bacterial contamination. The authors demonstrated that exposure of *P. aeruginosa* Xen 5 DHBA, electrospun into a nanofiber blend of poly(D,L-lactide) (PDLLA), and poly(ethylene oxide) (PEO), referred to as DF, for 8 h decreased biofilm formation by approximately 75 %. Moreover, their findings indicated that DHBA electrospun into nanofibers inhibits cell growth for at least 4 h, which is equivalent to the time required for all DHBA to diffuse from DF. This is the first indication that DF can be developed into a wound dressing to treat topical infections caused by *P. aeruginosa*.

Finally, instead of using chemical reactions or including the antimicrobial molecules within the precursor solution, Choi et al. [39] explored the possibility to incorporate antibiotics within the fiber structure by sorption. In particular they used two antibiotics, doxycycline (Doxy) and ciprofloxacin (Cipro), that were applied under a variety of conditions to wool and to hydrolyzed wool at 40 °C and nylon (used as a control). The authors evidenced that depending on the antibiotic employed the sorption process differs. As a result, Doxy was much higher in wool than in nylon, whereas sorption of Cipro was similar in both fibers. More interestingly, a drastic increase in sorption of antibiotics by hydrolyzed wool was observed and could be attributed to an increase in polar functional groups by peptide scission and in oxidized sulfur groups by cystine oxidation. As a result, both sorption and zone of inhibition (ZOI) values were improved by hydrolysis of wool. In particular, wool hydrolyzed for 20 or 40 min at 40 °C and dyed with Doxy at 45 °C for 3.5 h maintained around 30 mm of

ZOI after 24 h of challenge by a simulated flow of blood. Wool hydrolyzed for 60 min at 40 °C and dyed with Cipro at 45 °C for 3.5 h also maintained its antibiotic activity for an extended time.

7.4 Hybrid Organic–Inorganic Nanofibers with Antimicrobial Properties

The incorporation of inorganic nanoparticles or their precursors, well known for their excellent antimicrobial activity, is an alternative to provide antimicrobial properties to micro/nanofibers [40, 41].

In this context, several strategies have been employed. On the one hand, preformed nanoparticles have been incorporated in the solution prior to the fiber formation. For instance, one-dimensional polymer nanostructures have been employed as templates for the preparation of inorganic nanofibers with antimicrobial properties [42]. An example of this strategy was reported by Hwang et al. [43] that described the preparation of ZnO/TiO_2 composite nanofibers by electrospinning. As depicted in Fig. 7.3, the resulting nanofibers showed better antimicrobial activity against both Gram-negative *Escherichia coli* and Gram-positive *Staphylococcus aureus* under UV irradiation than in the absence of light. Interestingly, the combination of both ZnO and TiO_2 within the same fibers produced the best results.

Instead of incorporating nanoparticles formed in a separate step, several groups designed strategies to form the nanoparticles within the solution employed for the fiber construction. This alternative was employed by Abdelgawad et al. [44] that designed a green route to produce antibacterial nanofiber mats loaded with silver nanoparticles (Ag-NPs, 25 nm diameter) (Fig. 7.4). The first step for the fabrication of nanofiber mats is the preparation of colloidal dispersions of chitosan-based Ag-NPs blended with polyvinyl alcohol. Aqueous PVA solution was mixed with chitosan-based Ag-NPs at various weight ratios and electrospinned. As a result, nanofibers (150 nm average diameter and narrow size distribution) were obtained and cross-linked with glutaraldehyde. According to their findings, these fibers showed superior antimicrobial properties as a result of the synergistic combination of chitosan and Ag-NPs.

Instead of incorporating already formed nanoparticles several groups used nanoparticle precursors to, once impregnated in the fibers, fabricate the nanoparticles in situ [45, 46]. For instance, Pant et al. [47] fabricated silver-impregnated TiO_2/nylon-6 nanocomposite mats exhibit excellent characteristics as a filter media with good photocatalytic and antibacterial properties and durability for repeated use. More precisely, the strategy depicted by Pant et al. involves the incorporation of silver nanoparticles (NPs) in electrospun TiO_2/nylon-6 composite nanofibers. The silver NPs were obtained through the photocatalytic reduction of silver nitrate solution under UV-light irradiation. TiO_2 NPs present in nylon-6 solution were able to cause the formation of a high aspect ratio spider-wave-like structure during

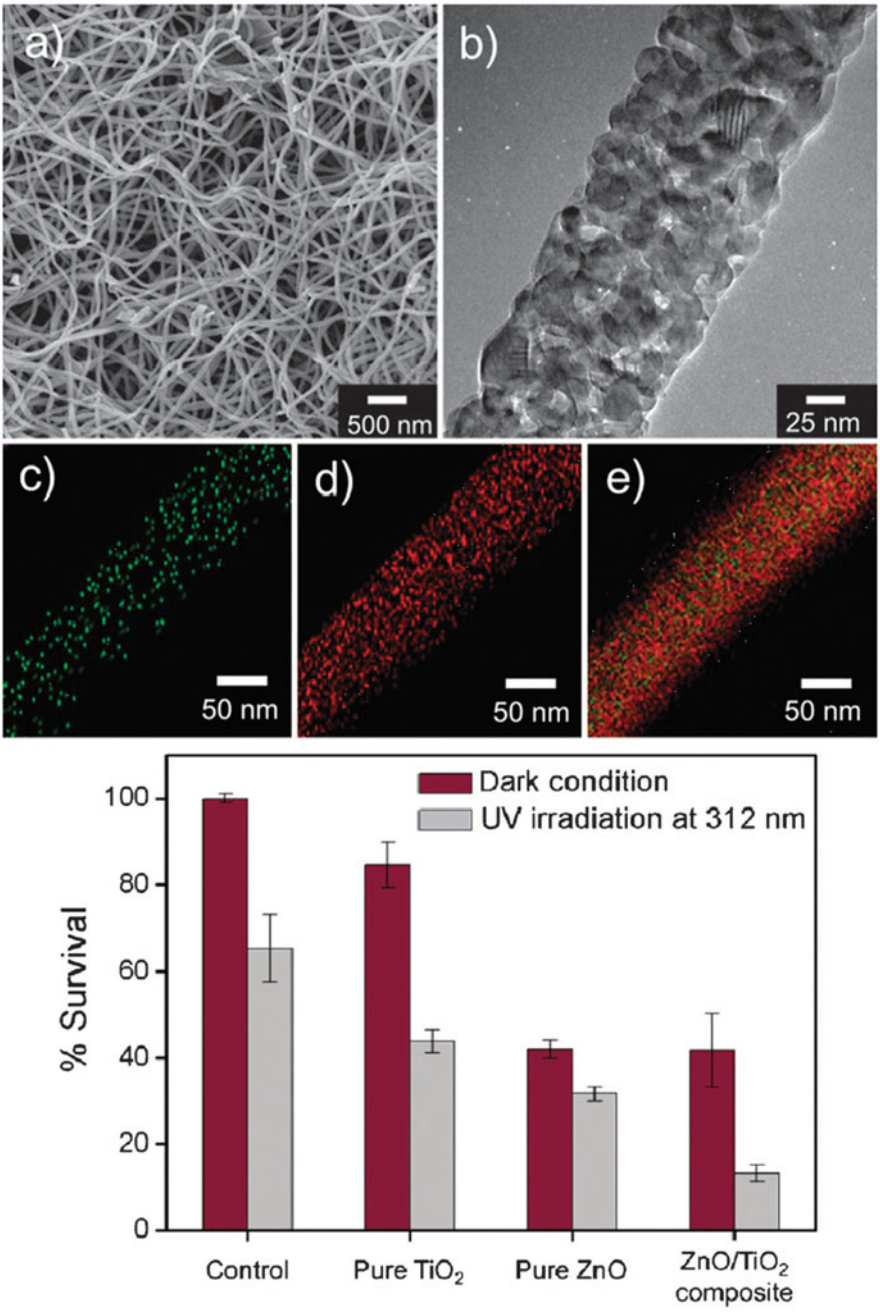

Fig. 7.3 *Above:* (**a**) FE-SEM and (**b**) TEM image of the fabricated ZnO/TiO_2 nanofibers. EDS mapping images of the composite nanofibers with (**c**) Zn element, (**d**) Ti element, and (**e**) Zn–Ti elements. *Below:* Graph of % survival of *S. aureus* after treatment with control, TiO_2 nanofibers, and ZnO/TiO_2 nanofibers in the absence and the presence of UV-light irradiation at 312 nm for 30 s. The number of bacterial colonies on the untreated Petri dish surface under the dark conditions was defined as 100 %. Reproduced with permission from [43]

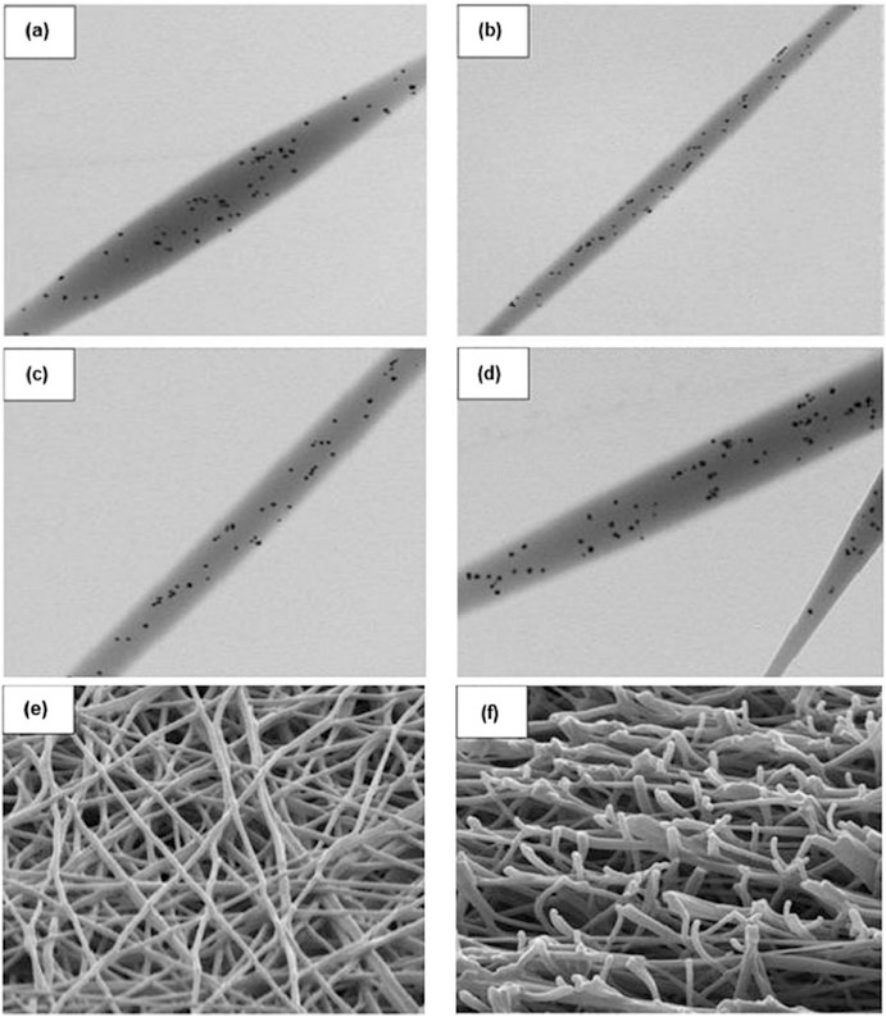

Fig. 7.4 TEM micrographs of e-spun fibers of 60/40 (weight ratio) PVA/CS-Ag-NPs, (**a**–**d**) micrographs show individual PVA/CS-Ag-NPs fibers loaded with Ag-NPs; (**e**) PVA/CSAg-NPs nanofiber mat top-view; and (**f**) cross-section of PVA/CS-Ag-NPs nanofiber mat. Reproduced with permission from [44]

electrospinning and facilitated the UV photoreduction of $AgNO_3$ to Ag. The antibacterial, efficacy tested against *Escherichia coli*, showed that TiO_2/nylon-6 nanocomposite mats loaded with Ag NPs are more effective than composite mats without Ag NPs.

A similar approach has been reported by Liu et al. [48] for the preparation of antimicrobial fibers. Their strategy involves three consecutive steps, i.e., prepolymerization, electrospinning, and finally photo-cross-linking process that leads

to water-stable cross-linked electrospun zwitterionic poly(sulfobetaine methacrylate) (PSBMA) fiber. The fibers were employed to construct a membrane that exhibited strong resistance to protein adsorption as well as cell attachment. Moreover, as depicted in Fig. 7.5, 3 h bacterial incubation results evidenced that the PSBMA electrospun membrane exhibited very little bacterial attachment for both P. aeruginosa and S. epidermidis in comparison with other electrospun fibers such as polycaprolactone (PCL) or using standard supports such as tissue culture polystyrene (TCPS) or glass. Equally, bacterial adhesion tests carried out during 24 h show that the PSBMA electrospun membranes still exhibited the lowest bacterial adhesion for both species. In addition to the antifouling properties observed in the PSBMA fibers, the authors explored the antimicrobial activity of the silver-incorporated electrospun PSBMA membrane. $AgNO_3$ was incorporated into the electrospun PSBMA membrane through ionic interactions and the antimicrobial activity of the Ag^+-impregnated membrane was determined using a zone-of-inhibition method. The authors found that the electrospun PSBMA membranes infused with silver nitrate inhibit the growth of both *P. aeruginosa* and *S. epidermidis*. The zone of inhibition was 6.3 mm for P. aeruginosa and 3.6 mm for S. epidermidis after 24 h of incubation. These membranes are promising materials among others for wound dressing purposes since they can prevent attachment and entry of the environmental pathogens to the wound. In addition to the protection capabilities, the dressing applied to the wound would not need an often replacement, which leaves less chance of introducing new bacteria with repeated exposure of the wound site to the environment.

Shi et al. [46] reported the synthesis in one-step approach of silver nanoparticle-filled nylon 6 nanofibers by electrospinning. They employed the electrospinning solvent (formic acid) as a reducing agent for in situ conversion of $AgNO_3$ into silver nanoparticles during the solution preparation. The resultant silver nanoparticle-filled nylon 6 hybrid nanofibers show a fibrous structure with diameter between 50 and 150 nm having narrow size 2–4 nm silver nanoparticles uniformly dispersed throughout the nylon 6 matrix. Interestingly, these silver nanoparticle filled nylon 6 nanofibers exhibit a steady and long-lasting silver ion release behavior, and robust antibacterial activity against both Gram-positive *B. cereus* and Gram-negative *E. coli* microorganisms.

Not only silver nanoparticles, also silver ions exhibited excellent antimicrobial properties when incorporated in nanofibers. An illustrative example of antimicrobial hybrid particles was reported by Bajpai et al. [49]. They focused on investigating the feasibility of using silver (I) ions loaded poly(acrylonitrile)-grafted silk fibers as antibacterial dressing material. The poly(acrylonitrile)-grafted silk fibers were loaded with silver(I) ions by equilibration method. The resulting fibers were investigated for their biocidal action against *E. coli*, by using zone inhibition and colonies counting method. The bacterial growth was suppressed to a great extent thus indicating that the fibers are very effective in killing bacterial cells.

Copper (II) oxide nanoparticles (CuO NPs) have also evidenced remarkable antimicrobial properties. Yalcinkaya et al. [50] employed these CuO NPs nanoparticles to test the antibacterial efficiency of nanofiber composite yarns. Instead of incorpo-

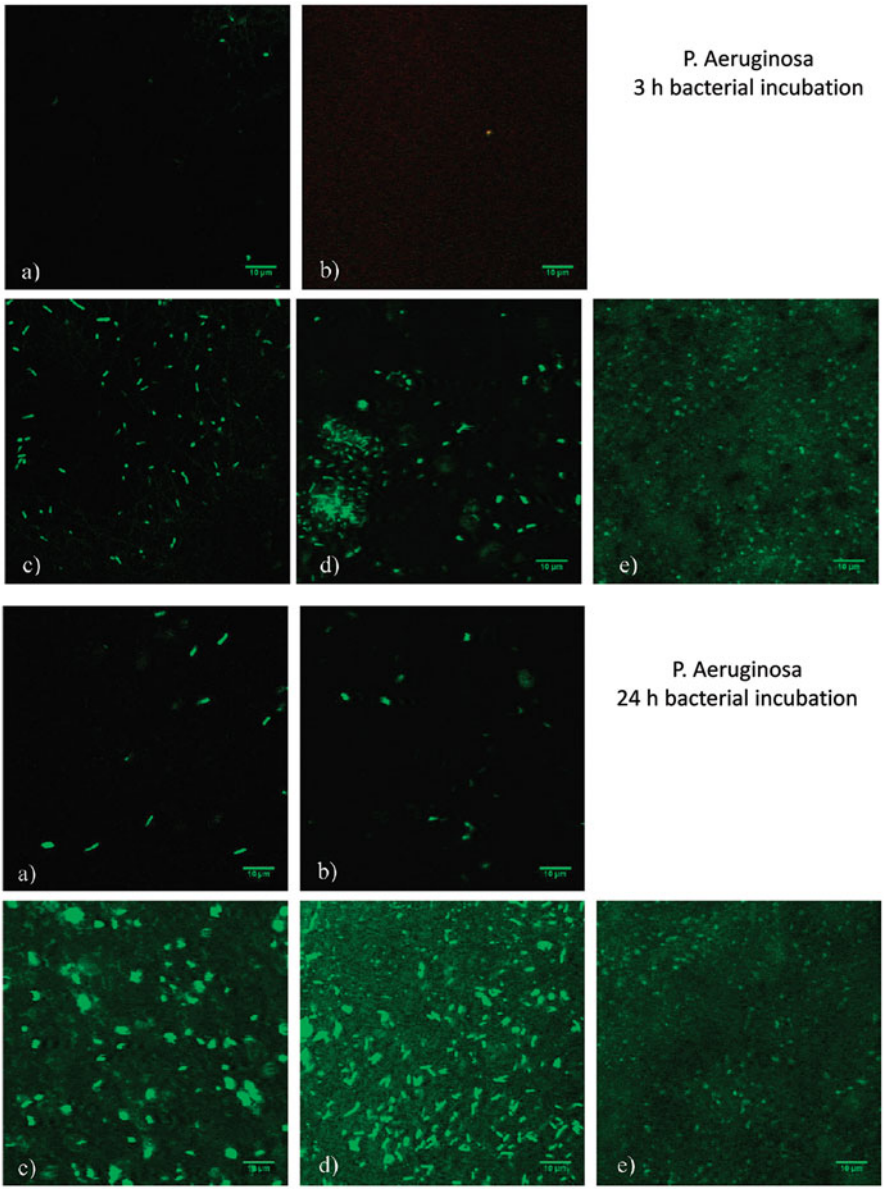

Fig. 7.5 Fluorescence microscopy images of *P. aeruginosa* attached onto electrospun PSBMA (**a**), PSBMA hydrogel (**b**), electrospun PCL (**c**), TCPS (**d**), and glass (**e**) at 3 and 24 h. Reproduced with permission from [48]

rating the NPs within the fibers, the resulting nanofibrous composite material combines the good mechanical properties of the core yarn with the high specific surface of the nanofiber shell to gain specific targeted qualities. Two polymers, polyvinyl butyral (PVB) and polyurethane (PU), were tested for the production of nanofiber composite yarns, and the antibacterial efficiency was evaluated against Gram-negative *Escherichia coli* and Gram-positive *Staphylococcus gallinarum* bacteria. According to the authors, PVB/nanofibers with a CuO antibacterial agent generally show significantly higher antibacterial efficiency compared to yarns covered with PU nanofibers. This can be directly related to the better uniformity of the antibacterial agent distribution caused by the reaction of CuO with acetic acid creating copper acetate.

7.5 Antibacterial Fibers with Covalently Bonded Biocides

While, as has been depicted above, most of the studies reported concern the release of a particular biocide to the environment, few works focused on the elaboration of "permanent" antimicrobial fibers by covalently immobilizing the biocide within the fiber structure. An interesting approach for the preparation of solvent-resistant antimicrobial fibers was described by Guo-Dong et al. [48]. The strategy reported is depicted in Fig. 7.6 and comprises a two-step synthetic approach by atom transfer radical polymerization (ATRP). The first step is the direct copolymerization of (2-dimethylamino)ethyl methacrylate) (DMAEMA) and glycidyl methacrylate (GMA) to fabricate an statistical copolymer poly[((2-dimethylamino)ethyl methacrylate)-co-(glycidyl methacrylate)] P(DMAEMA-c-GMA). This copolymer served as macroinitiator for the second polymerization step in which pentachlorophenyl acrylate (PPCPA) was employed as monomer to fabricate the second block. As a result, the authors fabricated a diblock copolymer having poly[((2-dimethylamino)

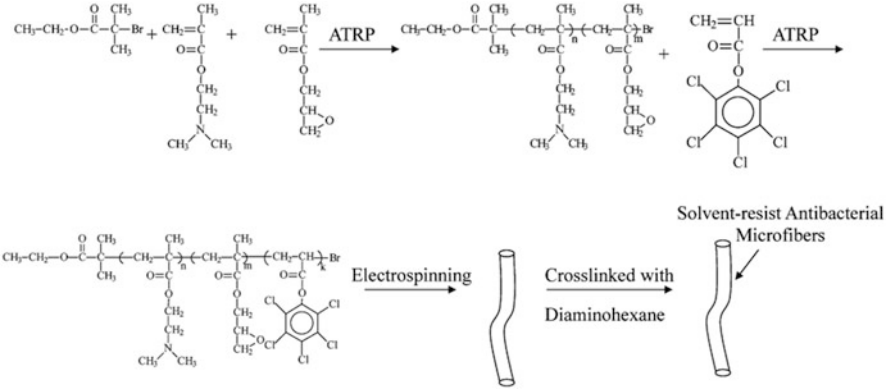

Fig. 7.6 Schematic illustration of the preparation of P(DMAEMA-c-GMA)-b-PPCPA microfibers via ATRP and electrospinning. Reproduced with permission from [48]

ethyl methacrylate)-co-(glycidyl methacrylate)] P(DMAEMA-c-GMA) block and a poly(pentachlorophenyl acrylate) (PPCPA) (P(DMAEMA-c-GMA)-b-PPCPA) block. Electrospinnning of P(DMAEMA-c-GMA)-b-PPCPA led microfibers with variable diameters 300 nm up to 1.3 μm. Taking advantage of the glycidyl groups, the authors improved the solvent stability the microfibers by reaction with 1,6-hexanediamine. In order to confer antimicrobial properties to these nanofibers, the authors carried out the modification of the tertiary amine groups of the P(DMAEMA-c-GMA) block and formation of quaternary ammonium salts (QASs). Upon evaluation of the antibacterial effect of the cross-linked microfibers against *E. coli* and *S. aureus* cultures, the authors concluded that 95 % *E. coli* and 97 % *S. aureus* were killed after 10 min contact with the P(DMAEMA-c-GMA)-b-PPCPA microfibers.

Another alternative explored involved the fabrication of fibers and their post-modification. For instance, Sun and coworkers attached *N*-halamine functional groups to cellulose to render textile materials biocidal [51, 52]. They fabricated a cyclic-amine monomer, 3-allyl-5,5-dimethylhydantoin (ADMH) that could be grafted in the presence of acrylonitrile onto cotton cellulose. After chlorine bleach treatment, hydantoin units in the grafted copolymers were easily transformed into *N*-halamine structures. These grafted samples exhibited potent antibacterial activity against *Escherichia coli,* and the functional properties were shown to be durable and regenerable [51]. They extended this concept to other commercially available fibers such as Nomex®, Kevlar®, Kermel®, or PBI® [52]. The chemical structure of the different fibers employed and the strategy employed to modify them is depicted in Fig. 7.7.

7.6 Fibers with Responsive Antimicrobial Activity

The elaboration of antimicrobial systems able to act under particular environmental conditions has been intensively pursued during the last decade. As a result, different strategies mainly involving stimuli-responsive polymers have been reported. Among the most extended stimulus employed, pH [10] temperature [53] or photoinduced [54] changes will be considered in this section.

Ionic interactions were employed by Son et al. [10] in the finishing to produce antimicrobial fabrics. They utilize the ionic interactions between anionic carboxylic end groups of polyamides and cationic quaternary ammonium salts in the chemical finishing of nylon fabrics to achieve desired durable antimicrobial functions. They studied nylon 6.6 fabrics treated with 2 % on mass of fabric (omf) of each of the cetylpyridinium chloride (CPC) and benzyldimethylhexadecylammonium chloride (BDHAC), hexadecyltrimethylammonium bromide (HTAB), and dodecyltrimethylammonium bromide (DTAB) solutions. In particular, the pH of the finishing bath was very critical in affecting the ionic interactions, the effect on bacterial reduction and thus exhaustion of the salts on the fabrics. As depicted in the table below After ten Launder–Ometer washes, the fabrics treated under neutral and acidic conditions, specifically the BDHAC-treated ones, dramatically lost their

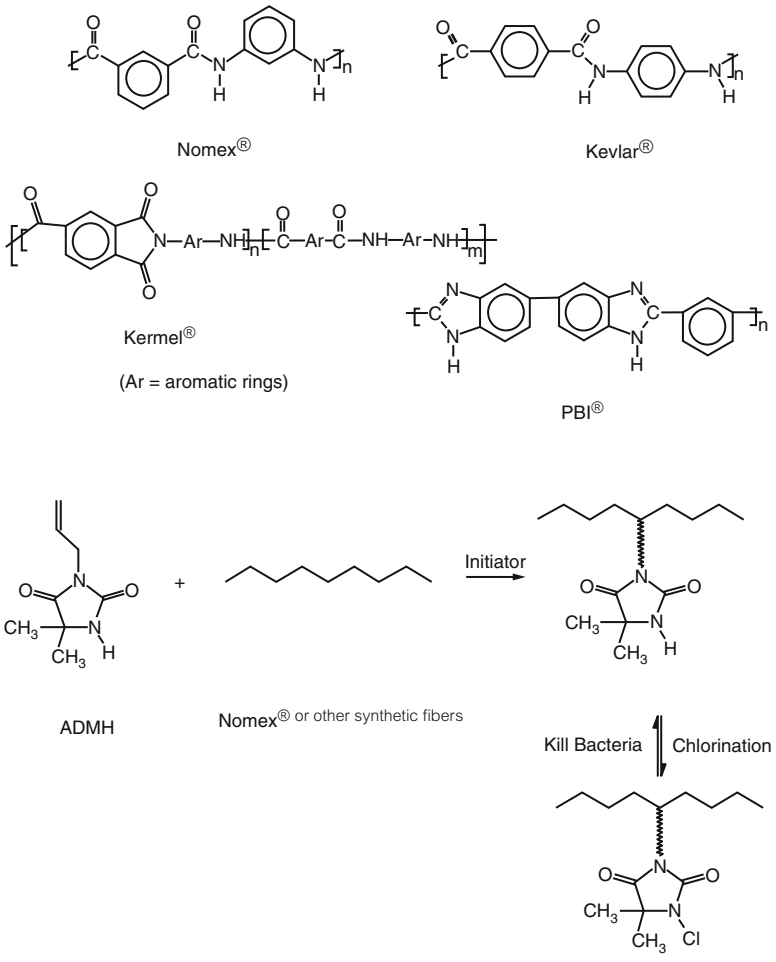

Fig. 7.7 *Above*: Chemical structures of the synthetic fibers. *Below*: ADMH grafting copolymerization and chlorination on the synthetic fibers. Reproduced with permission from [52]

biocidal properties. However, the finished products demonstrated excellent durability of antimicrobial functions at basic pH values (Table 7.2).

In addition to pH-sensitive antimicrobial fibers, temperature-responsive polymers have also been widely employed in the fabrication of antimicrobial fibers. Poly(*N*-isopropylacrylamide) is probably the most extensively employed thermoresponsive polymers with a phase transition at around 32 °C.

For instance, Liu et al. [53] studied the antibacterial activity of temperature-sensitive poly(*N*-isopropylacrylamide/polyurethane (PNIPAAm/PU) hydrogel grafted nonwoven fabrics with chitosan modification. They prepared series of temperature-sensitive hydrogel grafted nonwoven fabrics with different

Table 7.2 Effect of pH on bacterial reduction (%) to nylon 6.6. fabrics

	Bacterial reduction, *E. coli* (%)					
	CPC			BDHAC		
pH	1[a]	5	10	1	5	10
3.5	99.6	22.1	11.5	98.1	7.7	0
7	99.9	38.3	15.0	99.9	36.2	0
11	100	100	95.7	100	99.5	65.0

[a]After 1, 5, and 10 times Launder–Ometer washing; fabrics were treated with 2 % salt solution at 90 °C for 60 min; AATCC test method 100

N-isopropylacrylamide/polyurethane (NIPAAm/PU) feeding ratios. The resulting modified fibers were evaluated against *S. aureus* and *E. coli*. According to their findings, upon chitosan modification, the hydrogel grafted nonwoven cellulose fabrics demonstrate an antibacterial activity to *S. aureus* and *E. coli*, and the antibacterial efficiency is about 80 % within 1 h.

In another report, Chen et al. [55] fabricated chitosan wound dressings with temperature-responsive characteristics. Their strategy resort to the modification of polypropylene (PP) nonwoven fibers (NWF) by direct current pulsed oxygen plasma-induced grafting polymerization of acrylic acid (AAc). As a result, the hydrophilicity was improved due to the presence of carboxylic acid groups. These carboxylic acid groups were then employed to conjugate chitosan and poly(*N*-isopropylacrylamide) (PNIPAAm) using water-soluble carbodiimide as a coupling agent. The potential of these NWFs as wound dressings were evaluated using SD rat as the animal model. The authors evidenced that NWFs contained PNIPAAm were better than those contained only chitosan in wound-healing rates and the wound areas covered by PP-g-chitosan-g-PNIPAAm wound dressings healed completely in 17 days.

In those previous mentioned examples, the thermoresponsive characteristics of the polymer did not play any significant role. Some other approaches take advantage of the thermoresponsive PNIPAAm polymers to control the loading [56] or delivery [57] process of antimicrobial agents. An illustrative example was reported by Bajpai et al. [56] that employed the temperature induced alteration of the PNIPA swelling fabrics to induce the entrapment of silver nitrate [56, 57] (Fig. 7.8). In the first step, PNIPAAm fabrics are cooled below LCST allowed aqueous solution of silver ions to enter the swollen polymer network. Increasing the temperature forces entrapped water out of the matrix thus leaving only silver ions inside. These ions can be reduced to silver nanoparticles (AgNPs) using sodium borohydrate. Bajpai et al. [56] described the fabrication of modified cellulose fibers with PNIPAAm network produced in situ by photopolymerization using UV-radiation. Upon silver entrapment and nanoparticle formation the antimicrobial efficacy of the AgNP–PNIPAAm composites were evaluated against both Gram-positive and Gram-negative bacteria. The leaching of silver ions resulted in a clear zone of inhibition in the vicinity of the samples for both *E. coli* and *S. aureus* that depends on the amount of silver ions incorporated within the hydrogel (Fig. 7.9).

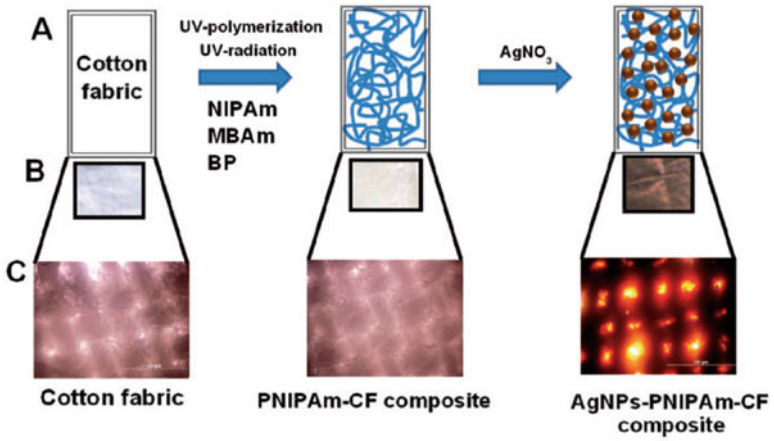

Fig. 7.8 Preparation of AgNPs loaded poly(*N*-isopropyl acrylamide) CF (AgNPs–PNIPAAm-CF) composite: (**a**) schematic representation of AgNPs–PNIPAAm-CF composite preparative route in two steps. Step 1 UV-radiation/photopolymerization of NIPAAm monomer in the presence of cross-linker and initiator on CF and Step 2 Silver nitrate entrapment using thermosensitive property of PNIPAAm and reduced with sodium borohydrate to embedded AgNPs on the CF via PNIPAAm chain attachment. (**b** and **c**) Photographs and optical microscope images of CF, PNIPAAm-CF composite, and AgNPs–PNIPAAm-CF composite, respectively. *AgNP* silver nanoparticle, *PNIPAAm* poly(*N*-isopropyl acrylamide), *CF* cotton fabric. Reproduced with permission from [56]

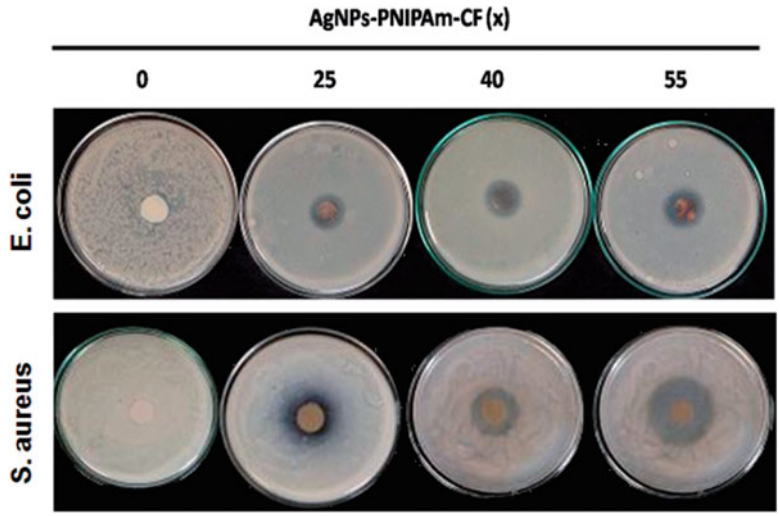

Fig. 7.9 Antibacterial activity of AgNPs–PNIPAAm-CF composites against *E. coli*. *AgNP* silver nanoparticle, *PNIPAAm* poly(*N*-isopropyl acrylamide), *CF* cotton fabric. Reproduced with permission from [56]

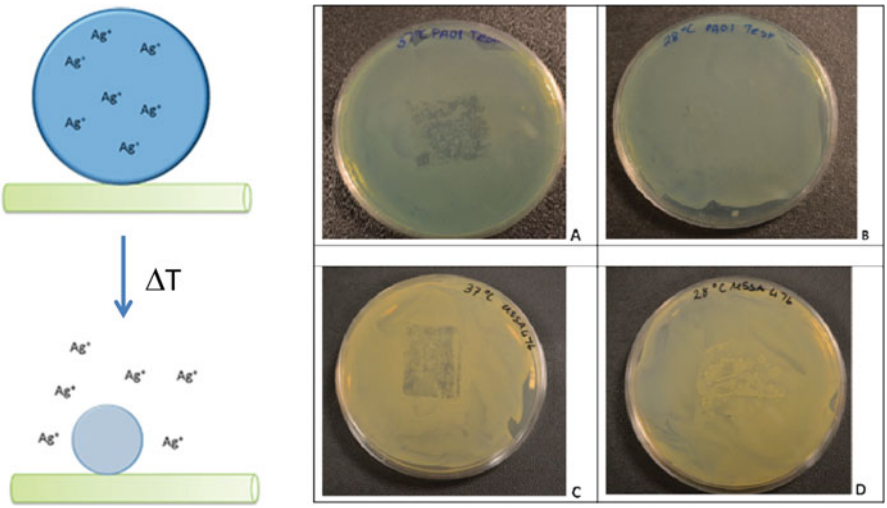

Fig. 7.10 Bacterial lawns of *P. aeruginosa* grown with silver nano-gel containing fabric at 37 °C (**a**) and 28 °C (**b**). *S. aureus* at 37 °C (**c**) and 28 °C (**d**). Reproduced with permission from [57]

Instead of using the changes induced by temperature to encapsulate the biocide, James et al. [57] fabricated nano-gels able to release the biocide at a particular range of temperatures. In their strategy, thermally responsive poly(*N*-isopropylacrylamide)-co-allylamine (PNIPAAm-co-ALA) nano-gels were synthesized and grafted onto nonwoven polypropylene (PP). The grafting process employed plasma reactions in the presence of maleic anhydride to functionalize the PP fibers. Immediately following formation of the maleic anhydride film (pp-MA) on fabric/polystyrene, the PNIPAM-ALA nano-gels containing silver nitrate were grafted to the pp-MA via amine nucleophilic attack from the ALA to the anhydride group on the film, forming amide linkages. Silver nitrate was incorporated into the nano-gels in their expanded state. The bacterial growth was measured before and after the lower critical solution temperature in order to evidence the role of the silver release on the antibacterial properties. As depicted in Fig. 7.10, below the LCST, the bacteria are able to grow while above the LCST bacterial growth was prevented or retarded.

Finally, several groups have been developed systems in which the antibacterial activity of the polymer fibers is regulated by the presence of UV-light [54]. It is well known that TiO_2 and ZnO nanoparticles [58–62] can effectively generate reactive oxygen species (ROS) on polymer surfaces under ultraviolet (UV) or day light exposure. The generated ROS can, in turn, provide light-induced antimicrobial properties employed in some cases to elaborate self-cleaning surfaces.

However, the presence of nanoparticles and the eventual possibility to come off from the surfaces of fibers and penetrate through skin and enter into the human body has raised several issues related to human safety. As an alternative to inorganic nanoparticles, different photoactive chemicals such as benzophenone derivatives have been incorporated onto cotton fabrics. These photoactive compounds can also

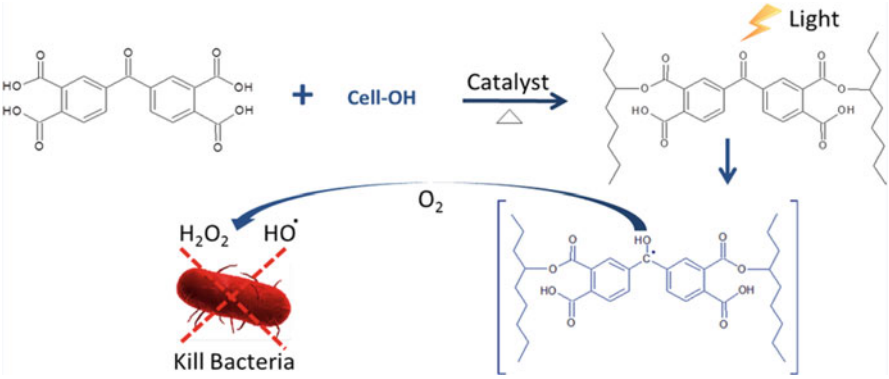

Fig. 7.11 Cross-linking reaction between cellulose and BPTCA and antibacterial activity in the presence of light. Reproduced with permission from [68]

generate ROS under UV irradiation, providing the fabrics with antibacterial activity [63]. The photoactive chemicals reported include porphyrin [64] and triazinyl porphyrin based [65], anthraquinone [66] and 3,3′,4,4′-benzophenone tetracarboxylic dianhydride (BPTCD). For instance, the latter has been proven to be effective as a light-induced antimicrobial agent on cotton fabrics [67].

In a recent report, Hou et al. [68] investigated the photoactive functions of benzophenone tetracarboxylic acid (BPTCA) treated cotton fabrics and the mechanism of the light-induced mechanisms provided by the incorporated benzophenone group (Fig. 7.11). The generated ROS, including hydroxyl radical and hydrogen peroxide, by the fabrics were measured. More interestingly, the modified fibers exhibited excellent antimicrobial activities against Gram-negative (*E. coli*) and Gram-positive (*S. aureus*) bacteria strains. These results indicate that the photoactive compound, BPTCA, a derivative of benzophenone, retains its photoactive property even after being covalently incorporated to cellulose.

7.7 Biodegradable Fibers with Antimicrobial Properties

Biodegradable fibers with diameters ranging from several micrometers down to tens of nanometers have found an increasing interest as a soft porous scaffold for tissue regeneration and wound-healing applications. However, eventual infection control and tissue repair involve an inevitable dynamic interaction of the fibrous mat with the wound environment including bacteria. In order to prevent bacterial contamination and biofilm formation, several studies involved the use of biodegradable polymers for the fabrication of fibers incorporating different biocides.

Poly (lactide-co-glycolide) (PLGA), an FDA-approved biocompatible copolymer, chitosan, and poly(vinyl alcohol) (PVA) are the most extended biodegradable polymers employed for the fabrication of fibers.

For instance, Said et al. [69, 70] prepared fusidic acid (bacteriostatic antibiotic)-loaded ultrafine PLGA fibers for wound-healing applications. Degradation of PLGA within bacterial culture allows for the release of fusidic acid. As a result, an increase in the bacterial colonization within a wound increased the PLGA degradation and, in turn, the antibiotic release. Furthermore, Said et al. showed effective wound healing in an animal model of fusidic acid-loaded ultrafine PLGA fibers. This study demonstrated early and persistent bacteria eradication in wounds heavily infected with *S. aureus* and wounds lightly infected with native skin flora when treated with fusidic acid-loaded fibers.

In addition to PLGA, also fibers constructed from chitosan have been explored as antimicrobial scaffolds [41]. Chitosan well known as a sustainable, biocompatible, biodegradable, antimicrobial, and nontoxic polysaccharide has been employed in many fields of application. Due to its abundance in nature and biocompatibility, the cationic polysaccharide chitosan is an excellent candidate to fabricate functional nanofibers. Moreover, chitosan has shown excellent antibacterial and antifungal activities and inhibits the growth of different bacteria, algae, and fungi [41].

Chitosan nanofibers have been employed as support for other biocides or combined with other polymers in which the antimicrobial activity is provided by the chitosan. Pure chitosan electrospun nanofibers have employed as carriers of model drugs such as potassium 5-nitro-8-quinolinolate [71] or by incorporation of biocide silver nanoparticles which are at a later stage released into the solution [72].

Other authors fabricated fibers from blends of chitosan with polymers, such as poly(vinyl pyrrolidone) (PVP) [73, 74], polyurethane (PU), or poly(vinyl alcohol) (PVA) [75] and evaluated the biocide properties of the electrospun fibers. In these cases, the polycationic nature of chitosan establishes electrostatic interactions with the negatively charged residues of the macromolecules at the cell membrane surface, resulting in the death of bacteria and fungi.

For instance, nanofibers containing quaternized chitosan (QCh) have been successfully prepared by electrospinning of QCh solutions mixed with poly(vinyl alcohol) (PVA) [75]. The average fiber diameter is in the range of 60–200 nm. UV irradiation of the composite electrospun nanofibrous mats containing triethylene glycol diacrylate as cross-linking agent has resulted in stabilizing of the nanofibers against disintegration in water or water vapors. Microbiological screening has demonstrated the antibacterial activity of the photo-cross-linked electrospun mats against *Staphylococcus aureus* and *Escherichia coli*. The obtained nanofibrous electrospun mats are promising for wound-healing applications.

Finally, also polyurethane–chitosan blended polymer was used by Shih et al. [76] to improve shrinkage and antimicrobial properties of woolen fabrics. The strategy involves, first the synthesis of polyurethane (PU) prepolymers from poly(ethylene glycol) (PEG) of different molecular weights. In the second step, the PU prepolymers were mixed with chitosan to form blended polymers. Shih et al. reported an improvement in both the shrink-proof and antimicrobial properties of the fabric with an increase in the temperature or duration of the heat treatment, as well as with an increase in the concentration of the processing agent.

7.8 Conclusions

This chapter describes the currently available strategies to fabricate micro- and nanometer size nanoparticles. Spinning techniques are among the most extended since permits the fabrication of continuous fibers from different polymers and blends. In particular, the possibility to incorporate biocide molecules or nanoparticles offers unique opportunities to produce fibers with antimicrobial properties. In this chapter, we summarized the alternatives reported to introduce antimicrobial moieties within fibers and the resulting activity against different bacterial strains.

More recently developed systems introduced the possibility to prepare active or nonactive antibacterial fibers that reversibly switch in response, for instance, to UV-light. Also nanofibers fabricated using pH or thermoresponsive polymers have been explored to direct the load and/or the release of the biocide in order to obtain materials able to precisely act in response to precise environmental changes.

Finally, some applications such as the use of fibers for wound dressing purposes require materials able to be reabsorbed. For this purpose, biodegradable polymers have been reported to be excellent candidates.

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Chapter 8
Antimicrobial Hydrogels

Abstract Hydrogels are usually defined as a class of materials fabricated from natural or synthetic polymers with, among others, two unique characteristics. On the one hand, they possess three-dimensional (3D) networks with variable physical properties composed of cross-linked hydrophilic polymer chains. On the other hand, they are able to incorporate an extremely large amount of water within the structure. These materials have found multiple applications such as drug delivery, surface coatings for implants, healing of chronic and traumatic wounds, encapsulation of cells for three-dimensional cell culture and tissue engineering. To mimic natural tissues, in addition to the mechanical and chemical properties, hydrogels require also cell biocompatibility. In this context, many current synthetic strategies focused on tuning the biological and physical attributes of hydrogels in order to reach specific interactions and responses from cellular systems. Nevertheless, for many of the above-mentioned bio-related applications, microbial infections still remain a serious limitation for the use of hydrogels. In this context, to overcome this issue, different approaches have been developed to fabricate antimicrobial/antiviral hydrogels.

This chapter aims to discuss the explored systems on the preparation of antimicrobial/antifungal hydrogels. Illustrative examples of the different methodologies will be presented as well. In particular, the antimicrobial hydrogels will be classified depending on their role as carrier or based on its inherent antimicrobial activity. Moreover, highly sophisticated systems in which the response to environmental conditions is at the base of the antimicrobial activity of the hydrogel will be discussed in detail as well.

Keywords Natural hydrogels • Synthetic hydrogels • Hybrid hydrogels • Peptide-based hydrogels • Chitosan • Antifouling/antimicrobial hydrogels

8.1 Introduction

Hydrogels are usually defined as a class of materials fabricated from natural or synthetic polymers with, among others, two unique characteristics. On the one hand, they possess three-dimensional (3D) networks with variable physical properties

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composed of cross-linked hydrophilic polymer chains. On the other hand, they are able to incorporate an extremely large amount of water within the structure [1]. Hydrogels have been, for instance, prepared from natural polymers such as polysaccharides (including dextran, alginate, and chitosan) or proteins (gelatin and fibrin). Equally, examples of hydrogels formed from synthetic polymers include poly(vinyl alcohol) (PVA), polyethylene oxide (PEO), and poly(acrylic acid) (PAA). A large number or excellent reviews have been devoted to the preparation and modification of hydrogels classifying the different types of hydrogels [2, 3].

These materials have found multiple applications such as drug delivery, surface coatings for implants, healing of chronic and traumatic wounds, encapsulation of cells for three-dimensional cell culture, and tissue engineering [2, 4–7]. To mimic natural tissues, in addition to the mechanical and chemical properties, hydrogels require also cell biocompatibility. In this context, many current synthetic strategies focused on tuning the biological and physical attributes of hydrogels in order to reach specific interactions and responses from cellular systems [7].

Nevertheless, for many of the above-mentioned biorelated applications, microbial infections still remain a serious limitation for the use of hydrogels. In this context, to overcome this issue, different approaches have been developed to fabricate antimicrobial/antiviral hydrogels.

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8.2 Types of Hydrogels

Hydrogels can be fabricated via different chemical methods as a function of the cross-linking strategy employed. Interestingly, independently of the chemical method used, the polymer engineer can modulate the parameters to achieve polymer networks with molecular-scale control over structure and with tailored properties, including biodegradation, mechanical strength, and chemical and biological response to stimuli [2].

The generally adopted classification catalog the hydrogels depending on the type of bond employed to create the 3D network. As a result, it is possible to distinguish between physical and chemical hydrogels. In the first case, the nature of the cross-linking process is associated to physical processes that include hydrophobic association, chain aggregation, crystallization, polymer chain complexion, and hydrogen bonding [3]. As a result, the hydrogels formed can be reversibly formed or disrupted. In the second case, the hydrogels are formed by covalent cross-linking. Chemical hydrogels exhibit permanent structures and reversible changes are

avoided. In addition to these two types of hydrogels, a third class of hydrogels, known as dual-network hydrogels can be eventually prepared combining physical and chemical cross-linking hydrogels.

Other classifications have been equally proposed depending on other hydrogel properties such as their response (physically responsive, chemically responsive, or biochemically responsive), their charge (negative, positive, uncharged), their source (natural or synthetic), or their degradability [3].

8.3 Hydrogels as Supports of Antimicrobial Agents

As mentioned in the introduction, hydrogels can be either loaded with antimicrobial molecules or can exhibit inherent antimicrobial activity [1]. This section will provide a thorough overview over the antimicrobial hydrogels prepared by encapsulation or immobilization of antimicrobial compounds.

8.3.1 Hydrogels Containing Antimicrobial Metal Nanoparticles

Silver and gold nanoparticles (NPs) have been extensively used in biomedical applications in particular due to their excellent antimicrobial properties against a large variety of microorganisms such as bacteria and fungi [8–12]. In the case of silver nanoparticles, while their action mechanism is still under examination, several studies evidenced that the antimicrobial activity is associated to the production of reactive oxygen species that bind to the bacterial cell membranes and provoke the membrane damage. Moreover, in addition the NP can release silver ions that can also have an antimicrobial action [10, 13–16]. For example, Sondi et al. [13] evidenced that the treated *E. coli* cells were damaged, showing formation of "pits" in the cell membrane of the bacteria, whereas the silver nanoparticles were found to accumulate in the bacterial membrane. As a result, the membrane with such morphology exhibits a significant increase in permeability, resulting in death of the cell.

In addition to silver nanoparticles, gold or zinc oxide nanoparticles have been equally explored for their antibacterial properties [9, 17]. For instance, Hernández-Sierra et al. [9] compared the bactericidal and bacteriostatic properties of silver, zinc oxide, and gold nanoparticles on *S. mutans*. They investigated the minimum inhibitory concentrations (MICs) and the minimum bactericidal concentrations (MBCs) for the three types of nanoparticles and concluded that higher antimicrobial effect against *S. mutans* of silver nanoparticles at lower concentrations than gold or zinc, which would allow reaching important clinical effects with a reduced toxicity.

More sophisticated approaches have been equally proposed in which the hydrogels were designed to incorporate more than one type of nanoparticle. For instance,

Reddy and coworkers [18] reported the fabrication of bimetallic hydrogels bearing simultaneously silver and gold in the structure. The hydrogels explored were based on acrylamide (AM) and 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS). Interestingly, they observed a synergistic effect, so that higher antibacterial activity was observed in comparison with hydrogels bearing exclusively either silver or gold NPs.

The incorporation of nanoparticles and in particular silver nanoparticles has been accomplished in both synthetic and natural hydrogels [4]. Within the first group, examples of nanoparticle loaded hydrogels include PVA [19], PVP [20], and poly(acrylamide-*co*-acrylic acid) [21]. Silver nanoparticles have been equally embedded on natural gelatin [22] and alginate [23] hydrogels.

The incorporation of nanoparticles with antibacterial properties can be achieved mainly using the following alternative strategies:

(a) *Loading nanoparticles onto a preformed hydrogel.*
The use of the breathing-in/breathing-out (BI-BO) method (depicted in Fig. 8.1) was employed by Thomas et al. [24] to prepare a poly(acrylamide-*co*-*N*-vinyl-2-pyrrolidone) hydrogels loaded with silver NPs. In this BI-BO process, a nonionic hydrogel is exposed to changing solutions that cause it to sequentially swell and shrink. During the swelling process, the NPs are present in the media are able to diffuse into the hydrogel. A second, shrinking process allows to finally encapsulate the particles into the gel's network. The resulting silver containing hydrogels exhibit antibacterial activity against *E. coli*.

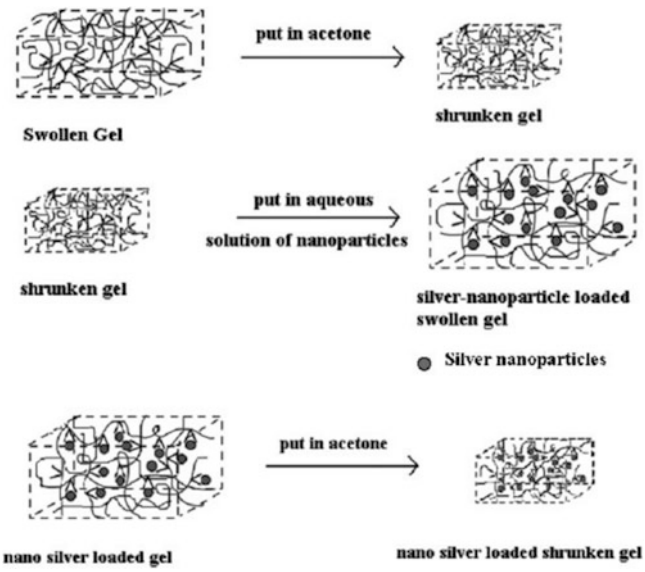

Fig. 8.1 Formation of silver nanoparticles within the hydrogel. Reproduced with permission from [24]

Moreover, this activity is directly related to the number of BI-BO cycles the gel had been subjected.

The incorporation of nanoparticles into a preformed hydrogel has been equally obtained by using freeze–thaw cycles. For instance, Yu et al. [20] prepared poly(vinyl alcohol)/ poly(*N*-vinyl pyrrolidone) (PVA-PVP) hydrogels containing silver nanoparticles by using this approach. They varied both the silver content in the solid composition was in the range of 0.1–1.0 wt% and the silver particle size was from 20 to 100 nm while the weight ratio of PVA to PVP was maintained constant at 70:30. This strategy permitted the formation of the hydrogels during the freezing–thawing treatment without aggregation of the silver nanoparticles. More interestingly, water absorption properties, release of silver ions from the hydrogels as well as the antibacterial effects of the hydrogels were evaluated against *E. coli* and *S. aureus* proving that the nanosilver-containing hydrogels had an excellent antibacterial ability.

Silver nanoparticles have also been incorporated to covalently attached soft poly(vinyl alcohol) (PVA) hydrogel films on biodegradable poly(L-lactic acid) (PLLA) [19]. The multistep procedure to fabricate the hydrogel, depicted in Fig. 8.2, involves oxygen plasma treatment, UV-initiated graft polymerization, and chemical grafting methods. In the first step, the surface of the PLLA film samples is treated with oxygen plasma that creates functional groups required to

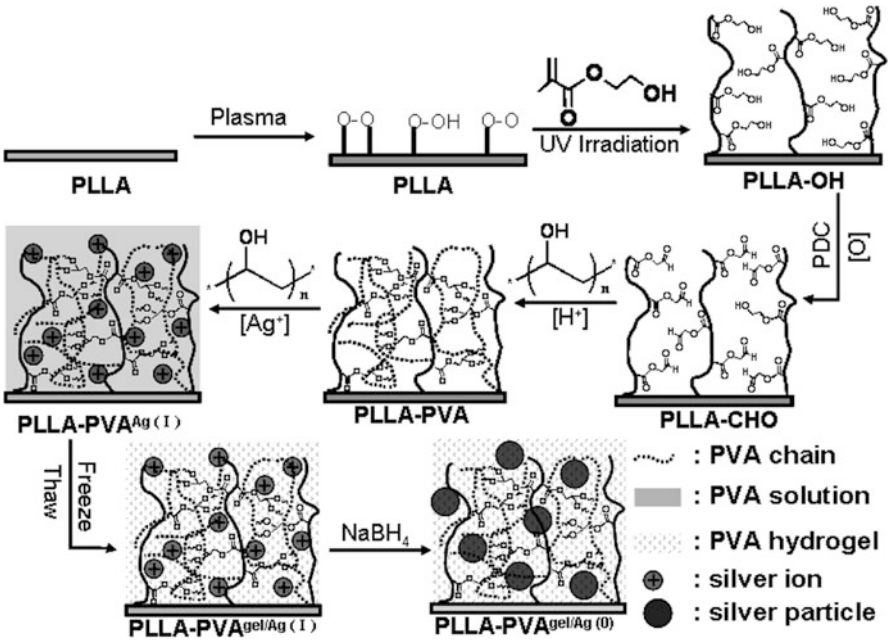

Fig. 8.2 Process for fabricating PLLA-PVA gel/Ag(0) film involving oxygen plasma treatment, UV-initiated graft polymerization, and chemical grafting methods. Reproduced with permission from [19]

graft 2-hydroxyethyl methacrylate (HEMA). Then, the alcohol functionalities in the grafted polyHEMA chains were oxidized using pyridinium dichromate to obtain an aldehyde-rich surface. These aldehyde groups served to anchor PVA chains that upon freeze–thaw cycles form a PVA hydrogel layer. The hydrogel film, i.e., PLLA-PVA gel, was doped with silver ions, which were reduced to silver nanoparticles using $NaBH_4$. PLLA-PVAgel/Ag(0) hydrogel films exhibit both antibacterial and reduced cell adhesion properties. The antibacterial properties are provided by the silver nanoparticles and the hydrogels with high water content prevented cell adhesion.

In addition to the approaches depicted above using silver NPs, antibacterial hydrogels bearing nanoparticles have also been developed through the incorporation of gold NPs into their networks [18, 25, 26] or by using a combination of more than one type of nanoparticle [18].

(b) *Formation of the hydrogel in the presence of NPs.*
The formation of hydrogel nanocomposites using preformed nanoparticles has been achieved using different strategies. For instance, Yu et al. [20] described the synthesis of poly(vinyl alcohol)/poly(*N*-vinyl pyrrolidone) (PVA-PVP) hydrogels bearing silver nanoparticles fabricated by repeated freezing–thawing treatment. This strategy was employed by Travan et al. [27] to prepare silver NPs in the presence of a chitosan-derived solution. In particular, they employed a lactose-substituted chitosan, 1-deoxylactit-1-yl chitosan, short-named "Chitlac" (Fig. 8.3). Then, a Chitlac-NP solution was mixed with an alginate solution forming a hydrogel. After evidencing that the silver NPs were immobilized in the gel, the authors explored the antimicrobial activity of the resulting hydrogels against different bacteria *S. aureus*, *S. epidermidis*, *E. coli*, and *P. aeruginosa*. According to their results, the antimicrobial activity of the material is related to the destabilization of the bacterial membrane when bacteria come into direct contact with the material.

(c) *Reduce a nanoparticle precursor within a gel network.*
The formation of hydrogel–nanoparticle nanocomposites using this approach is typically accomplished by the immersion of a hydrogel in a silver nitrate solution and subsequent treatment with a reducing agent (for instance, sodium borohydride) to reduce the silver and form the NPs directly in the gel network. An interesting issue is related to the nanoparticle size that has a direct relation to the final actimicrobial activity [21, 28]. Vimala et al. [29] developed a strategy to prepare, in a controlled manner, silver nanoparticles in a hydrogel network.

In some cases, it is directly the gel network that template the formation of the NP, thus providing a means to control NP shape and size [30]. An interesting example of antimicrobial hydrogels prepared by this approach was reported by Thomas et al. [21]. They proposed a novel approach to incorporate Ag nanoparticles into a grafted polymer network, with a subsequent citrate reduction, to yield devices with Ag-impregnated antimicrobial surfaces. As depicted in Fig. 8.4, the procedure involves formation of silver nanoparticles within swol-

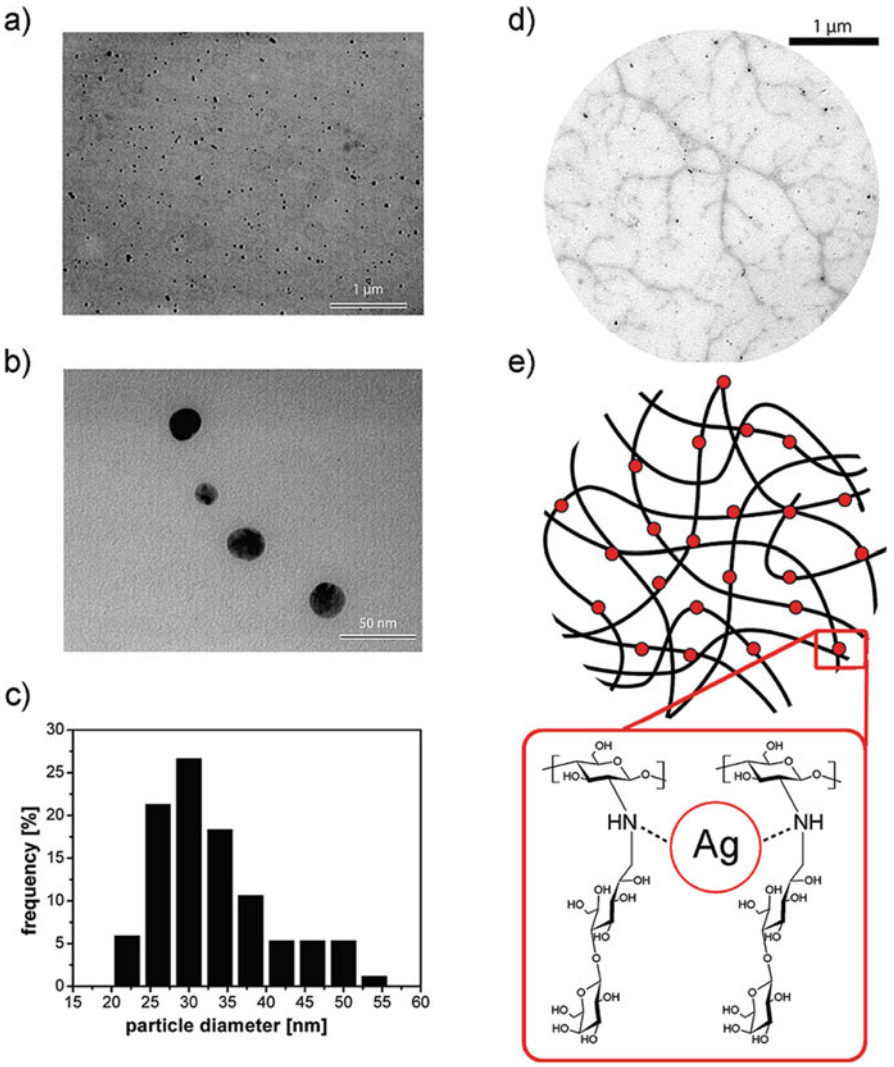

Fig. 8.3 (**a**, **b**) TEM pictures of silver nanoparticles disseminated in Chitlac at different magnifications; (**c**) silver nanoparticles size distribution histogram based on the TEM image in (**b**). The mean particle size measured was ~33.6 nm; (**d**) TEM image of silver nanoparticles formed on the polymeric chains of Chitlac; (**e**) schematic representation of the polymeric chains of Chitlac providing the nitrogen atoms for the coordination and stabilization of silver nanoparticles. Reproduced with permission from [27]

len poly (acrylamide-*co*-acrylic acid) hydrogels. The TEM of hydrogel–silver nanocomposites showed almost uniform distribution of nanoparticles with sizes of around 24–30 nm in size throughout the gel networks. More interestingly, the nanocomposites demonstrated excellent antibacterial effects on *E. coli*.

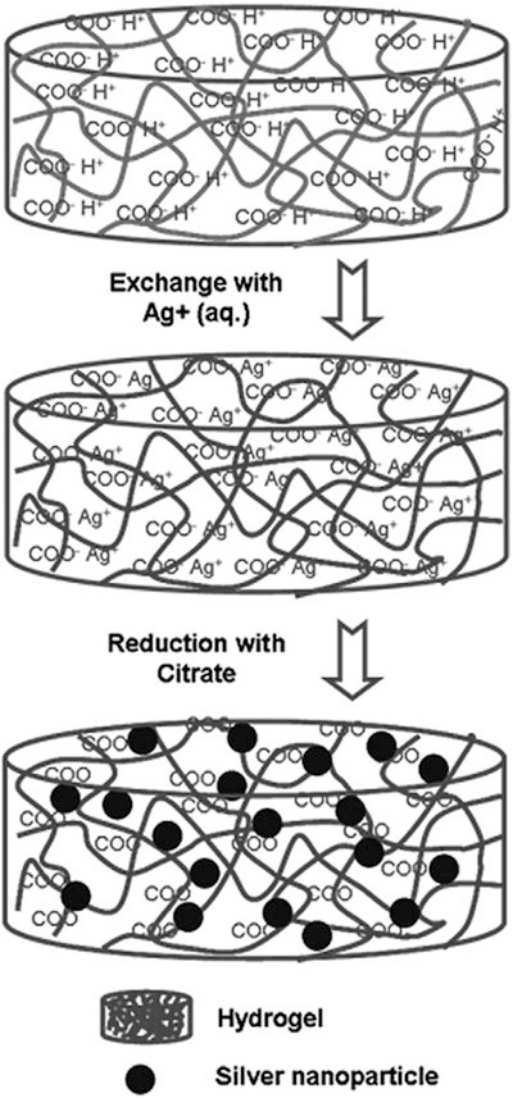

Fig. 8.4 Formation of silver nanoparticles within the swollen copolymeric network. Reproduced with permission from [21]

The antibacterial activity depended, among others, on the nanocomposites size, amount of silver nanoparticles, and finally the quantity of monomer acid present within the hydrogel–silver nanocomposites [21].

A similar strategy was developed by Rattanaruengsrikul et al. [22] to prepare gelatin hydrogel pads. This group reported the preparation of gelatin hydrogels from a 10 wt% gelatin solution that contained 2.5 wt% $AgNO_3$ in 70 % v/v acetic acid by using a solvent-casting methodology. The $AgNO_3$-containing gelatin solution was aged under mechanical stirring for variable

periods of time to allow for the formation of silver nanoparticles (nAgs). The reader can also find other examples reported using this strategy by Zan et al. [19] or Murthy et al. [31].

(d) *Simultaneous formation of both hydrogel and nanoparticles.*
Hydrogels containing NPs can also be prepared by the simultaneous fabrication of the hydrogel and nanoparticles [32, 33]. Fullenkamp et al. [32] take advantage of this strategy to prepare a silver-releasing antibacterial hydrogel that simultaneously allowed for silver nanoparticle formation and gel curing. For that purpose, they synthesized first water-soluble polyethylene glycol (PEG) polymers that contain reactive catechol moieties (Fig. 8.5). As precursor of the

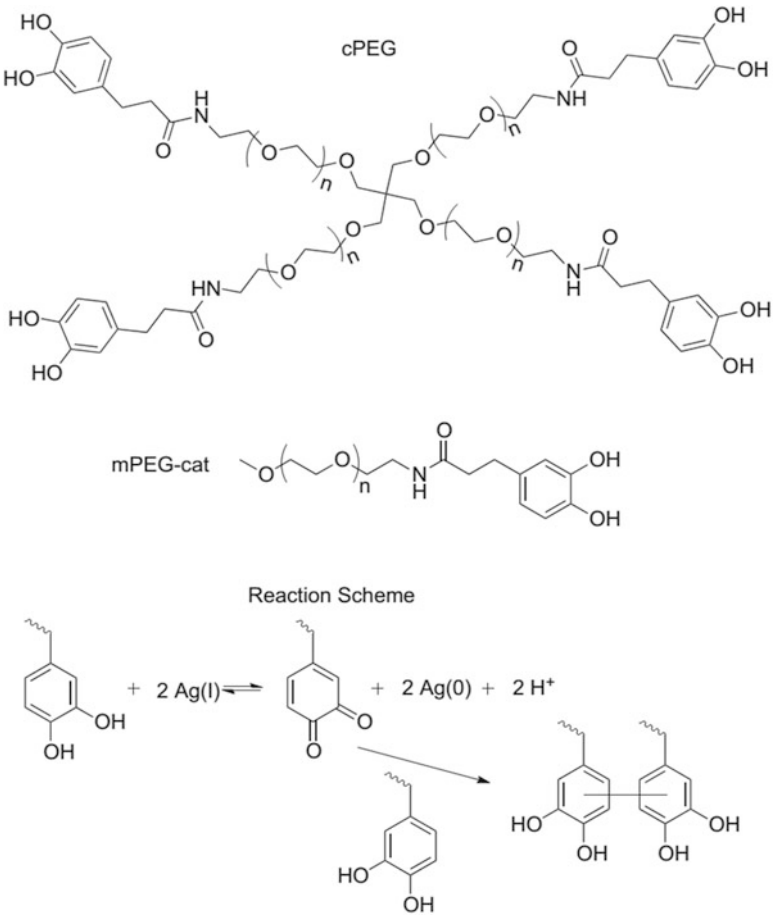

Fig. 8.5 Molecules employed and reaction scheme: cPEG was used to form hydrogels and mPEG-Cat was used for model GPC studies of the reaction. Catechol reduction of Ag(I) allows for quinone-initiated radical coupling to catechols as well as simultaneous silver nanoparticle formation. Reproduced with permission from [32]

silver nanoparticles, they employed silver nitrate that oxidize polymer catechols, leading to covalent cross-linking and, therefore, hydrogel formation with synchronized reduction of Ag(I). In these hydrogels, silver release was persistent during at least 2 weeks in PBS solution, and thus the hydrogels were found to inhibit bacterial growth while not considerably disturbing mammalian cell viability.

Independently of the strategy employed, other crucial aspects need to be considered in the preparation of antimicrobial hydrogels using nanoparticles. The first aspect is related to the improvement of the nanoparticle dispersion. Nanoparticle distribution and stabilization in water-insoluble, cross-linked hydrogel matrices has been usually achieved by modification of the nanoparticle surface functionality, thus, increasing the association between the hydrogel and the nanoparticles. For instance, Mukherji and Agnihotri [34] developed hydrogels containing chitosan as a cross-linker in a poly(vinyl acetate) gel matrix. They observed that an increase of the cross-linking agent in the gel increased both AgNP concentrations and porosity of the resulting hydrogel.

The second aspect is related to the cytotoxicity. The main objective is attempting to reduce AgNP toxicity to mammalian cells while maintaining antimicrobial activity. In this context, it has been demonstrated that the counterion of positively charged hydrophilic hydrogelators affected antimicrobial activity in AgNP hydrogels. Das et al. [35] described a direct relation between the counterion of a positively charged amino acid-based hydrogelator (they changed from chloride to a hydrophobic carboxylate) and the MIC against Gram-positive bacterial and fungal strains. More precisely, the MIC for Gram-positive *B. subtili* decreased from 10.0 to 2.0 μg/mL when the chloride was exchanged for n-hexanoate. Additionally, they found that the toxicity toward HepG2 and NIH3T3 mammalian cells decreased significantly.

8.3.2 Hydrogels Loaded with Antibiotics

Hydrogels, most of them hydrophilic in nature, provide excellent locations to load low-molecular weight antibiotics. Some examples of antibiotics integrated in hydrogel networks include: ciprofloxacin [36–38], gentamicin [39], teicoplanin [40], and amoxicillin [41].

For example, Marchesan et al. [38] reported the preparation of an antimicrobial hydrogel formed via the self-assembly of the hydrophobic tripeptide (DLeu-Phe-Phe). As depicted in Fig. 8.6, in their strategy assembly occurs in the presence of ciprofloxacin that takes an active part in the assembly process and is incorporated in the hydrogel's structure. The drug bound within the hydrogel by non-covalent interactions allows the hydrogel to retain its activity over a prolonged release timescale. The hydrogel showed antimicrobial activity against both *S. aureus*, *E. coli*, and a clinical strain of *K. pneumoniae* and low cytotoxicity toward human red blood cells or mouse fibroblast cell cultures [36, 38].

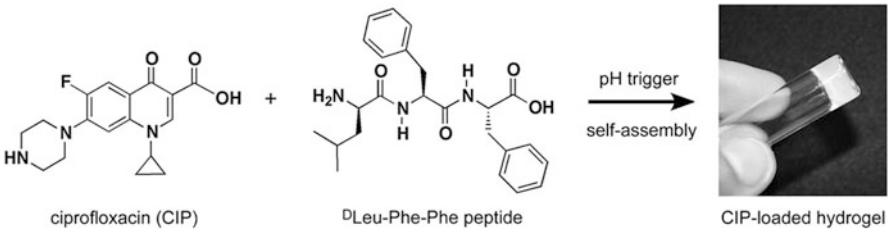

Fig. 8.6 Structures of ciprofloxacin (CIP) and peptide ^{D}Leu-Phe-Phe, which self-assemble into a hydrogel following a pH trigger. Reproduced with permission from [38]

Ciprofloxacin was also employed by De Giglio et al. [37] to modify hydrogels used as coatings on titanium implants in order to prevent implant-associated infections. They fabricated polyacrylic hydrogels, composed of poly(2-hydroxyethyl methacrylate) (PHEMA) and a copolymer based on poly(ethylene-glycol diacrylate) (PEGDA) and acrylic acid (AA) (PEGDA-AA) onto titanium substrates having the antibiotic ciprofloxacin by electrosynthesis. They evidenced that the PEGDA-AA hydrogel coating is able to release a greater amount of ciprofloxacin and showed better antibacterial activity than the PHEMA coating and is capable of inhibiting the growth of methicillin-resistant *S. Aureus* (MRSA).

8.3.3 Hydrogels Loaded with Antimicrobial Agents

Other strategies have been proposed in which the hydrogels were designed to deliver broadly acting antimicrobial agents that, in contrast to antibiotics, do not develop antimicrobial resistance [42–49]. For instance, nitric oxide or polyhexamethylene biguanide (PHMB) antimicrobials have been delivered from hydrogels and explored in the use in wound dressings. On the one hand, Halpenny et al. [42] fabricated pHEMA-based hydrogels have been developed for the release of nitric oxide. On the other hand, Jiang et al. [46] poly(*N*-isopropylacrylamide) (PNIPAAm)-based hydrogels have been developed for the release of PHMB. These hydrogels, when assessed in a wound model that included *P. aeruginosa* infection, were able to reduce the infection as well as expedite healing.

Antimicrobial peptides (AMPs) able to act through their general accepted mechanism that includes bacterial membrane disruption have also been incorporated in hydrogels. In the work of Laverty et al. [45], AMPs were integrated into PHEMA hydrogels as potential surface coatings for the prevention of biomedical device-related infections. In their work, three different AMPs, i.e., Maximin-4, H-Orn-Orn-Trp-Trp-NH_2, and C_{12}-Orn-Orn-Trp-Trp-NH_2, were employed and released from the hydrogel network. All the reported hydrogels were active against *S. epidermidis*; the ability of each gel to inhibit cell adhesion is directly related to the amount of AMP released.

Hydrogels to combat fungi (increasingly identified as major pathogens in bloodstream infections) have also been developed. To fight against fungi-associated

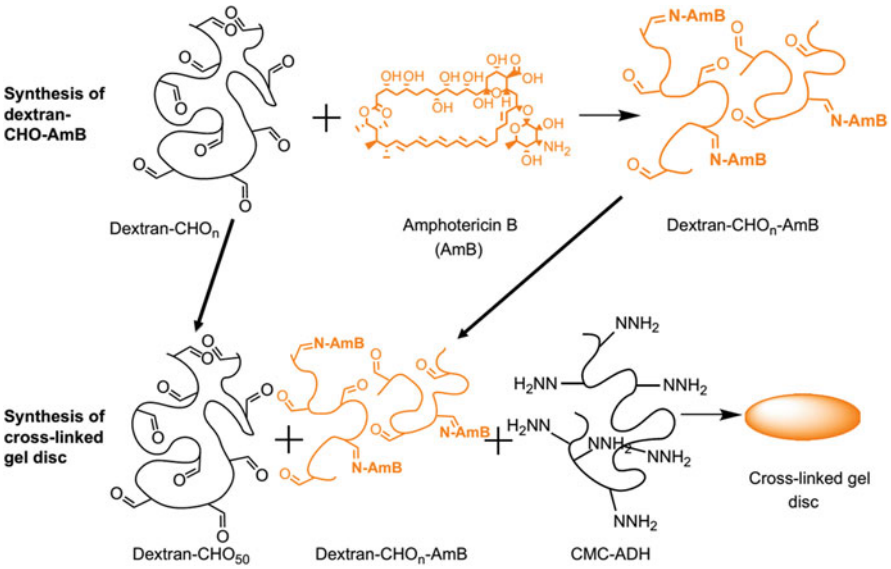

Fig. 8.7 Conjugation of AmB to oxidized dextran and the incorporation of the dextran-CHO–AmB into a CMC–Dextran gel. Reproduced with permission from [49]

infections, amphotericin B (AmB), a broad-spectrum antifungal agent often used to treat medical device-derived infections has been incorporated in hydrogels. Zumbuehl et al. [48] developed a dextran-based hydrogel called Amphogel in which amphotericin B was adsorbed. According to their findings, Amphogel kills fungi within 2 h of contact and still active for at least 53 days without losing its effectiveness against *C. albicans*. Moreover, the material is biocompatible in vivo and does not origin hemolysis in human blood.

Hudson et al. [49] reported an alternative strategy to prepare similar dextran hydrogels but bearing amphotericin B covalently anchored instead of physically adsorbed. In the synthetic strategy depicted in Fig. 8.7, the alcohol groups present in the AmB react with the aldehyde groups introduced in the dextran molecule. These hydrogels rapidly killed *C. albicans* by a mechanism involving direct fungi contact with the gel. Moreover, in vivo studies demonstrated the hydrogel's ability to prevent *C. albicans* infection in a mouse model.

8.4 Hydrogels with Inherent Antimicrobial Properties

Most of the reported antimicrobial hydrogels have been prepared by charging the hydrogels with different antimicrobial molecules (drugs, antibiotics, etc.) in a non-covalently manner. However, the lifetime for the above systems is limited to the drug diffusion time. Alternative approaches have been developed in which the

antimicrobial hydrogel is obtained by covalently linking an active agent onto a preformed hydrogel or using monomers that display antimicrobial activity to produce the hydrogel.

8.4.1 Antimicrobial Peptide-Based Hydrogels

The ultimate wound-healing scaffold must offer the appropriate physical and mechanical properties to prevent secondary infection, and an exceptional physiological environment to facilitate cell adhesion, proliferation, and/or differentiation. Synthetic cell-adhesive polypeptide hydrogels with inherent antibacterial activity have been explored for this purpose. In particular, antimicrobial peptides (AMPs) are characterized by both their cationic net charge and amphiphilic structure interact with the negatively charged cell membrane. Then, membrane disruption occurs by insertion of the peptides into the hydrophobic interior of the lipid membrane [50].

Illustrative examples of the use of polypeptides have been reported by Schneider and coworkers [51–53] that based on the structure and function of AMPs developed a family of self-assembling β-hairpin peptides that form hydrogel networks that display inherent antibacterial activity. The folded structure of these peptides resembles the amphiphilic, cationic nature of classical AMPs but, in contrast to AMPs, these peptides can self-assemble into fibrillar networks that constitute the formation of a hydrogel. The design of the peptide varied from a lysine-rich amphiphilic peptide, MAX1, [54–56] to an arginine-rich polypeptide [57–59]. The second series of AMPs resulted to be highly active against the multidrug-resistant bacteria, MRSA [52]. Upon optimization of the arginine content, they observed that the peptide containing six arginine residues, PEP6R [53] active against *S. aureus*, *E. coli*, and *P. aeruginosa*, while remaining noncytotoxic toward mammalian cells.

Zhou et al. take advantage of epsilon-poly-L-lysine (EPL), an AMP created by *S. albulus*, to fabricate antimicrobial hydrogels [55]. The synthesis of the hydrogels was carried out suing EPL graft-methacrylamide (EPL-MA) and a PEG diacrylate as a cross-linker. Interestingly, these hydrogels could be immobilized onto plastic surfaces in view of a possible use as coatings for medical devices. The antimicrobial activity of the EPL-MA hydrogels was evaluated and concluded that these hydrogels are broadly active against bacteria and fungi, for example, *P. aeruginosa*, *E. coli*, *C. albicans, S. aureus*, and *F. solani*.

Song et al. [56] also reported an interesting example of the use of peptide containing hydrogels for wound-healing purposes. They fabricated hydrogels using a series of polypeptides poly (Lys)x(Ala)y cross-linked with 6-arm PEG-amide succinimidyl glutarate. They changed the relative amount of alanine and lysine and evidenced that a particular formulation, i.e., poly $(Lys)_{60}(Ala)_{40}$ showed selective antibacterial properties with superior mammalian cell adhesion and cell proliferation activities while exhibiting significant antibacterial activity.

8.4.2 *Antimicrobial Hydrogels Prepared from Natural Polymers*

Among the different natural polymers Chitosan is overall the most extensively explored in the fabrication of antimicrobial hydrogels. Chitosan is a linear polysaccharide derived from the naturally occurring biopolymer chitin, one of the most abundant sugar-based biopolymer. Among its unique properties, Chitosan is biocompatible, exhibits low toxicity, and is biodegradable, hydrophilic, and cheap [60–62]. However, a crucial characteristic that imparts this polymer an intrinsic antimicrobial property is that Chitosan is weakly basic. As a result, the amino groups are readily protonated in acidic medium. As has been depicted, polycationic polymers favor the interaction with the bacterial cell membrane [63, 64]. The antimicrobial properties together with the wound-healing property make Chitosan hydrogels attractive materials for biomedical applications such as for wound and burns treatment [63, 65, 66] or surgical use [67].

The synthetic strategies explored using chitosan as antibacterial can be divided into the following major categories [64].

(a) First of all, chitosan can be immobilized on the surface of a particular material. For example, wound dressings were prepared by Chen et al. [68] immobilized chitosan onto PNIPAAm gel/PP nonwoven composites. They firstly used plasma-activation treatment and subsequently UV-light graft polymerization of NIPAAm gel to improve the PP hydrophilicity. Then, chitosan was grafted to the surface using the cross-linking agent, glutaraldehyde (GA). The authors showed that chitosan hydrogels displayed antibacterial ability to *E. coli* and *S. aureus*. The (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) (MTT) method indicated that the porous chitosan sponge were biocompatible when using fibroblast cells.

    Another example of the use of this strategy has been reported by Yang et al. [69]. In their study, they modified the surface of segmented polyurethane (SPU) catheters attempting to reduce friction and protein adsorption, minimizing catheter-related complications such as urethral trauma, encrustation, bacterial colonization, and infection. They employed a four-step surface modification method was developed to create a thin lubricious coating of chitosan/poly(vinyl alcohol) (PVA) hydrogel on the SPU catheter. Modification steps included oxidation of the SPU surface, functionalities modification, carbodiimide reaction and coupling, and hydrogel cross-linking. According to the authors, the protein absorption of the SPU catheter was significantly reduced by coating hydrogel. Moreover, the presence of chitosan in the hydrogel provides antimicrobial activity, and the hydrogel coating SPU samples showed antibacterial effects.

(b) Chitosan can be modified by means of the amino side groups present along the main chain.

    The principal strategy involves the quaternization of the amino side groups to enhance the antimicrobial properties of Chitosan. As depicted in Fig. 8.8, Li et al. [70] fabricated antimicrobial hydrogels based on quaternized ammonium

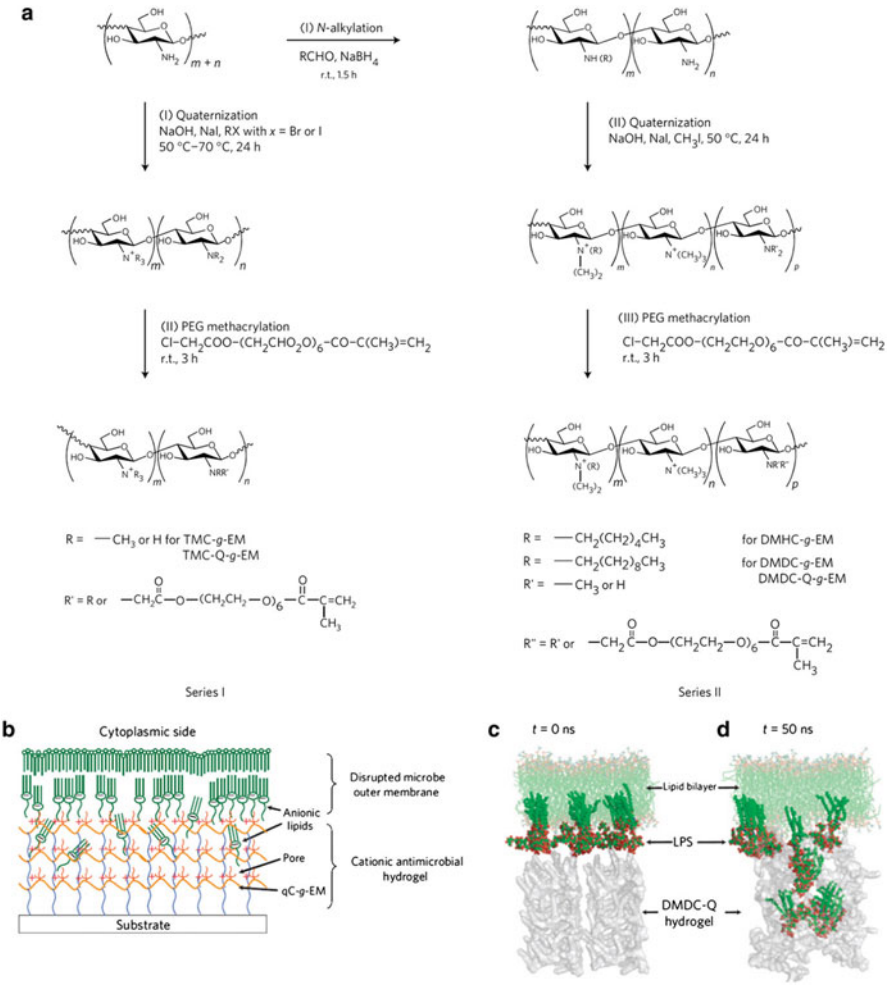

Fig. 8.8 (**a**) Scheme of the synthesis to fabricate modified chitosan antimicrobial hydrogels. (**b**) Schematic diagram of the "anion sponge" model showing parts of the negatively charged bacterial membrane being "suctioned" into the pores of the hydrogel. (**c**, **d**) Computer simulation of the killing mechanism showing the "suctioning" of *P. aeruginosa* bacterial membrane (lipid bilayer) LPS molecules into the hydrogel after 50 ns. Reprinted with permission from [70]

chitosan-graft-PEG methacrylate (qC-g-EM) differing both in their degree of quaternization as well as the type of alkyl chain employed. The hydrogel's activity was evaluated using *P. aeruginosa*, *E. coli*, *S. aureus*, and the fungus *F. solanis*. The authors found that by increasing the alkyl chain length of the quaternizing agent from trimethylammonium (TM) to dimethyldecylammonium (DMD), the efficacy selectively increases against Gram-positive *S. aureus* but not against Gram-negative bacteria *E. coli* or *P. aeruginosa*. More interestingly, an increase of the degree of quaternization leads to hydrogels with exceptional

antimicrobial efficacy against major classes of bacteria and fungus including Gram-positive (S. aureus), Gram-negative (*P. aeruginosa* and *E. coli*), and fungi (*F. solanis*). The author hypothesized that these cationic nanoporous hydrogels act via bacteria membrane disruption through ionic interactions with the anionic microbial surfaces (Fig. 8.8b, c). It is also worth mentioning that the contact-active hydrogels showed good in vitro and in vivo biocompatibility and are nonhemolytic.

(c) Chitosan has also been encapsulated and/or immobilized in the structure of a hydrogel.

Chitosan has been immobilized onto temperature-sensitive poly (*N*-isopropylacrylamide/polyurethane (PNIPAAm/PU) hydrogels by grafting using standard EDC-coupling strategies [71]. The authors examined the antibacterial behavior of the hydrogels before and after grafting and concluded that after chitosan modification, the hydrogel grafted nonwoven cellulose fabrics demonstrated an antibacterial activity to *S. aureus* and *E. coli* with an antibacterial efficiency of about 80 %.

Other authors [72] employed this strategy to prepare chitosan (CS)-grafted poly[(acrylic acid)-*co*-(2-hydroxyethyl methacrylate)] (CS-*g*-poly(AA-*co*-HEMA)). They varied the molar ratio of AA and HEMA, and additionally prepared nanocomposite hydrogels of CS-g-poly(AA-*co*-HEMA) with mica by radical copolymerization. While both CS-*g*-poly(AA) and CS-*g*-poly(AA-*co*-HEMA)/mica nanocomposite hydrogels exhibited high antiproliferative activity against *S. aureus* the presence of mica in the CS-g-poly(AA-*co*-HEMA) nanocomposite hydrogels did not affect the MIC.

Finally, Zhao et al. [73] resort to the use of blends as precursors of the hydrogel. More precisely, they combined poly(vinyl alcohol) (PVA) and carboxymethylated chitosan (CM-chitosan) and exposed the blend to an electron beam irradiation (EB) at 25 °C. By analyzing the product by both FTIR and DSC, the authors evidenced that grafting occurs between PVA and CM-chitosan molecules when irradiated. Finally, the antibacterial activity of the hydrogels against *E. coli* was also measured via optical density method. The blend hydrogels exhibited activity against *E. coli*, even at low CM-chitosan concentration below 3 wt%.

(d) Chitosan has also been employed as a component in the formation of polyelectrolyte complex hydrogels.

Tsao et al. [74] followed this strategy to fabricate a polyelectrolyte complex (PEC) hydrogel combining chitosan as the cationic polyelectrolyte and γ-poly(glutamic acid) (γ-PGA) as the anionic polyelectrolyte. The structure of these PEC hydrogels consisted of interconnected porous structures (pore size: 30–100 nm) in all of the chitosan-gamma-PGA formulations tested. Moreover, the chitosan-gamma-PGA PEC hydrogels were efficient against *E. coli* and *S. aureus*. In addition, in vitro cell culturing of three T3 fibroblasts revealed that all the chitosan-γ-PGA PEC hydrogels were effective in promoting cell proliferation, particularly those positively charged (chitosan-dominated).

(e) Finally, hydrogels based on chitosan were synthesized via a cross-linking reaction of chitosan [75–77].
For instance, Mohamed et al. [75] synthesized chitosan-based hydrogels via a cross-linking reaction of chitosan with variable concentrations of oxalyl bis-4-(2,5-dioxo-2H-pyrrol-1(5H)-yl) benzamide. The hydrogels antimicrobial activity was investigated against pathogenic fungi (*A. fumigatus* and *A. niger*) and five bacterial species (*B. subtilis*, *S. aureus*, *S. pneumoniae* as Gram-positive bacteria, and *S. typhimurium*, and *E. coli* as Gram-negative bacteria. The prepared hydrogels showed higher antimicrobial activities in comparison to the chitosan precursors. However, an increase of the degree of cross-linking in the hydrogels resulted in a weaker antimicrobial activity.

8.5 Dual Antimicrobial/Antifouling Hydrogels

The antimicrobial properties have been, in several reported examples, combined with antifouling behavior to prevent/limit the initial bacterial attachment [78, 79]. For instance, Liu et al. [78] described the fabrication of intrinsically antifouling polyethylene glycol (PEG)-based hydrogels bearing antimicrobial polycarbonate groups (polycarbonate containing quaternary ammonium groups, APC), thus leading to cationic PEG-APC hydrogels. For that purpose, a series of block copolymers thiol-terminated composed of PEG and cationic polycarbonate segments with varying amounts of randomly distributed quaternary ammonium groups and hydrophobic ethyl groups were fabricated via an organocatalytic ring opening polymerization (ROP) (Fig. 8.9a). The cationic PEG-APCs were then fabricated into hydrogels using a tetraacrylate functional PEG was reacted with the thiol-containing APC to yield a solution of star-shaped PEG that was partially conjugated with APC (Fig. 8.9b). Moreover, when tested against *S. aureus*, *E. coli*, and *C. albicans* they exhibit a 99.9 % killing efficiency. Also, against clinically isolated MRSA, vancomycin-resistant enterococci (VRE), *A. baumannii*, and *C. neoformans* exhibited excellent antimicrobial activity. In order to mimic the surface of a catheter, the gel was coated onto silicone rubber showing effective synergistic antifouling and antimicrobial activity against *S. aureus* and *E. coli*.

Zhao et al. [80] also synthesized hybrid poly(*N*-hydroxyethylacrylamide) (polyHEAA)/salicylate (SA) hydrogels with integrated antifouling and antimicrobial capacities and tested them as follows: first of all, the authors evaluated the antifouling efficacy of polyHEAA hydrogels to proteins, cells, and bacteria. Secondly, the antimicrobial activity of polyHEAA/SA hydrogels was investigated against both Gram-negative *E. coli* and Gram-positive *S. epidermidis*. In particular, polyHEAA/SA hydrogels displayed excellent resistance to protein adsorption, cell adhesion, and bacteria attachment.

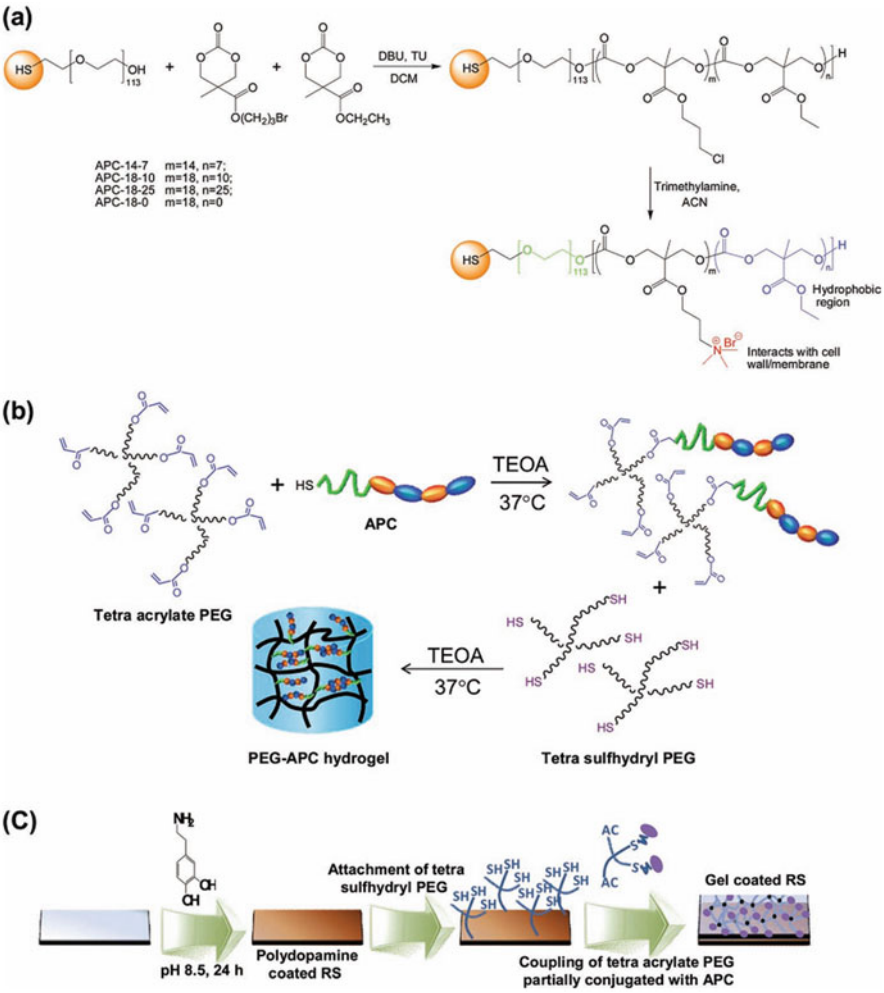

Fig. 8.9 Synthetic scheme of the fabrication of (**a**) aminated polycarbonates (APCs), (**b**) PEG-APC hydrogel, and (**c**) hydrogel coating onto silicone rubber surface. Reproduced with permission from [78]

8.6 Responsive Hydrogels with Antimicrobial Properties

The idea of introducing responsive moieties onto the antimicrobial hydrogels responds to multiple causes. In some cases, the response is justified by the required protection of the antimicrobial agent [41]. In other cases, a change in the hydrogel behavior can be employed to deliver the antimicrobial agent [81]. Hydrogels with response to different stimulus have been fabricated as follows:

(a) *pH-responsive hydrogels*

The most explored hydrogels are those that respond to changes in the environmental pH. For instance, Chang et al. [41] developed a strategy to fabricated chitosan/poly-γ-glutamic acid nanoparticles incorporated into pH-sensitive hydrogels were developed as an efficient carrier for amoxicillin delivery. Drug protection during transport is crucial to overcome the problems encountered when using antibiotics. The pH-sensitive hydrogels protect the nanoparticles from being destructed by gastric acid. The integration of amoxicillin-loaded nanoparticles within the hydrogel protects the drug from the actions of the gastric juice and enabled amoxicillin interaction precisely with intercellular spaces, the site of *H. pylori* infection.

Polyelectrolytes able to release or accept protons in the targeted pH environment (e.g., the acidic environment of stomach) are also excellent candidate to prepare pH-sensitive hydrogels [82]. In this context, chitosan is among the most extended natural polymers employed for this purpose since it is pH-sensitive with a pK_a around 6.3 [83]. This natural polymer was employed by Gupta et al. [84] to prepare interpenetrating hydrogels in combination with poly(acrylic acid) and poly(vinylpyrrolidone). Although, this group employed this hydrogel matrix for drug delivery purposes Risbud and coworkers [85] applied a freeze-dried version of the same matrix for the release of antibiotics. In particular, they encapsulated amoxicillin and evidenced that that the component was able to release around 73 % of the amoxicillin in 3 h at pH 1.0.

Finally, another example of hydrogels incorporating antifouling and antimicrobial moieties that varied as a function of the environmental pH has been reported by Cao et al. [79]. The authors synthesized two different hydrogels based on poly(2-(bis(2-hydroxyethyl) (2-(methacryloyloxy) ethyl) ammonio) acetate) (pCBOH2) and poly(2-((2-hydroxyethyl) (2-(methacryloyloxy)ethyl) (methyl) ammonio) acetate) (pCBOH1). These hydrogels are in a zwitterionic form at neutral or basic pH. However, in acidic media, these hydrogels become cationic and are able to bind to and kill bacteria by damaging the cell membrane. Interestingly, once the material has killed the bacteria a change in environmental pH back to neutral or basic conditions converts the material to its zwitterionic form. As a result, the dead bacteria previously adhered to the surface are now released from the gel. Therefore, these antimicrobial hydrogels are able to kill bacteria and prevent the accumulation of dead bacteria at the hydrogel surface (Fig. 8.10).

(b) *Thermoresponsive hydrogels*

In addition to pH-responsive hydrogels, thermosensitive polymers have also studied for the elaboration of antibacterial hydrogels. For this purpose, polymers or copolymers of poly(*N*-isopropylacrylamide) (PNIPAm) have been widely used in the fabrication of thermoresponsive coatings [86–89]. For the fabrication of thermoresponsive antibacterial hydrogels, thermosensitive monomers are usually combined with antibacterial monomers. For instance, quaternized methacrylamide (MA) combined with NIPA were copolymerized by Chen et al. [90] and Dizman et al. [91] The lower critical solution temperatures (LCSTs)

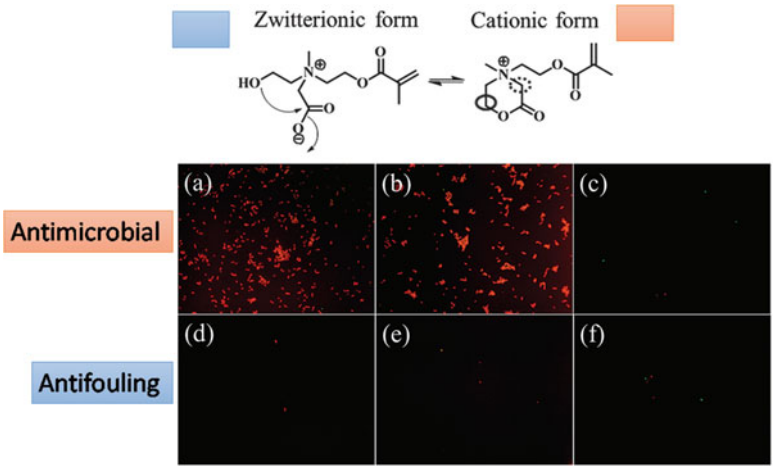

Fig. 8.10 Fluorescence microscopy images of bacterial attachment on pCBOH1 in cationic form (**a**), pCBOH2 in cationic form (**b**), and pCBMA (**c**) hydrogels before hydrolysis and on pCBOH1 in zwitterionic form (**d**), pCBOH2 in zwitterionic form (**e**), and pCBMA (**f**) hydrogels after 16 h hydrolysis in PBS. Cells with damaged cytoplasmic membrane are in red and cells with intact cytoplasm membrane are in green. Reproduced with permission from [79]

of the copolymers described varied between 25 and 42 °C. The variation of the LCST could be finely tuned depending on the ratio of MA to NIPA as well as the chain length of the alkyl group of the quaternary ammonium. At low temperature, the polymers were soluble and high levels of antibacterial activity against both *S. aureus* and *E. coli* were observed. This antibacterial activity is caused by the interaction of the polymer quaternary groups and the bacteria [90, 91]. By increasing the temperature above the LCST, the polymers become insoluble and the antimicrobial activity disappeared [91].

Other thermoresponsive antimicrobial hydrogels based on chitosan [81] or collagen [43] have been recently reported. For instance, injectable thermosensitive hydrogels based on chitosan, quaternized chitosan and alpha, beta-glycerophosphate (alpha,beta-GP) were reported by Ji et al. [81]. The gelation point of the hydrogel could be set at a temperature close to normal body temperature but also to any other temperature above 25 °C. This thermosensitive hydrogel exhibited high antibacterial activity toward two periodontal pathogens, i.e., *P. gingivalis* and *P. intermedia*.

Most of the hydrogels are designed to combat initial bacterial adhesion and prevent the formation of a biofilm. However, this appears to work during some period to of time and biofilms are finally formed. Temperature-responsive hydrogels may offer interesting alternatives to improve the antibacterial activity against biofilms. Li et al. [92] designed thermoresponsive hydrogels by stereocomplexation of triblock polymers, namely poly(L-lactide)-bPEG)-b-poly ((L-lactide) (PLLA-PEG-PLLA), poly(D-lactide)-bPEG)-b-poly((D-lactide)

(PDLA-PEG-PDLA), and the cationic triblock polymer poly(D-lactide)-*b*-cationic poly(carbonate)-b-poly(D-lactide) (PDLA-CPC-PDLA). Non-covalent interactions are involved in the gels formation providing them with shear-thinning properties. In addition to the antibacterial capabilities of these hydrogels against, for instance, MRSA, VRE, *P. aeruginosa*, *A. baumannii*, *K. pneumonia*, or *C. neoformans*, the authors demonstrated that these gels are able to remove microbial biofilms formed by *S. aureus*, MRSA, *E. coli*, and *C. albicans*.

(c) *Electrically responsive anti-adherent hydrogels*

Iontophoresis, i.e., the presence of ions diffusing in the hydrogels by applying an electric field, has been employed by Fallows et al. [47] to control the delivery and therefore the antimicrobial activity of a particular hydrogel. They prepared hydrogels based on hydrogels composed of the polyelectrolyte poly(methyl vinyl ether-*co*-maleic acid) (PMVE/MA) cross-linked with polyethylene glycol (PEG). They encapsulated photosensitizers within the hydrogel in order to investigate the potential of these hydrogels for photodynamic antimicrobial chemotherapy (PACT) that were delivered applying an electric field. The photosensitizers employed were meso-Tetra (*N*-methyl-4-pyridyl) porphine tetra tosylate (TMP) and methylene blue (MB). The authors demonstrated that the release concentrations were in excess compared to those required to induce complete kill of clinical strains of MRSA and *B. cepacia*. Thus, these results supported that the iontophoretic delivery of TMP and MB is a potential option in the rapid PACT treatment of infected wounds [47].

(d) *Multiresponsive hydrogels as antibacterial platforms*

Finally, the concept of incorporating different polymers sensitive to more than one stimulus in order to synergistically improve the antibacterial properties of the hydrogel has also been considered. Sui et al. [93] combined in the same hydrogel monomers that respond to temperature PNIPA with redox-responsive poly(ferrocenylsilane) (PFS) macromolecules. The thermoresponsive properties of the hydrogels were studied as a function of the PFS oxidation state. Interestingly, the redox activity of the PFS chains in the hybrid hydrogels could be used to prepare PFS–PNIPAM–Ag composites in a facile in situ process. Ag uniformly distributed nanoparticles were formed via reduction of silver nitrate by PFS. These composites showed strong antimicrobial activity against *E. coli* while maintaining a high biocompatibility with cells.

8.7 Conclusions

This chapter described recent strategies to fabricate antimicrobial hydrogels. As mentioned above, two main approaches have been employed. The first one involves the incorporation of the antimicrobial molecule within the hydrogel. These antimicrobial molecules can be either physically or covalently bonded to the hydrogel structure. In addition, some other hydrogels exhibit intrinsic antimicrobial properties. For instance, polymeric hydrogels constructed with cationic monomers,

i.e., opposite charge in comparison with the bacterial membrane act through non-stereospecific mechanisms that involve membrane disruption. This interaction mechanism has two main advantages. On the one hand, since the interaction is based on membrane–hydrogel interactions bacteria are unable to gain resistance. On the other hand, as has been reported, a major interesting improvement of these hydrogels in comparison with other antimicrobials is that they are active against current strains of multidrug-resistant bacteria. In addition, future bacteria with novel resistance mechanism are expected to be affected by this type of hydrogels as well. Moreover, combining inherent antibacterial hydrogels with polymeric systems can precisely act under the presence of a particular stimulus that can be an interesting additional element to determine the conditions in which one antimicrobial polymeric system has to be turned on and remain off the rest of the time.

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Chapter 9
Antibacterial Polymeric Membranes

Abstract Membranes have been typically defined as interfaces between two interfaces having as a major role to regulate the transport between two different compartment and act as selective barrier. Membranes are able to selectively allow the transport of one substance in the presence of other compounds without the use of additives or the use of elevated temperatures, thus reducing the energy consumption. They have found multiple applications in different areas ranging from separation processes but have also been employed in the fabrication of biomaterials, catalytic purposes, or even lab-on-chip devices.

Several major characteristics including the low operation cost, relatively small footprint, and complicity with environmental regulations have provoked that polymers have been extensively employed for the fabrication of membranes. Polymeric membranes do not require the use of additives. This permits these membranes to be active at low temperatures thus enabling a significant decrease of the energy employed for the separation in comparison with other processes. In addition, these membranes are easily formed and up-scaling and downscaling can be easily carried out.

This chapter will provide a brief description about polymeric membranes focusing on one of the major remaining issues, that is, their contamination by microorganisms and, in particular, by bacteria. Upon a concise analysis of the problem, the alternative approaches developed to produce antifouling/antibacterial membranes will be thoroughly analyzed. For detailed reviews on membrane fabrication and their applications, the reader is referred to the following publications.

Keywords Membrane fabrication • Microporous/macroporous membrane • Membrane biofouling • Membrane modification • Surface functionalization

9.1 Introduction to Polymer Membranes

Membranes have been typically defined as interfaces between two interfaces having as a major role to regulate the transport between two different compartment and act as a selective barrier [1]. As described by Ulbricht [2], membranes are able to

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Table 9.1 Classification of membranes as a function of the material employed, the membrane cross-section, the preparation method, and the module configuration

Classifications	Description
Membrane materials	Organic polymers, inorganic materials (oxides, ceramics, metals), mixed matrix, or composite materials
Membrane cross-section	Isotropic (symmetric), integrally anisotropic (asymmetric), bi- or multilayer, thin layer or mixed matrix composite
Preparation method	Phase separation (phase inversion) of polymers, sol–gel process, interface reaction, stretching, extrusion, track-etching, micro-fabrication, electrospinning
Membrane module configuration	Flat sheet, hollow fiber, hollow capsule

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selectively allow the transport of one substance in the presence of other compounds without the use of additives or the use of elevated temperatures, thus reducing the energy consumption.

Membranes have found multiple applications in different areas ranging from separation processes but have also been employed in the fabrication of biomaterials, catalytic purposes, or even lab-on-chip devices [2].

Several properties are desired in a membrane including high and stable filtration flux and low filtration pressure but also, for instance, in the case of water filtration to produce a high-quality water produced without thorough pretreatments. In view of these requirements, a large variety of membranes suited for technical applications [2] have been designed in which several aspects have been considered in its design. As depicted in Table 9.1, membranes can be classified depending on the membrane materials, membrane cross-section, preparation method, and the membrane shape. In this context, polymers are probably the most extended material employed for the fabrication of membranes. This is without any doubt due to three major causes. First of all, polymeric materials permit a better pore-forming control. Secondly, polymeric membranes can be fabricated at a lower cost in comparison to inorganic counterparts. Finally, there exists a wide range of monomers with variable functionalities that allow the preparation of membranes suitable for different separation process [1].

Another interesting classification proposed by Ng et al. [1] is depicted in Table 9.2. This classification takes into account the structure of the membrane that varies from nonporous to microporous membranes. Different separation processes through passive transport membranes can be found depending on the driving force employed. As a result, membranes with different gradients (e.g., concentration or pressure or by an electrical field) have been reported.

As introduced above, several major characteristics including the low operation cost, relatively small footprint, and complicity with environmental regulations have provoked that polymers have been extensively employed for the fabrication of membranes. Polymeric membranes do not require the use of additives. This permits these membranes to be active at low temperatures thus enabling a significant decrease of

Table 9.2 Membrane classification for the separation processes via passive transport

Membrane barrier configuration	Transmembrane gradient		
	Concentration	Pressure	Electrical field
Nonporous	Pervaporation (PV)	Gas separation (GS)	Electrodialysis (ED)
		Reverse osmosis (RO)	
Microporous pore diameter dp ≤2 nm	Dialysis (D)	Nanofiltration (NF)	
Mesoporous pore diameter dp = 2–50 nm	Dialysis	Ultrafiltration (UF)	Electrodialysis
Macroporous pore diameter dp = 50–500 nm		Microfiltration (MF)	

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the energy employed for the separation in comparison with other processes. In addition, these membranes are easily formed and up-scaling and downscaling can be easily carried out [1, 2]. As a result, polymeric membranes have found interest in many different applications such as drug delivery [3] or whey protein fractionation using polyether sulfone (PES) [4, 5], polysulfone [4, 6], and cellulose [7, 8] membranes on a laboratory scale.

This chapter will provide a brief description about polymeric membranes focusing on one of the major remaining issues, that is, their contamination by microorganisms and, in particular, by bacteria. Upon a concise analysis of the problem, the alternative approaches developed to produce antifouling/antibacterial membranes will be thoroughly analyzed. For detailed reviews on membrane fabrication and their applications, the reader is referred to the following publications [2, 9–13].

9.2 Contamination of Polymeric Membranes

As mentioned above, polymers are among the most favorable membrane materials mainly because their unique film forming ability, their mechanical strength, chemical and thermal stability, as well as both corrosion and oxidation resistance. In spite of this, a critical aspect in the design of polymer membranes is related to their inherently hydrophobic character. Hydrophobic materials exhibit important drawbacks associated to their increase in resistance to water permeation and, therefore, the energy consumption. In addition, colloids, organics as well as microorganisms present in the solution tend to be absorbed onto the membrane surfaces and, in the case of porous membranes, into pore walls, leading to membrane fouling. In summary, several kinds of fouling may occur in membrane systems, such as organic fouling, particulate and colloidal fouling, crystalline fouling, and also microbial fouling [12]. The fouling materials introduced an additional barrier that may even block the membrane pores limiting or completely preventing the solvent to be transported

through the membrane. In this situation, the transmembrane pressure increasing while the permeate productivity is reduced. Fouling is, thus, an undesirable process that finally produces a degradation of the membranes or at least a significant reduction of the membrane performances [14, 15]. This is particularly common in water and wastewater treatment applications [16].

Membrane technologies are paying special attention to this phenomenon attempting to design and fabricate membranes able to remove contaminants without production of any harmful by-products, especially in water and wastewater treatment processes. Nevertheless, even after decades of development, fouling still remains one of the major limitations of polymeric membranes that decline the flux to a large extent, particularly, in industrial wastewater treatment processes [17]. It is worth mentioning that severe membrane fouling may either require extensive chemical cleaning processes or, in the worst case, membrane replacement increasing the operation costs.

As will be depicted, the main approach for minimizing polymeric membrane fouling requires the prevention of the undesired both adsorption and adhesion processes. This will, completely prevent or, at least to some extent, difficult the accumulation of colloids, particles, or microorganism at the membrane surface.

9.2.1 Membrane Biofouling

While fouling is a general problem when using membranes, membrane biofouling which referees to dynamic processes of microbial adhesion and colonization as well as growth at the membrane surface [18, 19] is present in almost all aqueous media [20]. Biofouling remains, for instance, the most technical challenges in the desalination industry, since microbial adhesion decreases the permeate flux, shortens the lifetime of the membrane and, as a consequence, increases the operational costs [14, 15]. Similar to other polymeric surfaces (see Chap. 5), when the microorganism is established at the surface, they start to produce extracellular polymeric secretions (EPS). EPS comprise many different biomolecules including proteins, glycoproteins, lipoproteins, and polysaccharides among others [13] and is at the origin of the biofilm formation [21–23]. A crucial step is, therefore, the initial adhesion. Many different research groups have focused their efforts in the prevention and/or reduction of undesired interactions between foulants and the membrane surface.

In order to avoid fouling, different strategies have been proposed that, otherwise, usually resorts to the chemical modification of the membrane to either render the surface hydrophilic or to incorporate functional groups with either/both antifouling and antimicrobial properties [24]. Equally, reduce membrane surface roughness or the modification of the membrane surface charge with molecules that have the same electrical charge as the foulants have also been explored [19]. Hence, the following paragraphs of this chapter will describe recent advances in the devel-

opment of either antifouling or antimicrobial membranes through surface modification.

9.3 Strategies for the Modification of Polymeric Membranes

Various technical solutions have been proposed in order to overcome biofouling. These include chemical and physical membrane cleaning, pretreatment process installation, or ultrasonic entrenchment. Also physical cleaning techniques have been employed to limit biofouling. For instance, relaxation and backwashing (when permeate is used to flush the membrane backwards) is nowadays standard strategies incorporation in the operating process [25]. Nevertheless, the surface modification of membranes is nowadays one of the most important research areas since (as will be depicted) many different studies demonstrated that biofouling can be significantly reduced by fabricating functionalized (mainly hydrophilic) membranes [26]. In this context, the most extended procedures to functionalize membrane surfaces are:

(a) Membranes produced from polymer blends
Blending different polymeric materials or polymer with inorganic compounds is a convenient way to avoid complicated synthetic steps to prepare membrane materials with precisely defined hydrophilicity. This strategy was employed by Wang et al. [27] to prepare ultrafiltration membranes with enhanced protein-adsorption-resistant ability. They employed as the first component branched amphiphilic copolymers P123-*b*-PEGs, prepared by reacting Pluronic P123 with PEG400 using PCl_3 as a conjugation reagent. The second component of the blend is polyethersulfone (PES). The authors evidenced an enrichment of PEG segments at blend membrane surface directly related to the PEG arm number in the P123-*b*-PEG copolymers. Moreover, they observed that the protein adsorption amount was significantly decreased.

PEG was also employed by Mural et al. [28] as one of the components in combination with different polymers such as amine-terminated grapheme oxide ($GO–NH_2$), in situ formed polyethylene-grafted GO (PE-g-GO) and their combinations with maleated PE (maleic anhydride-grafted PE) to produce antifouling membranes. Upon finding the best blends with improved mechanical properties having a uniform dispersion of PEG, selected membranes were also tested for their antibacterial properties. In particular, they inoculated *E. coli* culture with the membranes and imaging at different time scales. They concluded that the developed polymeric membranes do not support live bacteria or bacterial growth and can act as an antibacterial membrane.

Wu et al. [29] employed inherently antimicrobial natural polymers as one of the components to fabricate the membrane. They blended chitosan known for their antimicrobial properties and cellulose by casting films from trifluoroacetic acid. Two interesting properties were found in these membranes. On the one hand, they present low water vapor transpiration rate, which prevented excessive

dehydration of the wound. On the other hand, chitosan/cellulose blend membrane was effective against *E. coli* and *S. aureus* [30]. Later, they developed membranes based also on chitosan in which another antimicrobial component $Ca_3V_{10}O_{28}$ was added in order to provide a synergistic effect. These membranes were prepared by self-assembly of $V_{10}O_{28}^{6-}$ and chitosan using the Ca^{2+} ion linkers. The complex membranes exhibited larger antimicrobial activity in comparison to the individual components against *S. aureus* and *E. coli* [31].

(b) Application of surface coatings and surface functionalization of membranes
In order to decrease the high susceptibility to fouling in commercial polyvinylidene fluoride (PVDF) ultrafiltration membranes (UF), Asatekin et al. [32] fabricated membranes coated with the amphiphilic graft copolymer poly(vinylidene fluoride)-graft-poly(oxyethylene) methacrylate, PVDF-*g*-POEM to create thin-film composite (TFC) ultrafiltration membranes. Reversible fouling occurring typically during the first hours was observed in these membranes during the first 10 days. Thus, the fouling performances of the membrane and, in addition, the effluent water quality were significantly improved in comparison to the base PVDF membrane. By using the atomic force microscope (AFM) colloid probe technique, the authors evidenced the presence repulsive steric interactions, which is, most probably the cause of the low adhesion of foulants to the membrane.

Poly(ethyelene glycol) (PEG) is by large the most extensively employed for the preparation of antifouling coatings on membranes. Another example of the use of this polymer has been reported by Ju et al. [33] that prepared cross-linked poly(ethylene glycol) diacrylate materials via free-radical photopolymerization of poly(ethylene glycol) diacrylate (PEGDA) solutions in water. These materials were employed as fouling-resistant coating in UF membranes. By varying the chain length of the PEGDA as well as the amount of water introduced in the initial feed, the authors varied the permeability of the membranes between 0.5 and 150 L μm/(m^2 h bar). In addition to the permeability, the fouling resistance of the membranes was characterized via static protein adhesion experiments. The authors evidenced that the membrane surfaces are more hydrophilic in samples prepared with a larger amount of water in the initial feed and with longer PEGDA chains and, therefore, exhibit less BSA accumulation.

Another interesting example of the fabrication of surface antifouling coatings has been reported by Sagle et al. [11, 34]. Similarly to the previous example, PEG was introduced for their antifouling properties but in this case forming part of hydrogel networks. They initially prepared three series of hydrogel using PEGDA as cross-linking agent and varied the monomer employed: acrylic acid (AA), 2-hydroxyethyl acrylate (HEA), or poly(ethylene glycol) acrylate (PEGA) as comonomers [11]. By modifying the cross-link density, both water uptake and water permeability for materials of constant chemical composition could be finely tuned. In addition, they identified that the incorporation of a comonomer reduced hydrogel cross-link density, and therefore increased the water sorption accordingly. These preliminary work demonstrated based on contact angle measurements that *n*-decane in water, oil exhibited a low affinity

for the surfaces of these polymers. In a subsequent study, the authors applied these hydrogels to commercial reverse osmosis (RO) membrane and provided a thorough study of the fouling properties of these membranes [34]. In particular, they have shown by Zeta potential measurements that the hydrogel coating slightly reduced the negative surface charge of the RO membrane.

Moreover by applying an oil/water emulsions model, they described that the surfactant charge played a major role in membrane fouling. More precisely, a strong decline in water flux was observed when using a cationic surfactant (dodecyltrimethyl ammonium bromide (DTAB)). On the contrary, little or no flux decline was measured in the case of an anionic surfactant (sodium dodecyl sulfate (SDS)). In spite of these differences, the coated membranes experienced low fouling in oil/water emulsions. For example, in the case of emulsions prepared from DTAB and *n*-decane, the water flux of the commercial RO membrane decreased down to 26 % of its initial value after 24 h. On the contrary, in the case of PEGDA-coated RO membrane the water flux remains in values of 73 % of its initial value.

(c) Incorporation of nanoparticles in membranes

The incorporation of nanoparticles in polymeric membranes has been the center of a large number of studies during the last decade in order to produce membranes with improved antifouling properties. Several strategies have been proposed to incorporate nanoparticles in polymeric membranes being the two most common [1]:

- *Direct casting* from solutions containing both polymers and nanoparticles in the solvent in a precise ratio [35–40]. Nevertheless, in some cases, the use of dispersants is a requirement in order to produce homogeneous particle distributions [41, 42]. This strategy has been employed by Yu et al. [36] to fabricate poly(vinylidene fluoride) composite membranes filled with different weight fractions of SiO_2 nanoparticles.
- An alternative to the direct blending in a solution methodology is the wet *phase inversion method*. The membranes are, in this case, fabricated by immersion of a glass plate into a coagulation bath of water at room temperature [43–46]. A large variety of nanoparticles have been employed to prepare hybrid membranes by this methodology including TiO_2 [47–49], SiO_x [50], CdS [51] ZrO_2, [52] or Fe_3O_4 [53]. The particles incorporated provide unique properties that, together with those of the polymeric material can produce membranes with tailor-made characteristics. For instance, it has been demonstrated that inorganic nanoparticles finely dispersed in a polymeric matrix significantly improved the membrane performance, among others, for ultra and nanofiltration [54–57] as well as for pervaporation and gas separation processes [10, 58].

An illustrative example of the superior performance of hybrid membranes has been reported by Bottino et al. [56]. This group reported the fabrication of organic–inorganic membranes composed of silica nanoparticles dispersed in poly(vinylidene fluoride). According to the authors, by increasing the amount

of silica nanoparticles the resulting membranes exhibit both higher permeate flux and lower retention. In addition, the addition of silica increases the viscosity of the casting solutions that simplifies the casting processes when using nonwoven supports.

Finally, it is worth mentioning that, in addition to inorganic charges, also polymeric nanoparticles have incorporated on membranes. For instance, Xu et al. [59] prepared a series of pyromellitic dianhydride (PMDA)/oxydianiline (ODA) polyimide (PI) membranes filling with polystyrene (PS) and poly(styrene-*co*-4-vinylpyridine) (PSVP)-nanoparticles.

(d) Functionalization by *grafting-from* and *grafting-onto* membrane surfaces

Polymerization from surfaces having immobilized initiators (*grafting-from* methodology) and the covalent attachment of preformed polymer chains onto surfaces with complementary functional groups (*grafting-onto* approach) have also been investigated to produce nonadherent membranes. The *grafting-from* approach was employed by Zhang et al. [60]. They prepared polyamide membrane surfaces grafted with a zwitterionic poly(sulfobetaine methacrylate) (pSBMA) via surface-initiated atom transfer radical polymerization. In comparison to the untreated membranes, these functionalized membranes displayed a remarkable increase in water flux (~65 %) while the amount of irreversible proteins adsorbed was considerably reduced by ~97 %. A similar strategy, i.e., surface initiated polymerization was also recently employed by Meng et al. [61] to fabricate responsive thin-film composite reverse osmosis (TFC RO) membrane. These easy-cleaning membranes were obtained by anchoring a zwitterionic poly (4-(2-sulfoethyl)-1-(4-vinylbenzyl) pyridinium betaine) (PSVBP) onto the surface of a polyamide membrane. The PSVBP was effectively grafted via redox-initiated graft polymerization. The polyamide-grafted-PSVBP (PA-*g*-PSVBP) demonstrated a significant increase in the salt rejection. However, a cross-flow protein fouling experiment for about more than 4 days evidenced that the PA-*g*-PSVBP membrane exhibit greater antifouling properties in the short term but lost the benefit for long-term operation.

The *grafting-onto* approach has been employed by Li et al. [62] to prepare zwitterionic-catechol conjugates by modifying a catechol molecule to introduce an initiator. Atom transfer radical polymerization (ATRP) of *N*-(methacryloxyethyl)-*N*,*N*-dimethylammonium betaine monomers (SBMA) was employed to produce catechol-containing zwitterionic polymers with narrow molecular weight distributions and precise molecular weights as shown in Fig. 9.1. Then, mild de-protecting conditions (using tetrabutylammonium fluoride) were employed to remove the catechol protecting groups before covalently attach the pSBMA-catechol onto the modified surface. In order to control the amount of polymer anchored different binding experiments were carried out on surfaces, including methyl (CH_3), hydroxyl (OH), and amino (NH_2)-terminated self-assembled monolayers (SAMs) as well as unmodified gold. The authors observed that by optimizing the experimental conditions, the coated surfaces are extremely resistant to nonspecific protein adsorption independently of the complexity and variety of proteins present in the solution. In addition, the

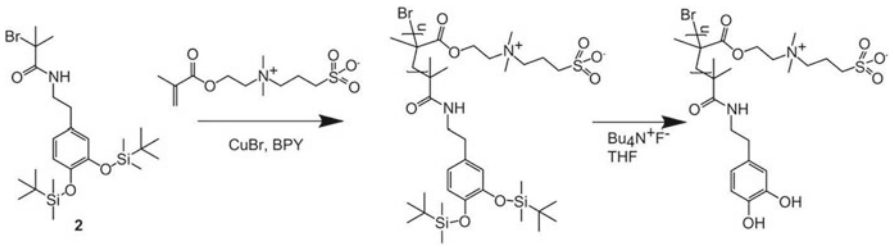

Fig. 9.1 Reaction steps for the grafting of pSBMA from the catechol initiator via ATRP and subsequent deprotection of hydroxyl groups before surface adhesion

authors explored the accumulation of *P. aeruginosa* during 3 days on the coated surfaces evaluating the amount of attached *P. aeruginosa* on the modified and non-modified surfaces. While, on the untreated glass surface, fast bacterial adhesion and subsequent biofilm formation of *P. aeruginosa* was observed, the adhesion of *P. aeruginosa* on the treated surface decreased by 99.6%.

9.4 Types of Antifouling/Antimicrobial Polymers Employed in the Fabrication of Membranes

9.4.1 Membrane Surface Modification with Anti-Adhesive Polymers

The most extended strategy to prepare antimicrobial/antifouling membranes involve the surface chemical modification introducing the appropriate functional groups [20]. Several functional groups can be attached to the surface to render the membrane surfaces anti-adhesive against bacteria.

(a) Incorporation of polyethylene glycol (PEG) at the membrane surface
Polyethylene glycol is a highly hydrophilic and neutrally charged polymer well known for its extremely low-fouling ability that, among others, prevents the nonspecific protein adsorption as well as significantly reduces cell adhesion [34, 63, 64]. In particular, this polymer forms hydrogen bonds in aqueous solutions that in addition to increase the surface hydrophilicity decreases the number of interactions with nonspecific proteins [63, 65]. The immobilization of PEG chains on surfaces, also known as PEGylation, has been explored to fabricate low fouling membranes by different research groups employing a variety of alternatives. In an exhaustive work, Gol et al. [65, 66] succeeded in the preparation of pegylated polyamides by in situ PEGylation of conventional poly(piperazineamide) thin-film composite nanofiltration (TFC NF) membranes. As depicted in Fig. 9.2, the authors explored three different alternatives to fabricate pegylated membranes

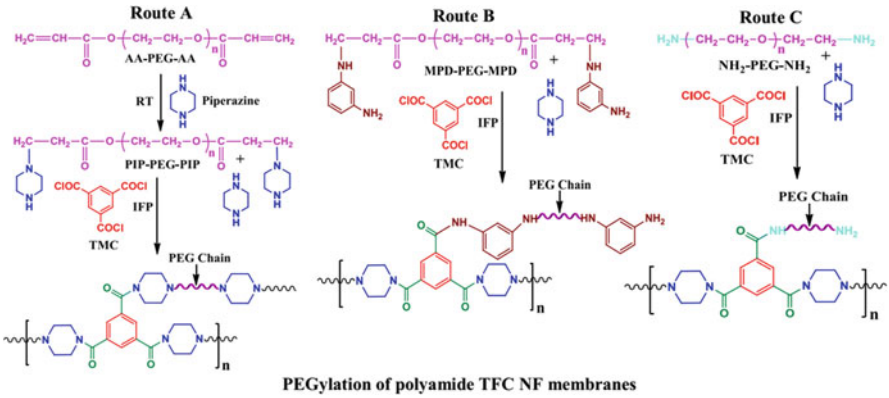

Fig. 9.2 PEGylation of TFC NF membrane via interfacial polymerization (IFP) between TMC and (**a**) PIP + PIP − PEG − PIP (in situ generated), (**b**) PIP + MPD − PEG − MPD, and (**c**) PIP + H_2N − PEG − NH_2 mixtures. Reproduced with permission from [65]

involving the interfacial polymerization between trimesoyl chloride (TMC) and (a) piperazine (PIP) + piperazine-terminated polyethylene glycol (PIP–PEG–PIP), (b) PIP + m-phenylenediamine-terminated PEG (MPD–PEG–MPD), and (c) PIP + alkyl amine-terminated-PEG (H_2N–PEG–NH_2) mixtures, respectively.

In comparison to the standard polyamide networks, these pegylated membranes significantly reduced the nonspecific protein adsorption probably due to the hydrophilization of the membrane network but also due, according to the authors, to two other important aspects. On the one hand, the authors reported a decrease of the surface roughness that limits the surface area and prevent the formation of any protein accumulation on eventually present micrometer size valleys. On the other hand, the steric hindrance of as a consequence of the incorporation of the PEG chains that is not present in the non-pegylated membranes [65, 66].

Microporous membranes prepared using the breath figures approach were reported by Martínez-Gómez et al. [67]. This approach permits the fabrication in one single step of hexagonally arranged porous surfaces with variable chemical composition by simply evaporating a polymer solution in a moist atmosphere. These authors prepared polyimide copolymers having pendant poly(ethylene oxide) (PEO) chains, that is, polyimide-g-PEO copolymers. The incorporation of PEO side chains enhanced the solubility of the polymers in chloroform (solvent employed for the breath figures approach due to the high volatility) and permits a particular orientation of these chains toward the inner part of the pores. As a result, PEO would work as antifouling compound to avoid the adhesion of microorganisms onto the porous films. The authors established that surface modified polyimide membranes exhibited a high resistance to biofouling against *S. aureus*. As depicted in Fig. 9.3, the antifouling performance is directly related to the amount of PEO chains within

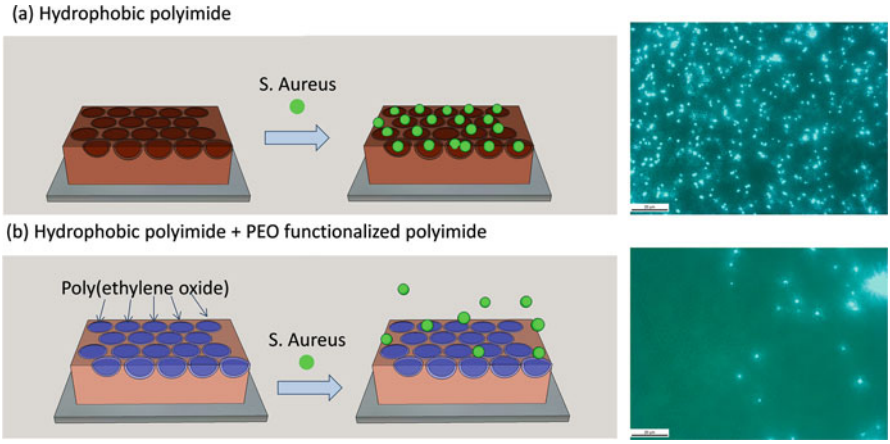

Fig. 9.3 Bacterial adhesion on honeycomb structured films. The presence of PEO groups reduced the amount of *S. aureus* that adhere to the porous films. Adapted from [67]

the pores. In particular, the authors evidenced an increase in the amount of PEO in the blend employed to prepare the porous films produced a reduction in the bacterial adhesion.

Nevertheless, serious limitations in the use of PEG are still trying to be resolved being the most relevance the effect of oxygen and transition metal ions on the PEG chains that oxidize the structure and finally degrade the polymer [64, 68, 69].

(b) Incorporation of natural hydrophilic polymers

Probably *sericin* is a natural, water-soluble protein bearing polar side groups: carboxyl, amino groups and hydroxyl [70, 71] extensively employed to functionalize polymeric membranes. For instance, *sericin* has been coated on the surface of commercial thin-film composite membrane for reverse osmosis (TFC-RO) membranes and covalently anchored by chemical cross-linking with glutaraldehyde (GA) [71]. The *sericin*-coated membrane presented reduced water permeability (as a result of the additional hydraulic resistance), but on the other hand improved salt rejection as a consequence of the enrichment of surface negative charge. More interestingly, the resistance of these membranes to BSA fouling was enhanced based on the combination of three important features: improved surface hydrophilicity, high surface negative charge, and smoothed surface morphology [71].

Also, Zhou et al. [70] employed *sericin* to, upon reaction with trimesoyl chloride (TMC) in an interfacial polymerization process, produce antifouling membranes. The fouling test confirmed that the sericin-TMC composite membrane has improved the fouling resistance to sodium alginate (SA) and BSA in comparison to homologous commercial membranes. In agreement with

other reports, the authors hypothesize that this phenomenon is basically due to the greater electrostatic repulsion between the *sericin*-TMC membrane (negatively charged) and the foulant molecules [70].

(c) Coating membranes with hyperbranched polymers
Polymers with a high density of hydrophilic end groups, hyperbranched polymers or dendritic, have also been employed to impart protein resistance to polymer membranes [72, 73]. For instance, Nikolaeva et al. [72] employed hydrophilic hyperbranched poly(amido amine) (PAMAM) to modify TFC membranes. PAMAM is a low cost material that can be produced in a simple one-pot polymerization step and can be easily purified. They fabricated RO membranes by interfacial polymerization (IP). More precisely, a thin polyamide separation layer was coated onto a porous poly(ether sulphone) support employing *m*-phenylenediamine (MPD) and trimesoyl chloride (TMC) as reactants for the IP. The acid chloride groups that remained non-converted during the interfacial polymerization are, in turn, employed to covalently anchor PAMAM to the PA layer forming amide bonds between TMC groups of the PA layer and amine groups of PAMAM dendrimer. The modification was achieved by spraying a solution of PAMAM onto the membrane surface either using methanol (PAMAM1) or water (PAMAM2). In contrast to the unmodified membranes, independently of the solvent employed both strategies led to membranes with a substantial increase in water flux. However, taking into account the required salt rejection and protein adsorption, PAMAM2 was preferred over the use of methanol (PAMAM1). This is mainly due to the creation of supplementary hydrophilic PAMAM layer, which behaves similar to a hydrogel layer when in contact with water (Fig. 9.4) [72].

(d) Surface membrane functionalization with zwitterionic polymers
Polymers bearing zwitterionic functional groups have gained special attention as a new group of fouling-resistant materials [60, 61, 74]. Zwitterionic functional groups incorporate both positive and negative charged units and are able to establish strong electrostatic interactions with water (even stronger than standard hydrophilic materials). Azari et al. [74], based on the unique adhesive proteins found in mussel, fabricated a zwitterionic amino acid, L-DOPA (3-(3,4-Dihydroxyphenyl)-L-alanine) that was effectively anchored on the membrane surface in order to resist protein fouling. Due to the functional groups contained in L-DOPA such as acid groups, carboxylate, hydroxyl, or amino [74] after L-DOPA immobilization a significant increase in membrane hydrophilicity was observed. The water flux increases accordingly to the surface hydrophilicity while the salt rejection remains unaffected. More interestingly, during filtration tests with BSA and alginic acid solution, the authors reported that in the unmodified membrane only 62 % of its initial flux was measured while the modified membrane retained about 82 % after 16 h [74].

Other zwitterionic groups employed as antifouling in membranes include poly(sulfobetaine methacrylate) (pSBMA) that was grafted onto the polyamide membrane surface via surface-initiated atom transfer radical polymerization [60, 75] or (4-(2-sulfoethyl)-1-(4-vinylbenzyl) pyridinium betaine) (PSVBP) anchored onto the polyamide surface [61].

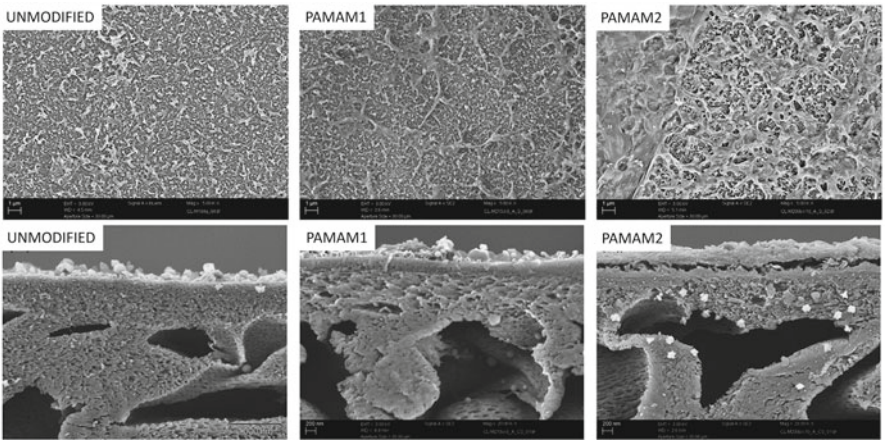

Fig. 9.4 SEM images of membrane surfaces (*above*) and cross-sectional profiles (*below*) of unmodified, TFC PAMAM1, and TFC PAMAM2

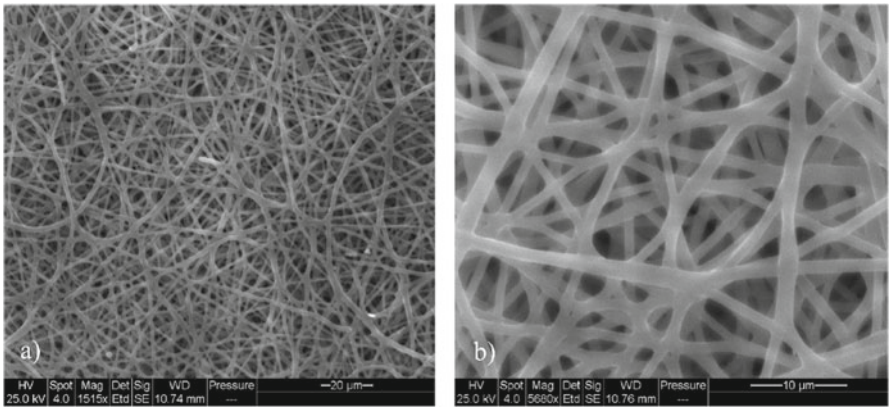

Fig. 9.5 Scanning electron micrographs of PSBMA electrospun membranes. Reproduced with permission from [75]

For instance, Lalani et al. [75] employed zwitterionic PSBMA known for its superhydrophilic and ultralow biofouling properties to fabricate water stable electrospun membranes (Fig. 9.5). They described a three-step involving a polymerization, an electrospinning step and finally a photo-cross-linking process. As a result, the electrospun membrane showed strong resistance to protein adsorption and cell attachment. Equally, bacterial adhesion studies using Gram-negative *P. aeruginosa* and Gram-positive *S. epidermidis* revealed that the PSBMA electrospun membrane was also highly resistant to bacterial adhesion. More interestingly, the authors fabricated Ag^{+}-impregnated electrospun PSBMA membranes in order to confer antimicrobial properties to the membrane.

These membranes exhibit antimicrobial activity against both *S. epidermidis* and *P. aeruginosa*. According to the authors, such electrospun PSBMA-based membranes are excellent candidates for novel nonadherent, superabsorbent, and antimicrobial wound dressing.

Using a similar strategy Liu et al. [76] reported the preparation of antimicrobial fibers. Their strategy involves three consecutive steps, i.e., pre-polymerization, electrospinning, and finally photo-cross-linking process that leads to water-stable cross-linked electrospun zwitterionic PSBMA fiber. The fibers were employed to construct a membrane that exhibited strong resistance to protein adsorption as well as cell attachment. Moreover, as depicted in Fig. 9.6, 3 h bacterial incubation results evidenced that the PSBMA electrospun membrane exhibited small bacterial adhesion for both *P. aeruginosa* and *S. epidermidis* in comparison with other electrospun fibers such as polycaprolactone (PCL) or using standard supports such as tissue culture polystyrene (TCPS) or glass. Equally, bacterial adhesion tests carried out during 24 h show that the PSBMA electrospun membranes still exhibited the lowest bacterial adhesion for both species. In addition to the antifouling properties observed in the PSBMA fibers, the authors explored the antimicrobial activity of the silver-incorporated electrospun PSBMA membrane. $AgNO_3$ was incorporated into the electrospun PSBMA membrane by means of ionic interactions and the antimicrobial activity of the Ag^+-impregnated membrane was determined using a zone-of-inhibition method. The authors found that the electrospun PSBMA membranes infused with silver nitrate inhibit the growth of both *P. aeruginosa* and *S. epidermidis*. The zone of inhibition was 6.3 mm for *P. aeruginosa* and 3.6 mm for *S. epidermidis* after 24 h of incubation.

These membranes are promising materials among others for wound dressing purposes since they can prevent attachment and entry of the environmental pathogens to the wound. In addition to the protection capabilities, the dressing applied to the wound would not require often replacement, thus decreasing the probability of further contamination by introducing bacteria upon exposure of the wound site to the environment.

9.4.2 Antimicrobial Biocides and Polymers Incorporated in Polymeric Membranes

In addition to the use of microbial repellent molecules several groups have focused in the incorporation of biocidal groups able to kill those bacteria upon contact with the membrane surface. Some of the most relevant antimicrobials employed to functionalize membrane surfaces are depicted below:

(a) Polydopamine
Polydopamine (PDA) has been straightforwardly employed for the preparation of antimicrobial and antifouling membranes by a simple dip-coating process.

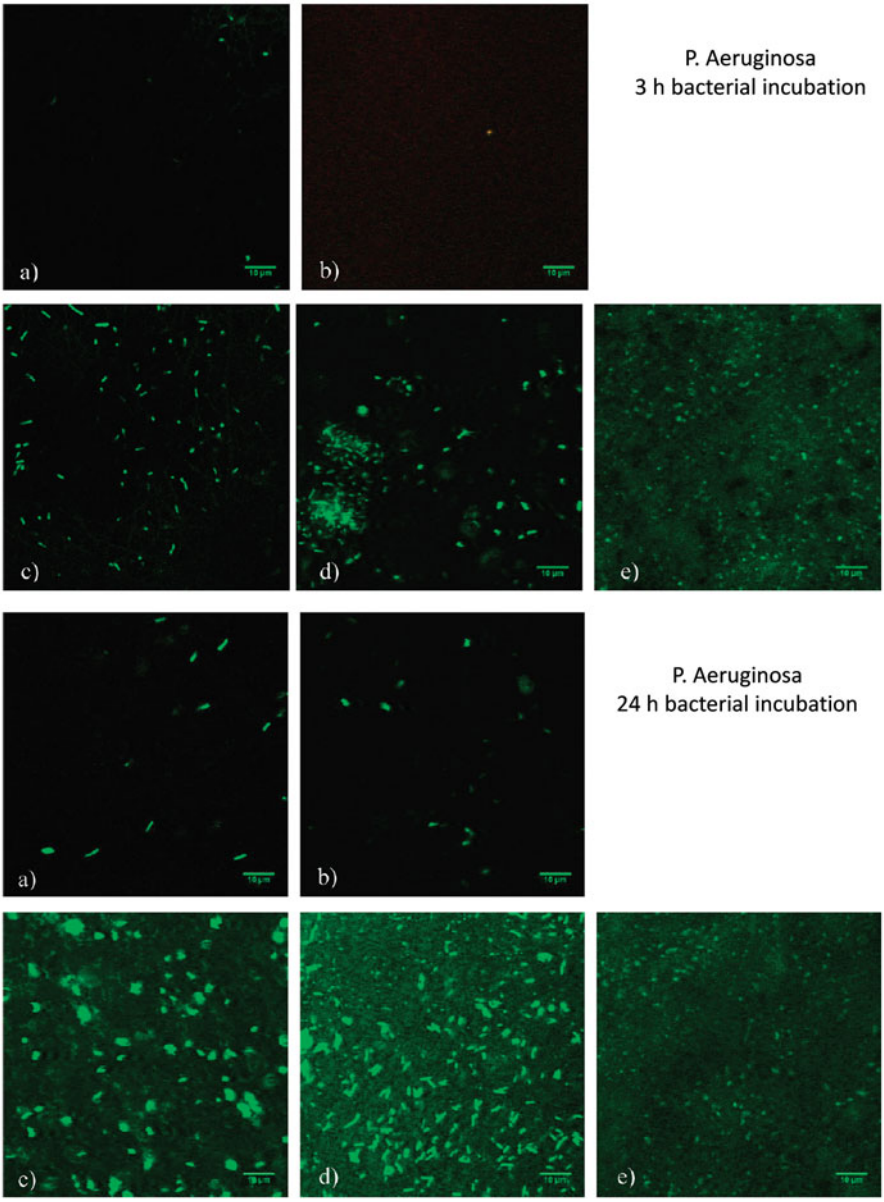

Fig. 9.6 Fluorescence microscopy images of *P. aeruginosa* immobilized onto electrospun PSBMA (**a**), PSBMA hydrogel (**b**), electrospun PCL (**c**), TCPS (**d**), and glass (**e**) at 3 and 24 h. Reproduced with permission from [76]

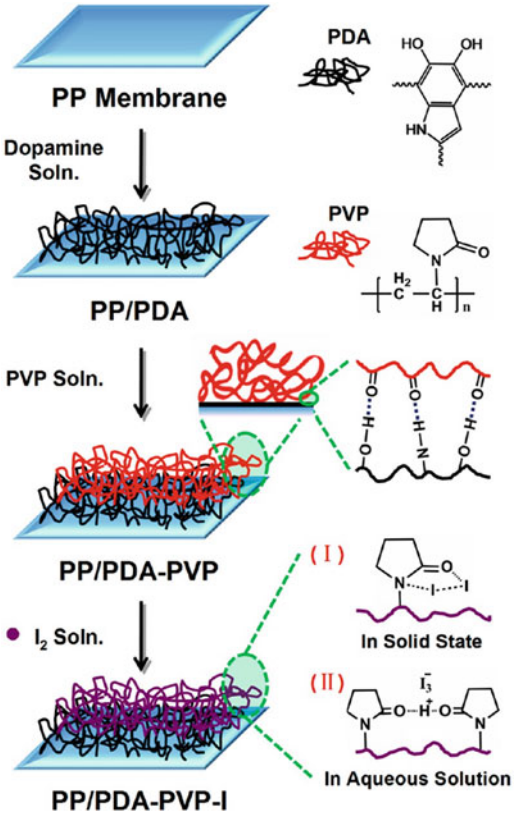

Fig. 9.7 Scheme of coating polydopamine (PDA) on a PP membrane as well as subsequent PVP and iodine complexation. Reproduced with permission from [24]

PDA forms strongly adherent PDA layer over an extensive variety of material surfaces by dipping the polymeric material on dopamine aqueous solution. Jiang et al. [24] employed this strategy (see Fig. 9.7) to coat hydrophobic polypropylene (PP) porous membrane with a PDA layer that served, in turn, to via hydrogen-bonding interactions between PVP and PDA anchor poly(*N*-vinyl pyrrolidone) (PVP). The PVP layer anchored on the membrane surface exhibit long-term stability because of the strong non-covalent forces between PVP and PDA coating. As a result, and based on the well-known anti-adherent properties of PVP, the permeation fluxes and antifouling properties of the membranes were improved as evaluated in protein filtration, adsorption tests, and oil/water emulsion filtration.

Additional antimicrobial activity was achieved by iodine complexation with the PVP layer. In order to evaluate the activity against bacteria the authors employed *S. aureus* and found that the sum for viable colonies considerably diminished after contacting with PP/PDA-PVP-I membrane for 24 h. Moreover, the relative viability of the *S. aureus* was lower than 0.1 % and the log decrease achieved more than 3 for the PP/PDA-PVP-I membrane (99.9 % of the *S. aureus* were killed).

(b) Membranes bearing antimicrobial polymers
Antimicrobial polymers immobilized on the surface of TFC membrane surface have been employed to prevent both biofilm growth and (bio)fouling. A large variety of antimicrobial polymers have been explored including polylactams, polymers containing *N*-halamines [77, 78] or tertiary and/or quaternary ammonium groups, and polyamino acids [79].

Membrane degradations by biofouling and free chlorine oxidation are the main problems for the extensive applications of aromatic polyamide RO membranes. *N*-halamine precursors were employed by Wang et al. [77, 80] to fabricate TFC membrane with enhanced chlorine resistance and anti-(bio)fouling property. For that purpose, the authors employed a commercial RO polyamide membrane and modified the surface by free-radical graft polymerization of 3-allyl-5,5-dimethylhydantoin (ADMH). The ADMH-functionalized materials can be chlorinated and lead to the well-known antimicrobial *N*-halamines [77, 78]. The antimicrobial tests exhibited that the chlorinated membranes possessed better antimicrobial efficiencies than the non-treated membranes, and the antimicrobial functions could be successfully regenerated by chlorination. According to the author's findings, upon chlorination, the decrease *E. coli* present in at the surface of the grafted membrane was above 90 % in comparison to the unmodified membrane [77].

Quaternary ammonium groups are also recognized by their unique antimicrobial properties. For instance, Ni et al. [81] prepared hydrophilic random copolymers based on poly(methylacryloxyethyldimethyl benzyl ammonium chloride-r-acrylamide-r-2-hydroxylethylmethacrylate) (P(MDBAC-*r*-Am-*r*-HEMA)) by simple free-radical copolymerization (Fig. 9.8). The terpolymer was later employed to coat a commercial RO membrane and anchored to the surface by glutaraldehyde (GA) cross-linking. The large hydrophilicity of the coated membranes considerably retains its flux under BSA filtration in comparison to that of pristine membranes. Interestingly, the coated membranes showed excellent antimicrobial activity to *E. coli* and inhibit bacterial growth [81].

(c) Covalent Binding of Single-Walled Carbon Nanotubes to Polymer Membranes
Single-walled carbon nanotubes (SWNTs) have been proposed to impart nanomaterial-specific properties to the surface of thin-film composite membranes. In particular, the immobilization of SWNTs at the membrane surfaces can provide additional biocidal properties. An illustrative example of the biocidal activity of SWNT on membranes has been reported by Tiraferri et al. [82]. Prior to the immobilization of the SWNTs, they first require the purification and modification (e.g., by ozonolysis) to provide the SWNT with sidewall functionalities. These functional groups will improve the cytotoxic properties and, simultaneously, improve the dispersion in aqueous solution. As depicted in Fig. 9.9, a three-step reaction protocol was established to create covalent amide bonds with the functionalized SWNTs. The first reaction step, involves the activation, with *N*-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride of the carboxylate groups of the membrane. In the second step, the carboxylic activated groups react with ethylenediamine to provide membranes surfaces reach

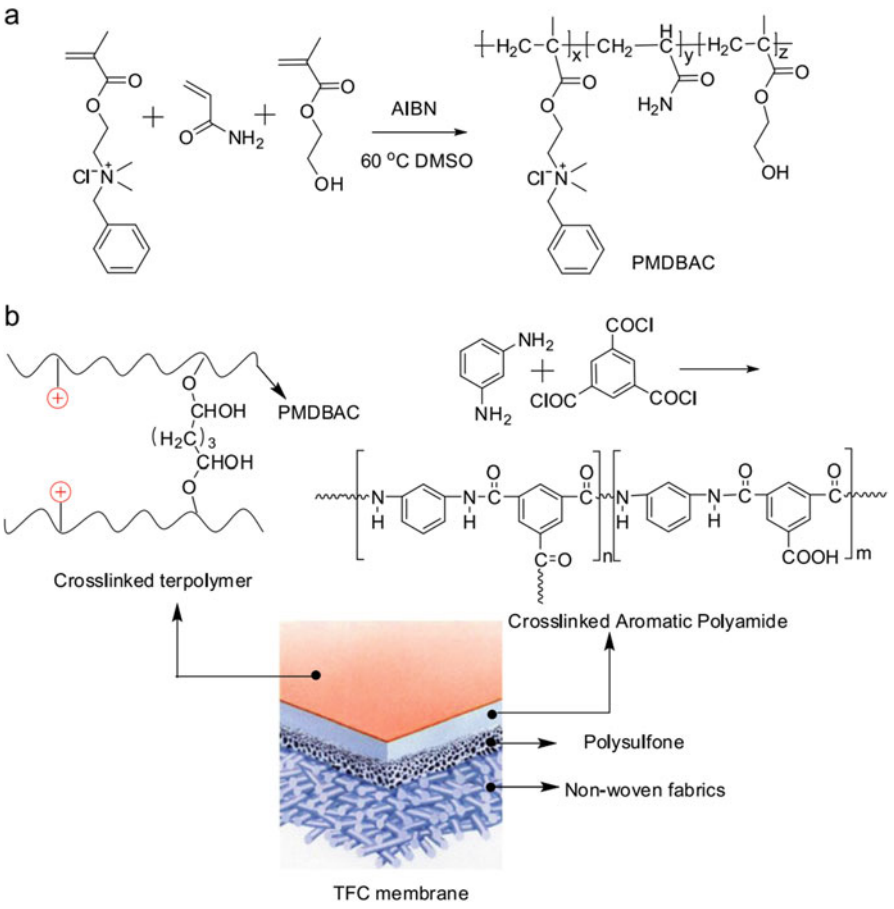

Fig. 9.8 Schematic diagram for (**a**) synthesis of the terpolymer P(MDBAC-r-Am-r-HEMA) and (**b**) surface modification of RO membranes. Reproduced with permission from [81]

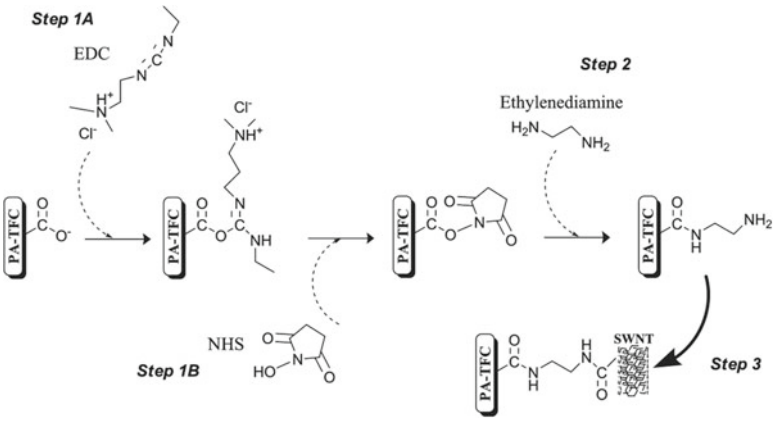

Fig. 9.9 Procedure to covalently bind single-wall carbon nanotubes (SWNTs) to the membrane surface

in amine groups. Finally, the amine groups were employed to form amide bonds with the carboxylic acid functionalized SWNTs. The stability of the covalently anchored SWNTs was confirmed by sonication of the membranes. The authors confirmed the antimicrobial activity of the membrane surfaces against *E. coli* cells evidencing an enhanced bacterial cytotoxicity for the SWNT-coated membranes. The SWNT membranes achieved up to 60 % inactivation of bacteria anchored to the membrane within 1 h of contact time.

(d) Polymeric membranes impregnated with antibacterial nanoparticles

As has been mentioned above, the incorporation of nanoparticles in polymeric membranes increases the several membrane properties such as selectivity, permeability, mechanical strength, and, in some cases also the hydrophilicity [1, 35, 83, 84]. Examples of this behavior include the case of poly(vinylidene fluoride) membranes combined with silica nanoparticles that exhibit higher selectivity, higher diffusivity and higher temperature [36], or polysulfone membranes incorporating silica nanoparticles that showed improved gas permeability [85].

Together with these mentioned advantages, it is worth mentioning that the integration of nanoparticles into polymeric membranes has some drawbacks. Probably, the most important restrictive factor is the distribution of the nanoparticles within the polymers. Particularly difficult to disperse are nanoparticles with less than 100 nm in diameter due to the extremely large amount of surface interactions. Moreover, the causes of the agglomeration inside polymeric membranes remain controversial. Authors such as Yu et al. [86] proposed that an increase of the nanoparticle concentration favors their agglomeration. On the other hand, Benjamin et al. [87] remarked that, in addition to the nanoparticle concentration, pH and ionic strength of the solution clearly direct the agglomeration between nanoparticles.

Within this context, provided an appropriate nanoparticle dispersion and the required membrane properties (mechanical, permeability, etc.) different groups have focus on the fabrication of membranes with antibacterial properties by using, among others Ag, TiO_2, CuO, or ZnO nanoparticles.

Zodrow et al. [88] prepared polysulfone membranes (PSf) impregnated with silver-based nanoparticles (nAg) fabricated using the wet phase-inversion process [9]. For that purpose, silver nanoparticles (1–70 nm) were dispersed polysulfone membrane in the casting solution prior to the dissolution of the polysulfone resin. Zodrow et al. [88] found that polysulfone membranes with 0.9 wt% nAg (nAg–PSf) exhibit similar permeability and surface charges to the pure polysulfone membranes and did not significantly vary the membrane structure. However, the incorporation of nAg (0.9 % by weight) considerably reduced the amount of E. coli grown on the membrane surface upon filtration (Fig. 9.10). In spite of the improved properties exhibited by the membranes, some aspects still require improvement. The most important aspect is related to the leaching of Ag^+ out of the membrane with a lost about 10 % of total silver (i.e., the silver leached from the membrane mainly in ionic form). It is worth mentioning that the Ag^+ loss was mainly occurs from the surface, precisely in those areas where membrane-bacteria and membrane-virus interactions occur [88].

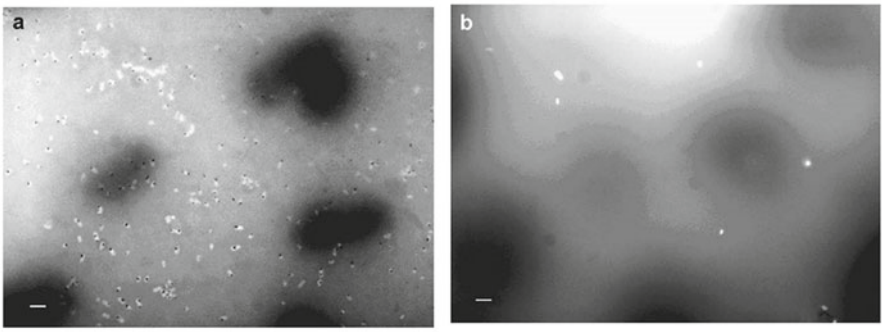

Fig. 9.10 Attachment of *E. coli* suspended in MD medium to membrane surface on (**a**) PSf and (**b**) nAg–PSf membranes. Cells were stained with DAPI and viewed with a fluorescence microscope. Scale bar indicates 5 μm. Reproduced with permission from [88]

This phenomenon has two major related drawbacks. On the one hand, leaching of silver from the membranes produced a significant decrease of the performances of the membranes as a function of time, and therefore they could be not appropriated to be used during long periods of time. On the other hand, leaching of silver nanoparticles might additionally pose the danger of water contamination if the membranes with silver nanoparticles are expected to be used in drinking water decontamination processes.

The synergistic effect of antimicrobial polymers and nanoparticles was explored by Li et al. [89] to produce chitosan/zinc oxide nanoparticles membrane displaying good mechanical properties and high antibacterial activities. The chitosan/ZnO nanoparticle (CS/nano-ZnO) composite membranes were fabricated by the sol-cast transformation method. The ZnO nanoparticles, homogeneously dispersed in the chitosan matrix, significantly improved the mechanical properties of CS/nano-ZnO composite membranes. Equally, the antibacterial activities of CS membranes against *B. subtilis*, *E. coli*, and *S. aureus* were largely enhanced by the incorporation of ZnO. In particular, composite membranes with as low as 6–10 wt% ZnO exhibited high antibacterial activities.

In addition, titanium dioxide (with similar band-gap and antibacterial activity than zinc oxide) alone or in combination with other nanoparticles has been equally employed for the fabrication of antimicrobial membranes. For example, Pant et al. [90] prepared silver-impregnated TiO_2/nylon-6 nanocomposite mats with exceptional characteristics as a filter media with simultaneously photocatalytic and antibacterial properties. For this purpose, silver nanoparticles (NPs) were incorporated in electrospun TiO_2/nylon-6 nanofibers by photocatalytic reduction of silver nitrate solution under UV-light irradiation. More importantly, the antibacterial activity of a TiO_2/nylon-6 composite mat bearing Ag NPs was evaluated against *E. coli*. In all cases, the authors evidenced that TiO_2/nylon-6 nanocomposite mats charged with Ag NPs exhibit a larger activity than those

mats without Ag NPs. Thus, the prepared material may find potential interest in the preparation of economically friendly photocatalyst and water filter media.

Finally, copper (II) oxide nanoparticles (CuO NPs) have also demonstrated notable antimicrobial properties. Yalcinkaya et al. [91] employed these Cu NPs to evaluate the antibacterial effectiveness of nanofiber composite yarns in order to potentially employ the composite nanomaterial in antibacterial filtration. The copper (II) oxide particles were immobilized at the polyurethane and polyvinyl butyral (PVB) nanofiber components of a composite yarn during the experimental tests. The antibacterial effectiveness was assessed against Gram-positive *S. gallinarum* bacteria as well as Gram-negative *E. coli.* The authors showed that the composite yarn with polyvinyl butyral nanofibers bearing CuO NPs exhibited better antibacterial efficiency compared to the yarn containing the polyurethane nanofibers. More precisely, with an amount of 5 % wt of CuO immobilized in PVB nanofibers displayed an antibacterial efficiency of 99.99 % at a production rate of 200 m/min.

9.5 Responsive Membranes

The possibility to control the membrane properties depending on the environmental conditions offers new potential alternatives to precisely control their behavior on demand.

An interesting example of responsive membranes was reported by Liu et al. [92] that employed biodegradable polymers, e.g., poly(lactic-*co*-glycolic acid) (PLGA) for the fabrication of bioresponsive membranes for wound-healing applications. Based on the PLGA/collagen wound dressing membranes that have been shown to accelerate wound healing, the authors studied the early stage open wound healing in rats. The results evidenced that electrospun PLGA/collagen membranes promoted early stage wound healing. The pictures of histological analysis showed that PLGA/collagen nanofiber revealed superior wound-healing influence in comparison to gauze and commercial dressing. After 1 week, there was no clear difference between histological sections of wounds treated by gauze, PLGA/collagen, and commercial dressing. All the tissues show inflammatory cell infiltration, granulation tissue formation, and ulcerated surface. However, after 3 weeks, the wound cured with PLGA/collagen nanofiber was almost healed, while the wounds treated with either gauze or commercial dressing, showed prominent inflammatory cell infiltration and incomplete re-epithelialization.

The salt-responsive property of polyelectrolyte membranes provides an interesting force to additionally force the release of protein foulants. Meng et al. [61] fabricated salt-responsive reverse osmosis (RO) membranes by tethering (by surface-initiated free-radical polymerization) a zwitterionic polymer poly (4-(2-sulfoethyl)-1-(4-vinylbenzyl) pyridinium betaine) (PSVBP) onto a commercially available RO membrane. Covalent grafting of PSVBP provides a negative charge to the membrane surface and, therefore, significantly improved membrane surface hydrophilicity and improved the rejection

from 98.0 to 99.7%. The functionalized membranes exhibit higher antifouling response in the short term (less than 100 h) but lost the advantage for long-term operation. However, the PA-g-PSVBP membrane can recover 90% of the initial flux by simply rinsing with a concentrated salt solution (brine). The salt-responsive property of the PSVBP membranes is assumed to be at the origin of the driving force for the release of protein foulants.

9.6 Conclusions

Microorganism biofouling and contamination, as well as biofilm formation, on polymeric membranes still currently a major issue limiting the use of these materials. In order to limit the adhesion of microorganism several strategies have been developed in which either antifouling or antimicrobial molecules have been incorporated within the membranes. Equally, the surface modification has been extensively explored. The incorporation of antifouling polymers such as polyethylene oxide, zwitterionic moieties, or even antimicrobial polymers such as polydopamine have significantly improved the efficiency of these membranes and enlarged their lifetime.

The incorporation of inorganic nanoparticles embedded in polymeric membranes is also a currently investigated alternative. Their incorporation has two interesting effects on the material. On the one hand, the improvement of the mechanical properties of the membrane and on the other hand the antimicrobial properties obtained when using, for instance, silver or TiO_2 nanoparticles. However, leaching still among the major problems to be faced in this case that both limits the antimicrobial activity of the membrane and could lead to the contamination of the membrane environment.

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Chapter 10
Environmental and Safety Issues

Abstract The use of antimicrobial molecules has, unfortunately, side effects that may limit their final use. Therefore, in addition to the antibacterial performance, the evaluation of environmental and safety issues is a requirement. According to the Directive 98/8/EC of the European Parliament relative to the use of biocidal products, it has been pointed out that several conventional biocides need to be replaced. Moreover, the use of antimicrobial substances, for instance, in food-related applications requires following the FDA requirements. In particular, the ISO 10993 is related to the biocompatibility and safety standards aiming to server as framework for selecting tests to evaluate biological responses. These include cytotoxicity, primary skin irritation, dermal sensitization, and systemic toxicity. In addition to the toxicity of the material, it is also crucial to determine if there exist leachable substances and eventual degradation products. In this context, antimicrobial polymers can provide alternative solutions to current microbial contamination and biofouling issues while respecting the environmental and health regulations.

This chapter will briefly describe the environmental problems that need to be considered when using polymers in particular in those cases, where the antimicrobial employed is leached from the polymeric material. The cytotoxicity associated to the nonselective performance of antimicrobials will be discussed as well. Finally, illustrative ongoing works for the fabrication of nontoxic antimicrobial polymeric materials will be analyzed.

Keywords Antimicrobial safety • Environmental issues • Biocide releasing • Nonleaching polymers • Cytotoxicity • Antimicrobial toxicity

10.1 Introduction

The use of antimicrobial molecules has, unfortunately, side effects that may limit their final use. Therefore, in addition to the antibacterial performance, the evaluation of environmental and safety issues is a requirement. According to the Directive 98/8/EC of the European Parliament [1] relative to the use of biocidal products, it has been pointed out that several conventional biocides need to be replaced. One of the principal concerns is related to the environmental contamination related to the

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use of biocides in particular for pest control and preservatives. For these uses, novel and more environmentally friendly alternatives need to be developed.

For the use of antimicrobial substances, for instance, in food-related applications requires following the FDA requirements. In particular, the ISO 10993 is related to the biocompatibility and safety standards aiming to server as framework for selecting tests to evaluate biological responses. These include cytotoxicity, primary skin irritation, dermal sensitization, and systemic toxicity. In addition to the toxicity of the material, it is also crucial to determine if there exist leachable substances and eventual degradation products.

In this context, antimicrobial polymers can provide alternative solutions to current microbial contamination and biofouling issues while respecting the environmental and health regulations.

This chapter will briefly describe the environmental problems that need to be considered when using polymers in particular in those cases, where the antimicrobial employed is leached from the polymeric material. The cytotoxicity associated to the nonselective performance of antimicrobials will be discussed as well. Finally, illustrative ongoing works for the fabrication of nontoxic antimicrobial polymeric materials will be analyzed.

10.2 Using Small Biocides Released from the Polymer

In order to prevent microorganism growth and proliferation, the most extended approach involves the use of low-molecular weight biocides. In general, the strategy involves the construction of polymers that gradually release small amounts of the biocidal active molecules/ions. The encapsulated biocide is able to migrate to the surface and is delivered to the environment, where the microbes need to be killed. Provided the optimization of the release kinetics, these antimicrobial polymers are able to deliver the biocidal active molecule continuously at low concentrations which is a prerequisite from a toxicological point of view. Nevertheless, even at low concentrations, there still remains a drawback since toxic biocides are delivered into the environment. Moreover, these compounds can be particularly toxic and/or irritant when they contain either heavy metals or halogens in their structure and are still a menace especially for sensitized persons and children. As a result, in general, the use of conventional antimicrobial agents is connected to the problems of remaining toxicity of these agents that can finally cause additional severe problems to the environment [2]. An illustrative example of this problem is the case of the use of triorganotin-based formulations (e.g., tributyl tin methacrylates) extensively employed in the fabrication of antifouling paints [3, 4]. Tributyl tin (TBT) successfully inhibits the growth of water organisms on the ship hull by gradually leaching into the seawater. While showing an excellent activity, the TBT leachates produce important toxic effects in sea dwellers. As a consequence, the use of TBT has been totally banned in the fabrication of antifouling paints from January 2008, and later efforts have been focused attempting to bind the active organic biocides to a polymer.

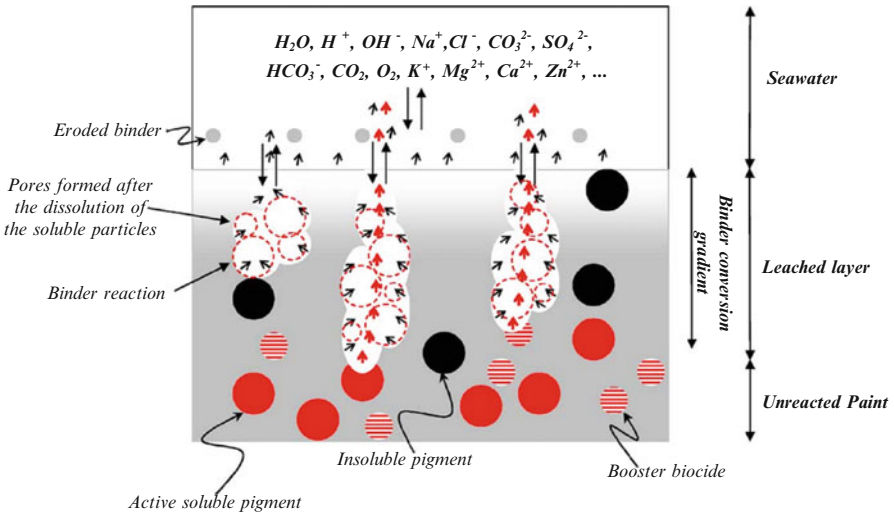

Fig. 10.1 Schematic illustration of the behavior of a biocide-based antifouling system exposed to sea water. Reproduced with permission from [7]

Food packaging is another application area that has also limited the use of small biocides, and many different groups are currently investigating other alternatives. In particular, in this case, these agents may diffuse into the food, can be ingested, and thus cause problems of different nature [5, 6].

Finally, for water treatment purpose, the most extended strategy to disinfect and sterilize water resorts to chlorine and other related chemicals. In order to release the biocide, water penetrates into the paint or coating, dissolve such biocides, and diffuse out into the bulk phase again (see Fig. 10.1) [7]. As a result, residues of these chemicals can be concentrated both in the environment and in the food chain. It could also be possible that halomethane analogues, suspected of being carcinogenic, can be formed. Therefore, these biocides should be equally avoided for this application [5, 8]. In particular, for aquaculture applications [9], some investigations have assessed the toxicity of biocides on nontarget species and concluded that most of them are growth inhibitors for freshwater and marine autotrophs [10], affecting key species, including corals [11] and sea grasses [12]. These studies revealed a clear impact of these compounds on the aquatic ecosystems [13].

The widespread use of TBT-based chemicals in public health applications and agricultural and industrial purposes introduced a dilemma. Initial efforts focused in a better understanding of how to control and utilize the unique properties of organotin compounds [3]. However, triorganotin-based formulations have been gradually replaced by other alternative tin-free biocides including copper and organic compounds have been developed [4]. Copper is typically employed in the form of copper oxide (Cu_2O) [7] either alone or in association with, for instance, inorganic zinc which in combination with copper enhances the overall toxicity of the formulation

and improve the leaching process [14]. In addition to inorganic molecules, other organic biocides, such as dichlofluanid, Sea Nine 211®, chlorothalonil, Irgarol 1051®, or Zineb have also explored, in particular to enhance the antifouling properties of paints [15].

Equally, within this context, one of the protective strategies to decrease the risk of catheter-related blood stream infections (CRBSI) involves the modification of the catheter surface since the biomaterial/environment surface are perfect areas for microbial colonization that finally may lead to bloodstream infections [16]. In order to reduce CRBSI, anti-infective agents have been incorporated into the catheter polymer or simply coated on the polymer. The principal biocides employed include heparin, chlorhexidine/sulphadiazine, silver ions, or antibiotic substances [17, 18]. Biocides such as chlorhexidine and other antibiotics usually leach from the catheter. However, leached chlorhexidine and sulfadiazine silver can sensitize patients, producing life-threatening anaphylaxis on subsequent contact [19–22].

In addition to patient-related problems, antibiotic resistance can also occur after continual contact to, for instance, minocycline and/or rifampicin-impregnated catheters. This occurs when bacteria have been exposed to subinhibitory concentration of antibiotics that were unsuccessful to remove these microorganisms. Raad et al. [22], Tambe et al. [23], and Sampath et al. [24] are few of the authors that observed in vitro resistance upon frequent use of catheters to leachable rifampicin or rifampicin combined with minocycline.

10.3 Alternatives to Small Biocides: Nonleaching Polymer Materials

As mentioned above, early generations of antimicrobial polymers were based on antimicrobial systems releasing antimicrobials from the device into the surrounding tissue to prevent bacterial colonization and growth on the device [16]. However, in spite of their good antimicrobial activity, as depicted in Fig. 10.2, the negative side effects including resistance to bacteria, possible sensitization and environmental issues motivated new investigations to produce nonleaching antimicrobials.

Nonleaching systems were proposed to help to reduce the above-mentioned risks. The potential benefits of the substituting toxic biocides for antimicrobial polymers include no leaching out of toxic or irritating ingredients, no migration, and wide-range efficacy against algae, bacteria, and fungi. Simultaneously, antimicrobial polymers can exhibit very low toxicity toward humans. Finally, by blending these polymers with standard polymers, it is also possible to fabricate an extensive variety of polymeric materials with antimicrobial surfaces, while maintaining the mechanical properties.

Antimicrobial polymers that do not release low-molecular weight biocides were first fabricated by covalently binding the active organic biocide to a polymer. In an interesting work, Bruenke et al. [16] reported a direct comparison between the

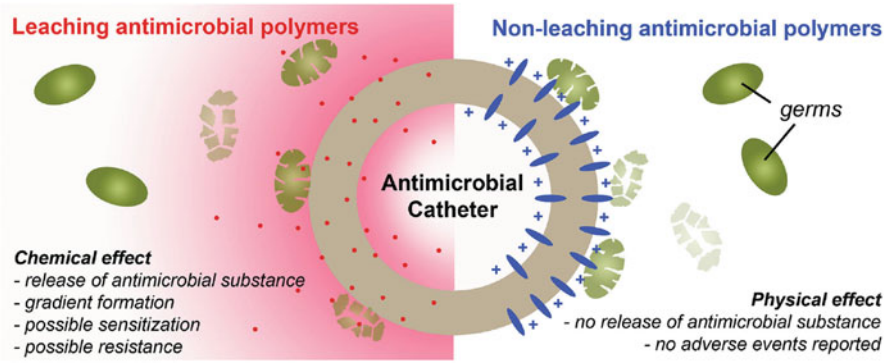

Fig. 10.2 Illustrative representation of the action mechanism of leaching versus nonleaching antimicrobial polymers. Leaching antimicrobial polymers (*red dots*) are released from the polymer to the environment to facilitate the antimicrobial effect by a chemical interaction with the germs (*green*). However, concentration gradient (*pink gradient*) is formed inducing the development of resistant pathogens in sublethal concentrations of the additive. Moreover, some additives can produce also sensitization reactions. In the case of nonleaching antimicrobial polymers, the antimicrobial agent (*blue rods*) is immobilized at the polymer surface (usually positively charged) that mediate the antimicrobial effect by a physical effect. For this, the germs need direct contact with the materials surface. So far, no adverse events are reported. Reproduced with permission from [16]

antimicrobial activity of leaching and nonleaching antimicrobial materials focusing on central venous catheters (CVCs). In particular, catheter-associated contaminations develop fast into general bacterial infections in day-to-day clinical environments. As depicted in Fig. 10.3, the antimicrobial efficacy of nonleaching CVCs is similar to conventional leaching CVC systems. The antibacterial evaluation was carried out using different germs usually associated with CVC-related infections. In Fig. 10.3 are included the results found for the case of the most relevant bacteria *S. epidermidis* and multiresistant *S. aureus* (MRSA). These interesting data revealed that there are no differences in the use of leaching and nonleaching strategies and that the effectiveness is related to the biocide employed. Thus, while the CVCs treated with ionized silver partly failed, the rest of the biocides employed produced a germ reduction of $\geq$99.9 %. In summary, nonleaching antimicrobial polymer maintain the activity of the leaching homologues and can thus help to reduce both loss of antimicrobial activity and health-associated risks due to biocide leaching.

As a result of the aspects commented above, we can summarize the following advantages and disadvantages of using polymeric leaching and nonleaching materials.

(a) First of all, it is worth mentioning that antimicrobial polymers display, in general, a broad spectrum of activity while maintaining a low toxicity to mammals. More importantly, the mechanism of action related to the interaction with the bacterial membrane and therefore nonspecific is expected to prevent the development of resistant microorganisms.

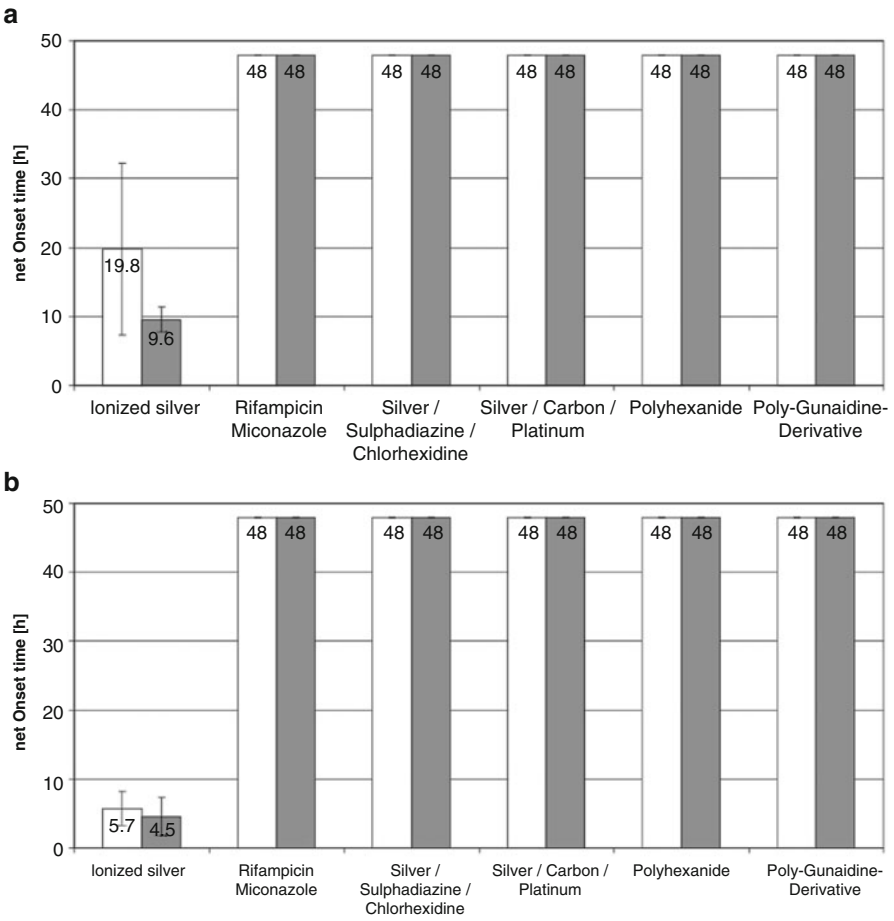

Fig. 10.3 Comparison of the antimicrobial efficacy of leaching and nonleaching before (left column) and after (right column) plasma preincubation. By using the Certika test, the antimicrobial efficacy was evaluated for (**a**) *S. epidermidis*, and (**b**) multiresistant *S. aureus* MRSA. The plasma preincubation did not play a significant role on the final antimicrobial activity. While the CVCs treated with ionized silver comparatively failed to mediate antimicrobial activity, the rest of the systems explored produced a germ reduction of ≥99.9 %. Reproduced with permission from [16]

(b) In addition to the environmental benefits of no leaching antimicrobial polymers, maintaining the active molecules within the material structure has also economic advantages. In effect, the active element is not consumed or released to the environment. Therefore, nonleaching polymers represent a sustainable strategy.

(c) On the contrary, one of the disadvantages of using exclusively surface-active biocides concerns the contact-limited action of these systems. Non-migrating antimicrobials will not diffuse into the microbes and eventual biofilm formation on top of the active surface will significantly reduce the efficacy, thus restricting the possible application.

(d) Another current limitation that still needs to be overcome in the use of polymer biocides is the durability in comparison with commercial formulations in which copper has been incorporated as antifouling agent. The commercial antifouling containing copper oxide materials typically remain clean from microorganism for several months. On the contrary, the antifouling test carried out using antimicrobial polymer shows very little fouling after 1 month but, in general, a bit later fouling started quickly.
(e) Finally, the third important limitation is related to the incorporation of polymeric active substances into coatings and plastics. In general, polymeric active substances are more difficult to incorporate than low-molecular weight biocides. This is mainly due to the limited solubility of polymers into each other. As a result, usually time-consuming optimization procedures can be required.

10.4 Safety Concerns Related to the Use of Different Antimicrobial Polymers: Cytotoxicity Against Mammalian Cells

Covalent incorporation of biocide functional groups within a polymer structure significantly increased the antibacterial efficacy. In effect, the constituent monomers isolated have in comparison with the final polymer a negligible biocidal activity [2]. In addition, as has been analyzed in Chap. 3, the macromolecular characteristics including density of biocidal groups, the molecular weight or polydispersity are crucial parameters that largely influenced the final activity. Moreover, polymeric antimicrobial agents display also additional advantages such as their low volatility, their chemical stability, and also their low permeability through the skin in humans as well as in animals. Finally, it is worth mentioning that polymers minimize the environmental problems related to the eventual residual toxicity of the antimicrobial agents and enlarge their lifetime. As a consequence, antimicrobial polymers are receiving increasing interest at the academic level as well as from the industrial sector [5, 25–29].

While it is true that functional polymers bearing biocides are expected to significantly reduce the environmental and health-associated issues, the eventual cytotoxicity can be crucial on the final use of a particular antimicrobial polymer. As a result, there is an increasing interest in the design and fabrication of selective antimicrobial polymers [2] whose potency against bacteria and non-toxicity toward mammalian cells can provide significant advantages over most polymeric biocides that are broadly poisonous [30–36].

Cytotoxicity refers to the capability of a particular antimicrobial to produce a toxic effect on cells, and in particular on human cells [37]. It is widely accepted that none of the existing drugs are completely free from toxicity and a usual reason for withdrawal of approved drugs is related to their adverse drug reactions [38, 39]. In this context, there is an optimum balance between the requirement for treatment and

the toxicity produced at therapeutic levels. Among the existing classes of drugs, antimicrobials present particular issues related to cytotoxicity since their final role is to provoke microbial cell death [40]. For instance, in the antimicrobial therapy the antimicrobial concentration needs to be precisely optimized. It is well known that antimicrobial peptides can provide benefit at lower antimicrobially active concentrations in the prevention of infected wounds, but may exhibit cytotoxicity at larger concentrations that finally affect wound healing unfavorably [41]. Similarly, the use of antiseptic agents pose problems for therapeutic usage since they exert a detergent-like effect, that far from being selective compromises both microbial and mammalian cell membranes simultaneously [42]. The cytotoxic effects are multiple and can vary from small irritations at the site of exposure to serious vascular injuries [40, 43].

10.4.1 General Mechanisms of Antimicrobial Toxicity

As depicted by Mandell [40], five main mechanisms of antimicrobial toxicity can be distinguished, i.e., unexpected interactions between drugs, direct effects of the drugs on tissues and organs, drugs producing hypersensitivity, changes in microbial flora produced by antimicrobials, and release of toxic products after microbial lysis. These mechanisms applied to antibiotics and drugs can be extended to the use of antimicrobial agents. A brief description of each mechanism is provided below.

10.4.1.1 Unexpected Interactions Between Drugs

The simultaneous consumption of more than one drug can produce unexpected adverse reactions. Two principal effects have been reported. On the one hand, one drug may reduce the effect of the other, for instance, by interfering with its absorption. On the other hand, in some cases, drugs can show synergistic toxicity, producing negative events that would not be produced using the drugs separately. For instance, in the case of consumption of tetracyclines or fluoroquinolones and antacids, the chelation with cations can significantly reduce the absorption of the antimicrobial drug. Another example of toxicity includes the nephrotoxicity of cephaloridine when this antibiotic is used together with furosemide or hypoglycemia produced by combination of chloramphenicol with tolbutamide [40, 44].

10.4.1.2 Direct Effects of the Drugs on Tissues and Organs

The use of antimicrobial agents can produce direct adverse effects on both tissues and organs. For instance, chloramphenicol has been associated to anemia processes. Similarly, amphotericin B is related to hypokalemia and aminoglycosides with eighth-nerve toxicity. While the precise mechanism still not completely

understood, in general, this adverse effect is related to the direct interaction between the drug or its metabolites and a particular tissue or organ in the body. An example of this is the myelosuppressive effects observed when using chloramphenicol. These effects are directly related to the inhibition of mitochondrial protein synthesis. Equally, irreversible aplastic anemia is believed to be associated to changes in stem cell genes [45, 46]. In other cases, the hypokalemia detected in some patients using amphotericin B is explained as the consequence of a decrease in renal blood flow [47]. Finally, aminoglycoside can damage either the inner hair cells of the organ of Corti or the sensory cells of the vestibular system. This produce in patients treated with aminoglycosides eighth-nerve damage, resulting in either deafness or vertigo [48].

10.4.1.3 Drugs Producing Hypersensitivity

Usual reactions to an antimicrobial substance produce gastrointestinal (GI) effects with either upset or diarrhea. However, these do not represent hypersensitivity reactions. The most important hypersensitivity is the type I since this type of hypersensitivity may proceed to anaphylaxis. In addition to type I, there are other adverse reactions associated to a hypersensitivity mechanism including Stevens–Johnson syndrome, serum sickness, Coombs' positive hemolytic anemia, and erythema nodosum [40].

10.4.1.4 Changes in Microbial Flora Produced by Antimicrobials

Studies on both human and animal have evidenced that during an antimicrobial therapy, in particular using broad-spectrum agents, can significantly reduce the host flora increasing the risk of colonization and possible infection by another pathogen. Illustrative examples of these changes include the vaginal Candida infection in women who have just finished an antimicrobial therapy or even the growth of fungal superinfections after finishing an antimicrobial therapy for a known bacterial infection.

10.4.1.5 Release of Toxic Products after Microbial Lysis

Another possible toxicity associated to antimicrobial therapy is related to the sporadic deterioration of a patient's clinical condition due to the release of toxic products upon microbial lysis. To this mechanism, two illustrative reactions are the Jarisch–Herxheimer reaction (observed in patients with syphilis of the brain treated with iv penicillin [40]) and the erythema nodosum leprosum (inflamed nodules that erupt over the skin that associated with fever). For instance, the latter is observed in around 50 % of the cases in which the patient has been treated with dapsone [49].

10.4.2 Cytotoxicity of Antimicrobial Polymers

One of the main factors that direct the cytotoxicity of an antimicrobial polymer is related to the type of functional group incorporated within the chain. For instance, as reported by Alamri et al. the cytotoxicity of antimicrobial polymers bearing amino groups against mammals is low [2]. More precisely, the polymers reported by these groups presented an acute oral and dermal toxicity in rats (LD50 value) above 2000 mg/kg. Moreover, the polymer is not irritating to the skin and only causes limited eye irritation. These groups are not sensitizing and did not show any effect in the in vitro gene mutation test, the in vitro chromosome aberration test, or the Ames test.

Antimicrobial polymers are designed to display an antimicrobial effect by interaction with negatively charged bacterial membranes that causes selective permeabilization [50]. However, this and other similar mechanisms can also be followed by polymers to interact with mammalian cells leading to cytotoxicity issues.

One of the most extended mechanisms occurs when the antimicrobial is used at large concentrations. In this case, the antimicrobial affect the membrane integrity and produce cell lysis. As a result, the cytoplasmic contents are released leading to a process known as necrosis. An alternative mechanism results when the antimicrobial is able to start the apoptosis process (i.e., genetically modified cell death process) in which both cell division and grow are stopped [51]. The apoptosis process can be easily detected since the refractive index of the cell changes during this process together with the disruption of the cell nucleus with cleavage of DNA into fragments as well as shrinkage of the cytoplasm [52]. As reported by Laverty et al. [50], these effects cannot be observed in the case of necrosis since the membrane destruction occurs rapidly, and there is no time for activation of apoptotic mediators [53].

Probably, one of the crucial aspects in the use of antimicrobials is therefore the differentiation between microbial and human cells. The objective must be to achieve a complete eradication of the infection while limiting the antimicrobial-related damage. Antimicrobial polymers may offer interesting alternatives to obtain the selectivity required, difficult to obtain with low-molecular weight antimicrobials.

10.4.3 Cytotoxicity of Hybrid Antibacterial Nanostructures

The use of nanotechnologies to reach bioactive biomaterials, in particular, in nanomedicine holds an unexpected and exceptional potential for both the prevention and treatment of human diseases [54]. For instance, the incorporation of antimicrobial nanoparticles into polymeric materials has been largely employed to combat bacterial colonization and biofilm formation. However, there is still a lack of knowledge about the toxicology of nanomaterials. Probably, the most important aspect limiting the progress on the toxicology of nanomaterials is related

to the lack of standardized experimental models to examine the toxicology of nanoparticles. Most of the current models have led to inconsistent results due to the lack of reproducibility [55].

Illustrative examples of controversial observations have been, for instance, published for the case of silver-based antibacterial nanostructured materials [56]. On the one hand, Albers et al. [57] observed local toxicity when using silver nanoparticles in a concentration range where antibacterial effects occurred. Similarly, Zhao et al. [58] evidenced that AgNPs integrated in a titania coatings had long-term activity against bacteria. However, these nanoparticles presented certain cytotoxicity provoking a diminished expression of alkaline phosphatase activity in the case of osteoblastoid cells. However, the studies reported by Liu et al. [59] concerning in vitro and in vivo effects of AgNPs incorporated in a PLGA coating concluded that the nanoparticles exhibit excellent antibacterial activity while preserving the induction of osteogenesis.

This controversial outcome can be, at least to some extent, explained by dissimilarities in Ag-NP coating/shapes, the type of cells employed, genotoxicity endpoint, intracellular dissolution, the cellular uptake, as well as the technique employed to expose the cells [60].

Other groups have also described the induction of apoptosis but also genotoxic effects as well as eventual translocation of NPs to tissues/organs with the possibility of systemic effects. According to the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) report [60], silver nanoparticles (Ag-NPs) can be distributed in different organs but are mainly localized in liver, spleen, and kidney. In the same report, the authors mentioned recent results indicating that persistence of silver can also occur in the brain and testes. Nevertheless, it is still unclear whether the silver distribution in the brain occurs in the brain tissue or is restricted to the endothelium of the brain. In effect, there are only few available studies on the in vivo genotoxicity of Ag-NPs they employed Ag-NPs of variable characteristics. For this reason, additional investigations are essential to determine whether Ag-NPs could be genotoxic in vivo.

One of the major limitations in assessing the toxicological effects of nanoparticles is related to the evaluation methods employed. As described in the SCENIHR report [60], only some of the conventional methods employed to evaluate Ag-NP solubility are capable to reveal the Ag^+ availability. On the other hand, evaluating the interactions between biotic receptors and Ag-NPs, together with the continued delivery of Ag^+ is a complex process that still need to the investigated. These aspects still require to be completely and thoroughly investigated, in particular in the case of using nanostructured antibacterial materials for routinary infection prophylaxis. In addition, it is also known that the type of nanoparticles employed (chemical composition), their shape, size, and concentration as well as their surface properties are important characteristics that can affect their toxicological properties as well as their selectivity against prokaryotic cells. These aspects still need to be well understood and precisely controlled in order to optimize the antimicrobial performance.

10.5 Environmental Friendly Non-Fouling Polymeric Materials

In view of the above depicted issues related to the use of antimicrobials, there is an urgent need to develop novel nontoxic polymeric materials and surfaces. In a recent review, Magin et al. [61] highlighted few of the alternatives to produce such materials.

10.5.1 Strategies Approaches Based on the Modification of the Surface Chemistry

It is today widely accepted that the chemical composition and the surface largely affects the initial microorganism adhesion, biofilm formation as well as the release of adhesion of fouling organisms to surfaces [61]. Therefore, by modifying the surface chemical composition and thus the surface energy it will be possible to reduce or completely avoid the microorganism adhesion to the polymer surface. The degree of biological fouling retention as a function of the surface tension of the substrate has been studied by Baier [62] As depicted in Fig. 10.4, a minimal fouling is

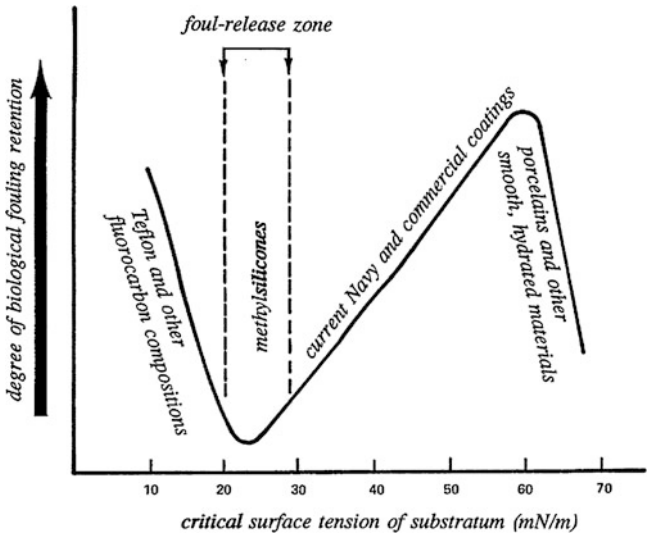

Fig. 10.4 Relationship between critical substratum surface tension and retention strength of attached biofouling organisms. This curve has been confirmed in different environments without significant changes. The minimum is always found in the zone between 20 and 30 mN/m although at different absolute levels depending upon the specific biological system, the time of contact, and the acting mechanical forces of removal. Reproduced with permission from [62]

achieved at a critical surface tension of around 22–24 mN/m. Thus, the optimal chemical groups for theta surface results are intrinsically hydrophobic, closely packed methyl (-CH_3) terminals or polyvinylidene fluoride (PVDF) with repeating CH_2CF_2 groups. In the case of polyethylene with repeating -CH_2- groups or polytetrafluoroethylene with consecutive -CF_2- groups are both less favorable since they have higher interfacial energy. Dispersive force-dominated critical surface tensions are 31 and 18 mN/m for polyethylene and polytetrafluoroethylene, respectively, and are clearly outside of the zone where the thermodynamic interfacial free energy function minimum.

Several groups have fabricated functional surfaces modification with different chemical groups and explored the ability of these surfaces to avoid the adsorption of biomolecules (such as proteins) but also microorganisms. Whitesides and coworkers [63] evidenced that functional groups that are electrically neutral, hydrophilic, and contain hydrogen bond acceptors, presented the best properties in order to resist protein adhesion.

One of the most extensively employed groups to prevent protein adsorption and biofouling is poly(ethylene glycol) (PEG) [63]. PEG, a biocompatible polymer [64], exhibits excellent protein resistance due to steric repulsion [65]. Also polymer bearing phospholipids [66–68], oligosaccharides [69], polyacrylates [70, 71], and zwitterionic polymers (with simultaneously positive and negative domains) resisted protein adsorption. Examples of zwitterionic compounds include phosphorylcholine [63] as well as sulfobetaine [72] just to mention two of them. Finally, bioinspired polymers attempting to mimic complex biopolymers that resist biofouling are currently being investigated. In particular, motivated by the unique properties of mussel adhesive proteins (MAPs) a great effort has been focused on the development of synthetic mimics of MAPs [73–75].

10.5.2 Fabrication of Nontoxic Antifouling Interfaces Based on the Surface Physical Properties

In addition to the modification of the surface with functional nonadhesive groups, another interesting alternative to avoid biofouling is related to the formation of micro and nanostructures at the surface [61]. Cells and bacteria respond to the surface topography in many different ways. For instance, cells are elongated when in contact with micro/nanofibers [76]. The possibility to prevent from contamination without the use of particular antimicrobials but exclusively based on the surface structure is on the one hand a great challenge but on the other hand an excellent opportunity to fabricate environmental friendly antimicrobial surfaces.

Based on these pioneer studies, different groups explored the role of the surface microstructuration in order to decrease or completely avoid biofouling. In this context, it has been demonstrated that surfaces with particular microtopographies can affect attachment of barnacles [77–79] or even prevent biofouling on mollusk shells [80, 81] and bacteria [82]. More recently, Carman et al. [83] investigated

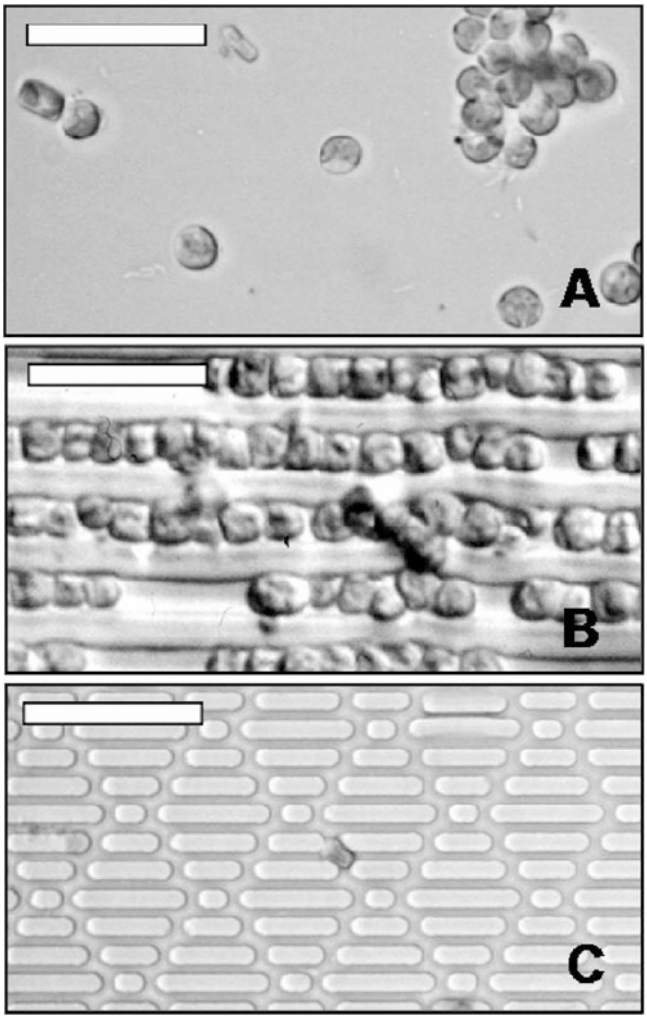

Fig. 10.5 Images of Ulva settlement on (**a**) a smooth surface; (**b**) 5 mm wide, 5 mm spaced, and 5 mm high channels; and (**c**) 4 mm high Sharklet AF TM in PDMS. Images were taken via light microscopy. Scale bars ¼ 25 mm. Reproduced with permission from [83]

how bioadhesion is influenced by microscale topography. For this purpose, the authors prepared polydimethylsiloxane (PDMS) surfaces with different micropatterns (i.e., channels, ridges, pillars, and pits that were 5 μm wide and spaced 2–20 μm apart) and compared the settlement of Ulva zoospores as a function of the surface pattern and with a smooth surface. They evidenced that the Ulva was significantly reduced when the dimensions of the patterns are smaller than the average diameter of the spores (i.e., 5 μm). As depicted in Fig. 10.5, the Sharklet AF™ topography, with dimensions smaller than the spore body, reduced settlement den-

sity by 86 % relative to smooth PDMS. When exposed to this structure, the spores avoided the 2 μm wide channels and were exclusively confined either in defects or wider spaces (~3 μm). Later, by using the same surface pattern, Chung and coworkers demonstrated that the topography can inhibit biofilm formation of *S. aureus* over a long period of time (~21 days) [84].

The types of surface patterns as well as the surface wettability (anisotropic or isotropic and enhanced/decreased due to microtopographical roughness) are two surface characteristics that require consideration in order to design surfaces with antifouling properties. It is outside of the scope to analyze this aspect since the employment of surface roughness to change the surface wettability in order to improve the antifouling properties has been extensively described. Readers interested in this topic are referred to the following references [85–88].

10.6 Particular Environmental and/or Safety Concerns Related to the Final Use and Conclusions

10.6.1 Particular Considerations in Polymeric Antimicrobial Packaging Systems

Active packaging has been designed to improve food safety as well as to help avoiding the development of resistant bacterial strains. Moreover, as depicted by Balasubramanian et al. [89] besides determining the occurrence of resistance in survivors of the treatments, a priority should also be the safety evaluations of both the antimicrobials and the packaging materials. While usually the materials employed for packaging purposes have been already approved for food uses the incorporation of antimicrobial compounds require a reexamination in order to follow the regulatory rules. For instance, several essential oils employed as antimicrobials belong, however, to the category of flavorings according to the EU legislation and are Generally Recognized as Safe (GRAS status) in the USA. Others have been banned in view of their toxicological effect since they can produce irritation, allergic, or even spasmodic reactions [90, 91]. For instance, eugenol, thymol, and menthol in the treatment of root canal provokes the irritation of mouth tissues. This is probably a consequence of both membrane lysis and tissue penetration. It is also interesting to mention that differences between in vivo and in vitro experiments have been reported. For example, while in vivo carvone, thymol, carvacrol, and cinnamaldehyde show minor effects, in vitro are potentially toxic at the cellular level [91].

At the European level, since the compounds released into the food are included in the category of food additives they must be evaluated according to those regulating laws. Moreover, when using nonleaching antimicrobials, i.e., the antimicrobial stays within the packaging material is considered as food-contact material constituent. In this case, regulations are focused on the prevention of undesirable migration into the food [92]. As an illustrative example, a limit of 10 mg/dm^2 was set for migration of active materials from packaging polymers in 2003 [93].

10.6.2 *Modern Approaches to Environmentally Effective Marine Antifouling Coatings*

Structures exposed to the marine environment such as ships or marine platforms requires protection from several elements such saltwater, biological attack, and temperature fluctuations as temperature fluctuations, saltwater, and also from biological attacks, i.e., biofouling [94]. Protective surface coatings are designed to offer these properties and have been largely employed among others in the shipping industry. In addition to these main functions, it is also desirable that the protective coatings also provide the characteristics summarized in Table 10.1.

As depicted in Fig. 10.6, from the initial TBT-based systems banned in 2003 the antifouling industry have been searching for other options [4, 95] such as biocide-free nonadherent surface coatings [96]. The main objective was then to find acceptable replacements with appropriate environmental behavior [4, 7, 95, 97]. During this period, the first candidates were also metallic species, including copper or zinc released using self-polishing copolymer delivery mechanism. Nevertheless, these metallic species presented difficulties during the preparation of controlled dissolutions of the antifouling compounds and, their toxicity still under investigation [98]. In effect, metals and in particular heavy metals are frequently toxic to both humans and marine organisms since they can divide metabolic functions. As a result, both heavy metals and TBT due to the improved legislation in terms of toxicity requirements were replaced in favor of other approaches. Some of these, extensively reviewed by Chambers et al. [94] are briefly summarized below:

(a) Booster biocides
One of the first explored alternatives was the incorporation of the so-called booster biocides. These have been typically introduced to improve the length and functionality of copper-based antifouling coating systems. Two illustrative examples of booster biocides are Irgarol 1051 and Diuron. However, these compounds are rapidly controlled by the UK Health and Safety Executive and

Table 10.1 Requirements for an optimal antifouling coating

Must be:	Must not be:
Anticorrosive	Toxic to the environment
Antifouling	Persistent in the environment
Environmentally acceptable	Expensive
Economically viable	Chemically unstable
Long life	A target for nonspecific species
Compatible with underlying system	
Resistant to abrasion/biodegradation/erosion	
Capable of protecting regardless of operational profile	
Smooth	

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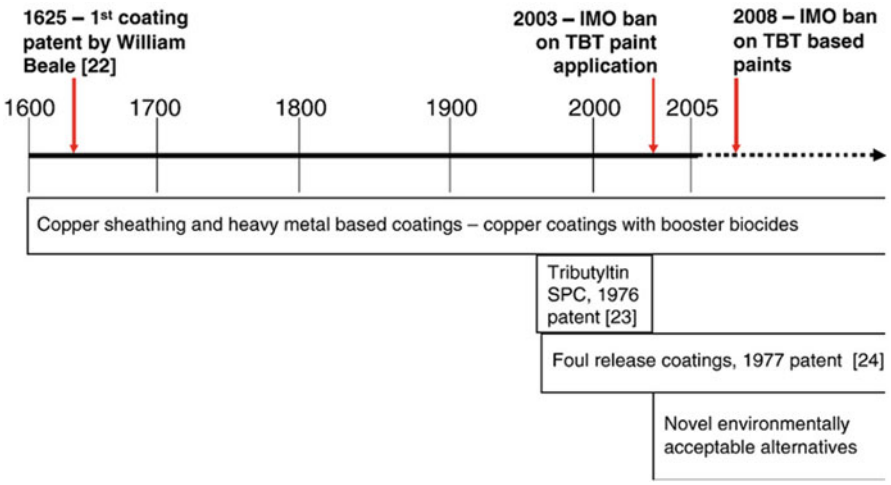

Fig. 10.6 Evolution of the antifouling generations. Reproduced with permission from [94]

whereas Diuron was directly banned, the use of Irgarol has been limited to the use in the case of vessels larger than 25 m in length [12, 99]. As a result, the use of booster biocides only provided an interim solution due to the large demands for effective antifouling strategies [4].

(b) Foul release coatings
Foul release coatings (FRCs) take advantage of the possibility of finely tune the surface energy in order to reduce the organism's ability to create a strong interfacial interaction with the surface. Moreover, these coating are rather smooth and permits that the anchored organisms to be dislodged when the vessel moves above a critical speed [100], which depending on the type of microorganisms can vary between 10 and 20 knots [7]. Thus, these surfaces help to remove fouling due to tensile and shear stresses by decreasing the thermodynamic work of adhesion [101]. Moreover, in addition to the appropriate surface energy, the combination with a low elastic modulus permits to easily create fractures between the organism and the coating surface and fail [100]. The most important families of FRCs are those prepared using fluoropolymers and those using silicon-based coatings. The share a low surface energy while the thickness of the coating is larger for silicone coatings (150 μm) than for fluoropolymer-based coatings (75 μm) [102].

(c) Nontoxic biomimetic coatings
Nature has been in many studies a source of inspiration to design surfaces with unprecedented properties. In particular, nature has been a model for engineering development of highly sophisticated surfaces, for instance, with hierarchical order [103]. In effect, there is interest in the use of natural microtopography [80, 81, 104] and the design of synthetic microtextured surfaces [77, 105–107]

based on those found in nature with antifouling properties. As has been already mentioned, it has been reported that some organisms can be settle or removed depending on the size and periodicity of the surface patterns. However, in order to fully understand the mechanisms regulating bioadhesion the surface properties of shells from both a chemical and a physical point of view are still under investigation [80, 108].

In addition to the surface structure, the functionality plays a key role on the development of non-foulant surfaces [103, 109]. In effect, the tailored microarchitecture [106] of materials, polar properties as well as the surface-free energy [110] have been explored with the objective of fabricating more performant and nontoxic antifouling surfaces. For instance, using biomimetic strategies several groups have reported the immobilization of protein-resistant polymers to surfaces. For that purpose, mussel adhesive proteins have been employed to achieve functional coatings with high density [111].

10.7 Conclusions

In this chapter, we have revised the most relevant environmental and safety issues related to the use of antimicrobial polymers. In contrast to small biocides that are usually released to the environment, the use of nonleaching polymeric materials offers important advantages decreasing the possibility of eventual sensitization and environmental issues. In effect, the use of antimicrobial polymers prevents leaching out of toxic or irritating ingredients and exhibit wide-range efficacy against algae, bacteria, and fungi. Polymers minimize the environmental problems related to the eventual residual toxicity of the antimicrobial agents and enlarge their lifetime.

Concerning the safety aspect, polymeric antimicrobial agents display also advantages such as their low volatility, their chemical stability, and also their low permeability through the skin in humans as well as in animals.

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Chapter 11
Applications and Current Status of Antimicrobial Polymers

Abstract The use of antimicrobial polymers has been extended to many different fields mainly due to their improved quality and safety benefits in comparison to traditionally employed biocides. In effect, low-molecular weight antimicrobial agents have important disadvantages including their toxicity to the environment and/or short-term antimicrobial ability. On the contrary, the use of antimicrobial polymers may enhance the effectiveness of some of the currently employed antimicrobial agents while reducing the environmental issues accompanying conventional antimicrobial agents (typically by decreasing the residual toxicity of the agents, increasing their efficacy and selectivity, and extending the life span of the antimicrobial agents).

Taking into account the important advantages that antimicrobial polymers offer, a wide range of classes and applications can be envisaged for these materials. As will be depicted in this chapter, areas that can benefit from the use of antimicrobial polymers include the fabrication of fibers, textile sector, the design of water filtration systems, food packaging, and biomedical and pharmaceutical industries. In particular, focusing in the biomedical field, these polymers can decrease the sufferings of people improving their recovery, therefore offering better life quality.

This chapter will summarize the most important areas of applications in which polymers are at this time playing an important role or can be of potential interest in the near future. Moreover, the current limitations as well as those aspects that require both further investigation and improvements will be depicted focusing in their use for food packaging and food storage as well as for biorelated applications including the fabrication of medical devices, hygienic applications, or surgery equipment.

Keywords Antimicrobial applications • Healthcare products • Food packaging • Textile products • Water treatment • Antimicrobial paints • Clinical status

11.1 Introduction

As has been evidenced throughout this book, the use of antimicrobial polymers has been extended to many different fields mainly due to their improved quality and safety benefits in comparison to traditionally employed biocides. In effect, low-molecular weight antimicrobial agents have important disadvantages including

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11.2 Main Areas of Application of Antimicrobial Polymers

In an excellent review Kenawy, Worley and Broughton [1], later Timofeeva and Kleshcheva [2] and more recently Jain et al. [3] described the basics of antimicrobial polymer chemistry as well as some of the most important applications for these materials. This section will be devoted to briefly describe the most important areas in which the use of antimicrobial polymers can be of interest.

11.2.1 Applications in the Fabrication of Medical and Healthcare Products

Probably, the most important application of antimicrobial polymers is associated to their use in the elaboration of medical products. The active agents used in pharmacologically are typically low-molecular weight biocides that are able to penetrate in the cells and are quickly eliminated from the body. Their use, however, poses important limitations. First of all, their rapid elimination forces the use of several doses in order to retain the therapeutic effect. In addition, these biocides usually possess low specificity against microorganism and produce different undesirable side reactions.

In contrast to the above-mentioned low-molecular weight biocides covalent immobilization of antimicrobial molecules to polymers offers important advantages such as a reduced elimination from the body and, as a result, a controlled release depending on the polymer characteristics. More precisely, precise delivery is first of

all required in order to enhance the efficacy of the therapy as well as to safely control the amount of drug delivered. Finally, it is worth mentioning that the use of polymer as drug carriers may provide also alternatives to increase the drug delivery for longer periods of time [4, 5].

One of the principal purposes of antimicrobial polymers in biomedical applications is to combat bacterial infections produced at the surface of medical devices including implants, catheters, etc. Implant-associated infections is, probably, the major leading cause of infections produced in hospitals. One of the most extended strategies to overcome this problem concerns the development antimicrobial materials bearing biocides such as silver, copper but also quaternary ammonium compounds, etc. that area delivered into the environment to kill the microbes. For instance, Hart et al. [6] fabricated aliphatic PE-PU polymers prepared from poly(lactic acid) diol (DLLA), poly(caprolactone) diol and 1,6-hexamethylene diisocyanate and blended with levofloxacin. This polymer shows a constant release pattern which reaches to plateau and is able to prevent the *S. aureus* growth for 40–66 days. Therefore, this polymer shows potential to avoid infection of implants in intra-operative models for more that 20–30 days post-implantation.

However, as has been mentioned in previous chapters, most of the impregnated polymers still poses environmental problems mainly due to contamination and, equally, exhibit only a short shelf life [3]. As a result, several groups reported novel alternatives in which the biocide is covalently anchored to the surface of different materials including glass [7, 8], silicon wafers, [7] metals, and polymers [9]. The concept behind this approach is to design functional surface able to kill bacteria upon contact. In addition to the surfaces with highly antimicrobial activity, another crucial aspect is the long-term stability of the antimicrobials in order to prevent the formation of biofilms. While the fabrication of surfaces with antimicrobial properties has been extensively explored, studies reporting long-term antimicrobial materials still a challenge. Illustrative examples of durable antifouling/antimicrobial surfaces were reported by Chen et al. [10] and Ye et al. [11, 12].

Cheng et al. [10] reported the use of zwitterionic poly(carboxybetaine methacrylate) (pCBMA) known for its excellent and durable ultra-low fouling properties to graft glass surfaces. They studied the long-term (over 24 h) colonization using two bacterial strains (*P. aeruginosa* PAO1 and *P. putida* strain 239) on a pCBMA surface at three different temperatures 25, 30, and 37 °C. They evidenced that pCBMA coatings significantly reduced the biofilm formation of *P. aeruginosa* up to 95 % at 25 °C during 240 h and for 93 % at 37 °C during 64 h. In the case of *P. putida*, biofilm formation was prevented during 192 h at 30 °C. In the two-step strategy developed by Ye et al. [11, 12], a prime layer cross-linked poly(dimethylaminomethyl styrene-coethylene glycol diacrylate) (P(DMAMS-coEGDA)) was deposited onto a substrate first and subsequently an in situ grafting of poly(dimethylaminomethyl styrene) (PDMAMS) was carried out from the reactive sites available on the prime layer. The hybrid coatings exhibit a durable bactericidal activity even after constant washing and displayed ~ 99 % bacterial killing against both Gram-negative *E. coli* and Gram-positive *B. subtilis*. Therefore, high as well as durable antibacterial efficacies are major requirements in order to fabricate materials for biomedical applications such as implants.

Stainless steel is one of the most widely employed materials for the fabrication of implants for orthopedic surgery. However, microorganisms are prone to adhere to these materials that can lead to undesired health problems. An alternative to overcome this problem is to coat these materials with antifouling or antimicrobial coatings. For this purpose, negatively charged as well as hydrophilic groups have been employed to reduce the interaction between microorganism and the substrate. An illustrative example of this strategy was reported by Ignatova et al. [13] that fabricated coatings using a "grafting from" methodology. They employed electrografting to anchor polyacrylate chains containing the initiator required for the polymerization step (atom transfer radical polymerization). The grafting from methodology was carried out using 2-(tert-butylamino)-ethyl methacrylate (TBAEMA) as monomer or by copolymerization of TBAEMA with either monomethyl ether of poly(ethylene oxide) methacrylate (PEOMA), acrylic acid (AA), or styrene. By following this strategy, the stainless steel modified surface with brushes of polyTBAEMA, poly(TBAEMA-*co*-PEOMA), and poly(TBAEMA-*co*-AA); the authors observed a decrease on the bacterial adhesion against *S. aureus* of 3–4 orders of magnitude in comparison with the untreated steel.

Chitosan has also been integrated into polyelectrolyte membranes (PEM) on metallic implants [14]. PEM with incorporated chitosan, heparin, and silver nanoparticles displays excellent antibacterial activity against *E. coli* [15, 16]. In order to improve their antimicrobial properties, chitosan has been chemically modified. One of the most important parameters is the density of positive charge that can be improved by addition of extra cationic charged groups to its backbone. For instance, cationic groups employed include acyl thiourea and chitosan-*N*-arginine (CS-*N*-Arg) [17, 18]. While these modifications reinforce the antibacterial activity of chitosan [17–20], more studies are still required to determine the potential of these antibacterial chitosan derivatives in their use as coatings on metallic substrates.

In addition to solve implant-associated infections, the use of antimicrobial polymers can also be interesting for other biomedical applications such as the preparation of anti-infective tissues. An interesting example of this application was reported by Liang et al. [21] that investigated the potential of different *N*-halamine siloxane and quaternary ammonium salt siloxane copolymers to prepare antimicrobial cotton. They fabricated different copolymers that were coated onto cotton and their antimicrobial activity was evaluated against *S. aureus* and *E. coli*. In both cases, *N*-halamine and quaternary functional groups were demonstrated to be effective against *S. aureus*. However, quaternary functional groups were not active against *E. coli*.

11.2.2 Antimicrobial Polymers in Food Packaging Applications

Contamination of food by microbes has two important consequences. On the one hand, contamination reduces the shelf life of foods. On the other hand, there is a related increase in the risk of food-associated illness. Moreover, as depicted by Appendini and Hotchkiss [22], there is an increasing demand for easily prepared,

minimally processed, and ready to eat "fresh" food products. This context together with the fact that today a worldwide distribution is required pose major challenges in order to safely deliver food with high quality. The interested reader is also referred to the review by Appendini and Hotchkiss, which presented a thorough discussion of the need for antimicrobial food packaging.

There are multiple alternatives in which antimicrobial polymers can participate in the design of antimicrobial packaging. These include:

1. Incorporation into the final package of reservoirs (sachets or pads) containing the volatile antimicrobial agents.
2. Blending the antimicrobial agent with the polymer.
3. Surface immobilization onto the polymer surface of the antimicrobials onto polymer surfaces either via covalent linkages or by applying coatings.
4. Some polymers do not require the use of antimicrobial agents since they are inherently antimicrobial.

In addition to synthetic polymers, biopolymers (i.e., those prepared from raw materials originating from agricultural and marine sources) have been extensively explored to prevent the contamination by food-borne microorganism. For instance, Jiang et al. [23] reported the use of chitosan (an inherently antimicrobial polymer) to maintain the quality of fruits. For this application, chitosan was coated on the fruit prior to storage. Also, chitosan was employed by other authors such as Caner et al. [24], Ouattara et al. [25], or Chen et al. [26]. However, chitosan has found some limitations in particular they resulted ineffective to protect food from *lactobacilli bacteria*. An interesting review concerning the use of biopolymers for antimicrobial packaging purposes was published by Cha et al. [27].

While most of the explored systems resort to the controlled delivery of a particular antimicrobial substance, more recently the concept of *active packaging* is gaining more attention. *Active packaging* refers to those systems in which the environmental conditions in a food package can be modified depending on a defined stimulus. Active packaging will be able, thus to improve the food quality by increasing the shelf-life as well as by rapidly reacting to the presence of microorganisms [28–30]. Nowadays, many different alternatives for the preparation of active packaging are available but all share the same principle, i.e., the active interaction between the packaging and the food product. As depicted in Table 11.1, active packaging involves approaches to scavenge O_2 and CO_2, systems to absorb moisture, emitting systems (CO_2 and ethanol), and systems bearing antimicrobials [27, 31–33].

11.2.3 Textile Products

The use of antimicrobial polymers for the fabrication of textile products is receiving also extensive attention due to the variety of products that may benefit from this knowledge [1]. As depicted by Varesano et al. [37], textiles with antimicrobial properties can be employed to fabricate good such as towels, undergarments, outdoor

Table 11.1 Active packaging approaches and potential food applications [30, 34, 35]

Active packaging techniques	Potential food applications
O_2 Scavenging systems	Roasted nuts, chocolates, meat sausages, chicken salami, cereals, cookies, beer, bread
CO_2 Scavenging systems	Coffee, poultry products
CO_2 Emitters	Nuts, cake, potato chips
Antimicrobial release systems	Bread, cheese, meat, cakes
Antioxidant release systems	Cereals, wine
Ethanol emitters	Cheese, fish, bakery products
C_2H_4 Emitters	Minimally processed foods
Moisture scavenging systems	All dry fruits and nuts, fish, bread, sea food, meat

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apparels, shoes, hygienic uses, furnishings, medical uses, hospital linens, wound care wraps, upholstery, or wipes. It has also become widely employed in sportswear to impart anti-odor or biostatic properties [38–40]. Moreover, textiles able to be self-sterilized have probable benefits to decrease disease transmissions among hospital populations or biowarfare protection among other purposes.

The use of antimicrobial treatments to finish many textile products is currently widely employed using typically, as depicted by Kenawy et al. [1] for three main purposes:

(a) to offer protection against microorganism including yeast, bacteria, or dermatophytic fungi in view of using these products in hygienic or medical purposes.
(b) to defend textile from biodeterioration. This is typically caused by mildew, mold, and rot-producing fungi.
(c) finally, antimicrobials can protect textiles from insects and pests.

In particular, antimicrobial polymers are excellent candidates to be employed in the fabrication of textile goods. Major advantages of using antimicrobial polymers include their environmental stability and since biocides can be covalently linked to the polymer backbone diffusion on the wearers' skin can be avoided. In addition, polymers exhibit low toxicity, good biocompatibility and do not produce skin irritation. Finally, corrosion of polymers is rather low and can have both long residence time and biological activity [37].

A large variety of antimicrobial polymers have been employed for the elaboration of different textile products; therefore, we will limit our discussion to two illustrative examples that involve the use of *N*-halamines incorporated in different fabrics [41, 42] or the use of natural polymers such as chitosan which is an appropriate eco-friendly material for the elaboration of textiles [43].

N-halamines were employed by Lee et al. [41] to render antimicrobial a cotton fabric. For that purpose, an *N*-halamine precursor, m-aminophenylhydantoin (m-APH) which was rendered antimicrobial upon contact to chlorine, was synthesized and was applied on cotton fabric using polycarboxylic acids as cross-linking agents (butanetetracarboxylic acid—BTCA). The efficiency of these textiles against

both Gram-positive and Gram-negative bacteria was excellent with a 6 log reduction of the bacteria concentration upon contact during 1 min. In addition to the efficiency also the durability (retain their antimicrobial efficacy up to ten washing cycles) as well as the possibility to recharge them make of these fiber interesting for textile purposes. More recently, Li et al. [42] used and analogous strategy in which the *N*-halamine precursor, was, in this case, 2,2,6,6-tetramethyl piperidinol (TMP), that was covalently anchored onto a cotton fabric by employing BTCA as cross-linking agent. The resulting cotton samples displayed great efficiency against *S. aureus* (100 % reduction with 7.1 log reduction with 5 min of contact) and *E. coli* O157:H7 (87 % reduction at 10 min of contact).

Chitosan is a versatile, biodegradable, and nontoxic polymer that, in addition, presents inherent antimicrobial properties. However, one of the most important drawbacks is related to the chitosan's poor adhesion to fabrics and its eventual gradual reduction of the antimicrobial activity, in particular, under alkaline conditions. In order to overcome this drawback, chemical modifications on chitosan have been employed to enhance the properties of chitosan. Fu et al. [43] fabricated three different water-soluble chitosan derivatives having double functional groups following the synthetic route depicted in Fig. 11.1. As a result, O-quaternized-*N*-benzylidene-chitosan O-(QCTSS), O-quaternized-*N*-benzyl-chitosans (OQCTS-Bn), and O-quaternized-*N*,*N*-bimethyl-*N*-benzyl ammonium chitosans chloride (O-QCTS-DEBn) were the three chitosan derivatives depicted.

In terms of morphology, the SEM micrographs depicted in Fig. 11.2 revealed that before the treatment (a), cotton fiber surface was smooth and flat, while the final (b) fiber surface exhibit small grains as well as the fiber mesh layer. Nevertheless, the treatments did not produce significant structural changes. The finished fabrics show strong antimicrobial with more than 99 and 96 % against *S. aureus* and *E. coli*, respectively. Moreover, the durability of O-QCTSS, OQCTS-Bn, and O-QCTS-DEBn-treated fabrics still maintain over 93, 78, and 79 % of bacterial reduction, respectively, after 20 wash times.

In addition to these selected examples, literature related to the fabrication of textiles with antimicrobial properties is extensive. In Table 11.2 are summarized in which other different polymers and biocides have been employed.

11.2.4 Water Treatment

Traditional strategies to sterilize water resort to the use of chlorine and other water-soluble disinfectants. Nevertheless, the use of soluble disinfectants poses important limitations related to environmental aspects as well as to health-related problems. On the one hand, these disinfectants are rather toxic and due to their solubility the residues are difficult to handle [57]. This is a serious limitation when they are incorporated in the food chain where they can be concentrated and supposes a more serious problem. On the other hand, some of these chlorine compounds can be transformed due to chemical reactions with other compounds present in the

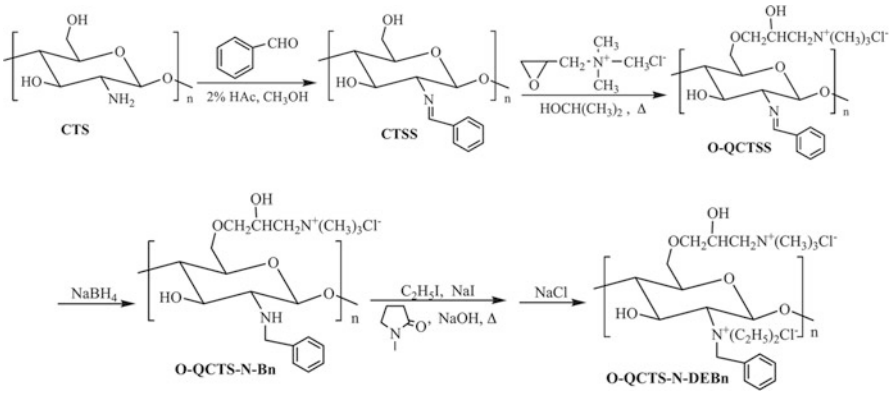


Fig. 11.1 Synthetic route and chemical structures of the chitosan derivatives. Reproduced with permission from [43]

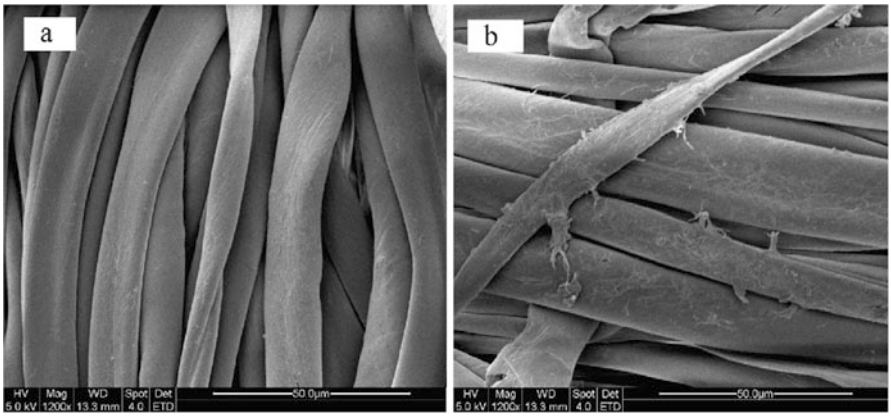


Fig. 11.2 SEM images of the surface of cotton fiber before (**a**) and after (**b**) finish with O-QCTSS. Reproduced with permission from [43]

environment leading to more toxic side-products (carcinogenic trichloromethanes and chloroacetic acids). In addition, it has been evidenced that the use of water-soluble disinfectants finally favors the emergence of chlorine resistant microorganism, and they exhibit short-term stability in aqueous solution. As a result, the covalent immobilization of disinfectants without releasing the active agent has emerged as the most promising strategy for water treatment [58]. In this context, water-insoluble polymeric materials have been applied for the fabrication of water filters, fibrous disinfectants, or water filters.

An illustrative example of the use of polymers for water purification purposes was reported by Tyagi et al. [59]. They prepared a water-insoluble copolymer using methyl methacrylate (MMA) and *N*-vinyl-2-pyrrolidone. To provide the antimicrobial properties to the polymer, the authors iodinated the copolymer and refill cartridge as depicted in Fig. 11.3. Iodine is a regularly used antimicrobial agent that has

Table 11.2 Results on antibacterial efficiency and fastness to washing of antimicrobial textiles

Polymer	Substrate	Bacteria	Efficiency (%)	Deposition method	Efficiency after washing	References
Chitosan and carboxymethyl chitosan	Cotton	*E. coli*	60[a]	Pad-dry-cure	Unknown	[44]
		S. aureus	75–79[a]			
Chitosan	Cotton	*E. coli*	~100[a]	Chemical grafting	83 % after 10 cycles at 60 °C for 45 min	[45]
		S. aureus	~100[a]			
Chitosan	Cotton	*S. aureus*	~100[a]	Chemical grafting	91 and 93 % after 25 laundering cycles	[13]
		K. pneumoniae	~100[a]			
N-alkyl-polyethylenimine	Cotton	*S. aureus*	98[c]	Chemical grafting	S. aureus:	[46]
		S. epidermidis	97[c]		98 % after one cycle in methanol and water	
		E. coli	99[c]		98 % after stirring in water at 55 °C overnight	
		P. aeruginosa	98[c]			
		S. cerevisiae	97[c]			
		C. albicans	96[c]			
N-alkyl-polyethylenimine	Cotton	*S. aureus*	100[b]	UV curing	Unknown	[47]
		E. coli	~100[b]			
Poly(dimethylaminomethylstyrene)	Nylon	*E. coli*	~100[a]	Chemical vapor deposition	Unknown	[48]
		B. subtilis	~100[a]			
Quaternized ammonium polymer	Polyester	*E. coli*	~100[a]	Pad-dry-cure	Unknown	[49]
Acyclic *N*-halamine polymer	Polyester	*E. coli*	100[d]	Coating	Unknown	[50]
		S. aureus	100[d]			
N-halamine polymer	Cotton	*E. coli*	~100[d]	Coating	Unknown	[51]
		S. aureus	~100[d]			

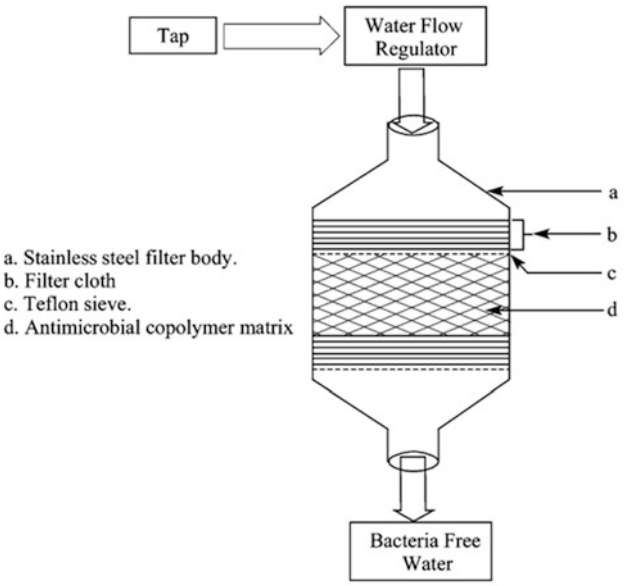
(continued)

Table 11.2 (continued)

Polymer	Substrate	Bacteria	Efficiency (%)	Deposition method	Efficiency after washing	References
Poly(hexamethylene biguanide)	Polyester/cotton	*S. aureus*	~100[c]	Pad-dry-cure	~100 % after 25 cycles AATCC 143–96	[52]
		K. pneumoniae	~100[c]		94 % after 25 cycles AATCC 143–96	
Poly(hexamethylene biguanide)	Wool	*E. coli*	~100[c]	Pad-dry-cure	67 % after 25 cycle A5 ISO 6330:2000	[53, 54]
Polypyrrole	Cotton	*S. aureus*	65[a]	Chemical deposition	Unknown	[55]
		E. coli	59[a]			
		C. albicans	73[a]			
Polyaniline	Cotton	*S. aureus*	95[a]	Chemical deposition	Unknown	[56]
		E. coli	85[a]			
		C. albicans	92[a]			

Reproduced with permission from [37]
Test methods:
[a]ASTM E 2149-01
[b]Spray methods (nonstandard method)
[c]AATCC 100–1999
[d]Sandwich test (nonstandard method)

(a)



(b)

	microbial density/100 L of water passed		
species	initial	up to 5000 L	5000–8000 L
S. aureus	2×10^5	nil	1 ± 1
E. coli	2×10^5	nil	5 ± 2
Candida	1.5×10^5	nil	1 ± 1

Fig. 11.3 (*Left*) Diagram of the refill cartridge reported by Tyagi et al. (*Right*) Microbial Counts with Respect to Water Flow through the Column. Reproduced with permission from [59]

fungicidal, viricidal, and also bactericidal properties [60]. The efficiency of the iodinated copolymer was tested using known concentrations of microbial cells composed of E. coli, *S. aureus*, and *Candida spp.* that were injected into the water reservoir and quantifying the percentage of microbes alive after passing through the column. According to their findings, no growth upon filtering 5000 L of water through the column was observed since as there was no growth of microbes on the culture plates.

Probably, one of the best antimicrobial functionalities for water purification applications is related to the use of cyclic *N*-halamines. These chemical groups exhibit improved long-term stability-free halogen are not released into aqueous solution and, they only work as biocides upon direct contact of the halogen atom with the membrane of the microorganisms maintaining high biocidal efficacy (better than quaternary ammonium salts). Another important advantage is to regenerate

the antimicrobial activity (the antimicrobial activity was loss upon halogen detachment) upon exposure of the molecules to additional free halogen [61].

One interesting strategy to water disinfection using water-insoluble matrices and without releasing active agents was reported Worley et al. [61, 62]. They took advantage of the excellent antimicrobial properties of *N*-halamines to prepare modified highly-cross-linked polystyrene beads for water disinfection. More precisely, they prepared biocidal polymers poly[1,3-dichloro-5-methyl-5-(4′-vinylphenyl) hydantoin] and poly[1,3-dibromo-5-methyl-5-(4′-vinylphenyl) hydantoin] and the monochlorinated derivative as insoluble porous beads. Usually, the use of granular solid particles is associated to a decrease of the flow rate due to the appearance of clogging problems. On the contrary, porous films prevent clogging to occur while maintaining outstanding biocidal efficacies. According to the authors, the porous beads provide inactivation times on the order of a few seconds for all pathogens tested, i.e., *S. aureus*, *E. coli* O157:H7 and MS2 virus.

In effect porous structures, for instance, in the form of particles or foams are interesting systems to significantly improve the antimicrobial properties. Gangadharan et al. [63] prepared porous cross-linked polystyrene-based beads by suspension polymerization and studied its application as a water treatment system. The porous beads were functionalized with a dendritic structure composed of di(chloroethyl)amine-type end group functionality (Fig. 11.4). These functional structures were evaluated both against Gram-negative and Gram-positive bacteria.

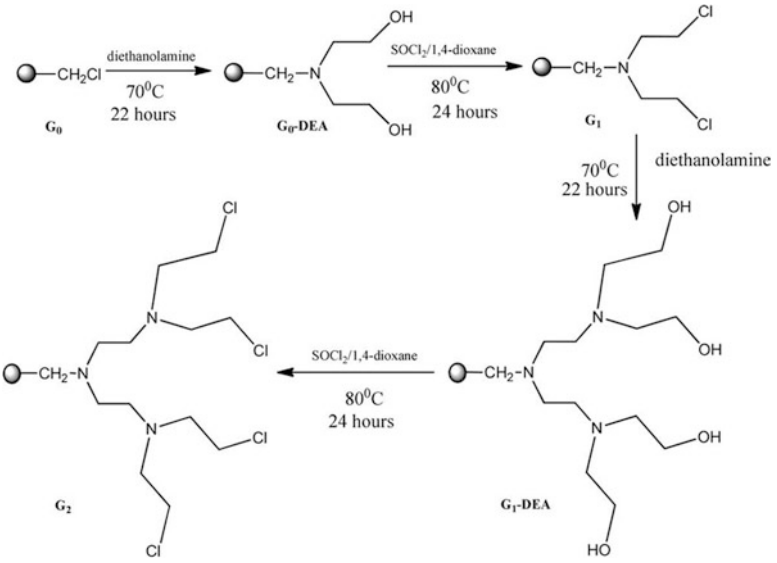


Fig. 11.4 Synthesis of dendrimers supported onto porous polystyrene beads

Interestingly, the number to nitrogen atoms in the dendrimer backbone is directly related to the activity of the particles. As a result, the dendritic microparticles bearing both amino and di(chloroethyl) groups exhibit a dramatic decrease in the bacterial count.

Other strategies proposed to fabricate antimicrobial materials for water cleaning purposes involve the use of polymers capable of forming foams having both large porosity and surface area are highly desired to improve the contact between the material surface and the microorganism. Polyurethane (PU) is one of the most extensively for the fabrication of foams with variable chemical composition, pore sizes, and mechanical properties. PU has been employed by Jain et al. [64] and Aviv et al. [65] to produce antimicrobial foams for water decontamination. Jain et al. [64] prepared polyurethane foams coated with silver nanoparticles and investigated their antibacterial activity at flow rates used in domestic water purifiers (0.5 L/min). As evidenced by the authors, upon 5 and 10 min contact time of the microorganism with the PU coated with silver nanoparticles, no bacteria could be identified in the treated water (Fig. 11.5). Independently of the *E. coli* strain employed (ATCC 25922 or MTCC 1302), the number of bacteria counted was zero for all the dilutions employed. More interestingly, similar results were obtained using a constant flow rate of 0.5 L/min, i.e., for input loads of 1×10^3 and 1×10^5 CFU/mL, there were no bacteria detected in the output water. As a result, no growth was observed below the PU coated with nanoparticles while a bacterial evolution was observed in the case of pure PU.

Instead of using silver nanoparticles, Aviv et al. [65] prepared iodine-loaded IPU sponges. For this purpose, the sponges were either exposed to iodine vapors or immersed in aqueous/organic solutions of iodine. The amount of iodine adsorbed in the PU was increased by the immersion method in comparison to the sublimation approach were adsorption only occurs at the surface. In addition, the authors applied an EVA coating on the IPU sponges that allowed a controlled and stable iodine release with an average of 25–30 ppm iodine release during the 250 L of water treatment. As depicted in Fig. 11.6 to estimate the efficiency of EVA-coated IPU as a component in a commercial water purification system, the sponges were positioned

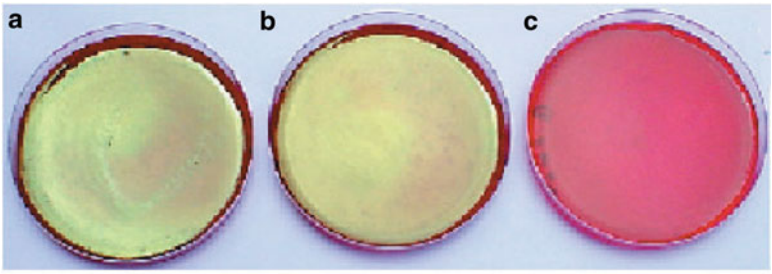


Fig. 11.5 Test tube result for *E. coli* MTCC 1302 for 10^{-2} dilution for 5-min exposure. (**a**) Initial count. (**b**) After exposure to pure polyurethane (PU). (**c**) After exposure to nanoparticle-coated PU. Characteristic metallic sheen shown by *E. coli* is clearly visible in **a**, **b**, while the bacterium count was zero in **c**. Reproduced with permission from [64]

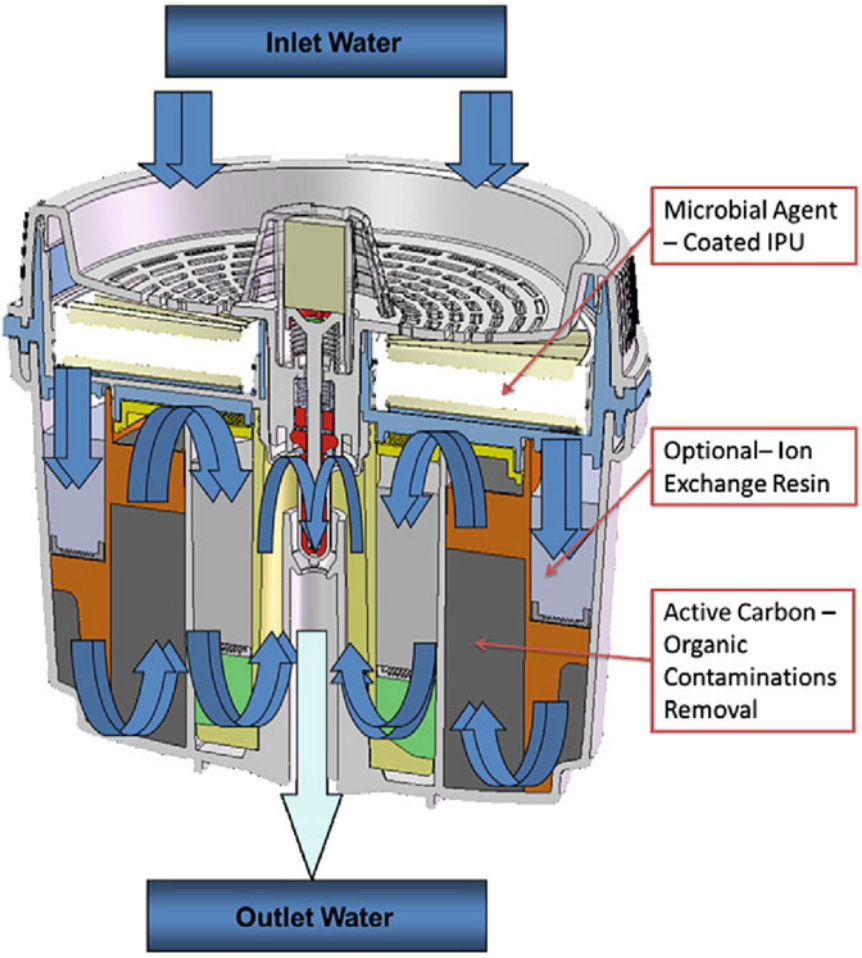


Fig. 11.6 Illustrative image of the device used by Aviv et al. to evaluate the EVA-coated IPU as an antimicrobial system for water decontamination; the foams were positioned in the unit with an additional activated carbon cartridge in order to collect iodine residues. Reproduced with permission from [65]

in a base filter purifier unit. This unit is complemented with an activated carbon cartridge that will collect eventual iodine residues. This promising approach produced a dramatic reduction of the *E. Coli* bacteria from 10^7 to 10^8 CFU/100 mL to bacterial concentrations below the detection limit during the 250 L of water treatment. On the other hand, the capability of these systems to inactivate MS2 bacteriophages was significantly lower than that of *E. Coli* bacteria. For this application,

pH, turbidity, or different ionic conditions that can significantly affect the relative resistance of bacteriophages to the iodine need to be precisely controlled. For instance, warm water at neutral pH are optimal conditions that favor the effect of iodine in virus while a higher amount of iodine will be required at low pH levels and at cold water temperatures.

11.2.5 Antimicrobial Paints

Another important industrial area that may benefit from using antimicrobial materials is the paint industry [66]. Different antimicrobial agents have been already employed for this potential use that include silver nanoparticles [67, 68], quaternary ammonium salts [69–72], acrylics [73], photoactivated metal oxides [74], or *N*-halamine materials [66, 75–77] among others.

N-halamine due to their rapid inactivation rates for pathogens [75, 76, 78–80], their low cost and the possibility to be recharged in situ once the oxidative halogen is exhausted is one of the most suited candidates for this application. One of the pioneer works using *N*-halamines of the preparation of paints was described by Worley et al. [76]. They fabricated a hydantoinyldiol monomer that was copolymerized with a commercial water-borne acrylic polyol and an isocyanate in order to fabricate polyurethane coatings. These coating were, in a second step, chlorinated to render the surface antimicrobial. Interestingly, the authors observed a complete inactivation of *S. aureus* (4.5-log initial challenge) within 2 h of contact. The same authors reported later two antimicrobial *N*-halamine hydantoinyl siloxane materials also studied [79, 81] as additives in the elaboration of paints based in polyurethane formulations. These materials did not significantly improve the results obtained previously. However, the incorporation in the form of emulsion of *N*-halamines for antimicrobial paints enhanced the antimicrobial activity. This strategy was employed by Cao and Sun [75] that showed that an emulsion of *N*-chloro-2,2,6,6-tetramethyl-4-piperidinylmethacrylate added to a commercial latex paint permits to obtain 8-log inactivation of several pathogens. The inactivation time depended on the *N*-halamine concentration. Thus, only few minutes of contact were required at high concentrations (20 wt%) while concentrations as low as 1 wt% 12 h were required.

Finally, more recently, Kocer et al. [66] prepared a series of homopolymers and copolymers containing units of hydantoinylacrylamide and the sodium salt of 2-(acrylamido)-2-methylpropanesulfonic acid. These polymers were added into commercial water-based latex paint formulations. Finally, upon drying, the painted surfaces were treated with the copolymers that rendered the surface antimicrobial. The chlorinated homopolymer did not displayed antimicrobial properties most probably due to its tendency to isolate into aggregates in the paint. On the contrary, the copolymers that were miscible with the commercial latex produced a 6-log inactivation of *S. aureus* and *E. coli* O157:H7 within 5 min of contact time.

11.3 Gap Between Lab-Scale and Reality

One of the still remaining challenges concerns the extrapolation of experimental achievements carried out at the lab scale to real uses. Lab experiments are carried out in controlled environments with only few variables [36, 82]. Nevertheless, in the real-world antimicrobials need to be effective in very complex systems. For instance, as reported by Malhotra et al. [36] and Rosenberg [82] for the case of antimicrobial food packaging, lab scale tests are carried out with food simulants. However, in a real food system many other molecules are available such as salt content, nutrients, fats, and proteins that can also interact with the antimicrobial [83–85]. In addition, other environmental aspects related to the real conditions in which antimicrobials will be exposed including temperature, or moisture can significantly modify their effectiveness [30, 86].

Another important aspect is related to the analytical, chemical, and microbiological tests. When those tests are carried out at the lab scale, it is possible to precisely characterize the process, i.e., it is possible to determine where the antimicrobial agent goes and in how much quantities [87, 88] On the contrary, the complexity of real systems impedes these accurate evaluations. In general, important differences in the evaluation of the antimicrobial activity are obtained during testing real foods and lab scale systems. For instance, testing real foods with antimicrobials explored Kim et al. [89] and Duan et al. [90] reported that antimicrobial packaging system was less effective in comparison with those antimicrobials tested at the laboratory scale [89, 90] Equally, when essential oils were employed as antimicrobial agents the tests carried out by Burt et al. [91] evidenced significantly higher levels of them were needed to achieve the desired antimicrobial effect. According to these authors, higher organic acid and trace metal content, greater availability of nutrients for cellular repair, and interactions with compounds in the food can, at least to some extent, inactivate the active substance.

11.4 Clinical Status of Antimicrobial Polymers

Among the large variety of systems developed at the lab scale, some of them are currently being clinically evaluated. In Table 11.3 are summarized some illustrative examples of antimicrobial systems from three different families: natural antimicrobial chitosan, polyethyleneimine and, finally, acrylate derivatives [3]. The purposes of these clinical assays are mainly related to the efficacy and safety in the use of a particular antimicrobial for a targeted application. Among the applications explored, several ongoing trials are focusing on the elaboration of biofunctional textiles, the preparation of hemostatic sealants, or the design of implants in cranial bone reconstruction.

Table 11.3 Clinical trials for antimicrobial polymers

SN	Clinical trial title	Condition	Delivery system	Comments	NCT no.	Phase	Status
Chitosan							
1.	Efficacy and safety of a biofunctional textile in the management of atopic dermatitis	Atopic dermatitis	Biofunctional textile	Purpose is to study the use of biofunctional textile coated with chitosan. Improves quality of life and diminishes skin colonization with Staphylococcus aureus and skin moulds like Malassezia	NCT01597817	2	Ongoing
2.	USF hemostasis: usage of HemCon for femoral hemostasis after percutaneous procedures	Coronary angiography	HemCon pad, a bandage made of chitosan	HemCon pad was tested after diagnostic percutaneous coronary angiography as an adjunct to manual compression to better control vascular access site bleeding and reduce time-to-hemostasis	NCT00716365	4	Complete
3.	Evaluation of the BioWeld1 system as a method for surgical incision closure	Surgical incision closure	BioWeld1: a novel medical device that consists of Chitosan film and BioWeld1 plasma ejecting device	Purpose is to assess the safety and performance of the BioWeld1 system procedure for surgical incision closure of the skin in women scheduled for elective C-section procedure.	NCT01709240	2,3	Ongoing
4.	Trial of a Novel chitosan Hemostatic sealant in the management of complicated epistaxis	Epistaxis	Chitosan-coated nasal packing (ChitoFlex used in conjunction with the HemCon Nasal Plug)	Purpose is to evaluate applicability of sealant in management of difficult spontaneous epistaxis and its healing effect on nasal mucosa			
				Any nondesirable effects of chitosan on the nasal cavity, such as the production of fibrosis and foreign body reaction were studied	NCT00863356		Complete

(continued)

Table 11.3 (continued)

SN	Clinical trial title	Condition	Delivery system	Comments	NCT no.	Phase	Status
Polyethyleneimine							
5.	The effects of a polyethyleneimine-coated membrane (oXiris) for hemofiltration versus polymyxin B-immobilized fiber column (Toraymyxin) for hemoperfusion on endotoxin activity and inflammatory conditions in septic shock—a randomized controlled pilot study	Septic shock	oXiris filter, a surface-treated AN69 membrane with polyethyleneimine	Hypothesis behind the study is that positively charged inner surface of the membrane allows absorbing negatively charged bacterial products such as endotoxin that leads to activation of pro- and anti-inflammatory mediators at the early phase of sepsis	NCT01948778		Not started
6.	A clinical study: the antibacterial effect of insoluble antibacterial nanoparticles (IABN) incorporated in dental materials for root canal treatment	Endodontic treatment Irreversible Pulpitis Health Pulp Infected Pulp	Insoluble alkylated polyethylenimine nanoparticles	The effect of antibacterial nanoparticles, incorporated in root canal sealer material and in provisional restoration to be examined	NCT01167985	2	Recruiting
Acrylate derivatives							
7.	Effect of provisional-crown surface coating on biofilm formation	Dental plaque	Coating of a dental restoration material (polymethylmethacrylate) with liquid polish resinlq245r	The effect of liquid polish coating or resin bonding coating on biofilm formation on poly methyl methacrylate provisional restorations (PMMA PRs) was studied and in vivo early biofilm formation on PMMA PRs with and without resin coatings was measured	NCT00254345	1	Complete

8.	Bioactive glass composite implants in cranial bone reconstruction	Bone substitute	Composite implant	Purpose is to study composites of bioactive glass and methylmethacrylate with glass fiber reinforcement in cranial bone defect reconstruction	NCT01202838	0	Unknown
9.	Evaluate the effectiveness of an experimental urethane dimethacrylate resin-based dental composite material	Dental caries	Dentsply Caulk: urethane dimethacrylate resin-based composite resin	The purpose of study is to compare the clinical success of two tooth colored resin composite dental filling materials TPH3 and an experimental urethane dimethacrylate resin-based composite resin for wear resistance staining and marginal seal using modified Ryge criteria to evaluate the posterior restorations for 24 months in duration	NCT02018822	–	Ongoing

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11.5 Conclusions

As highlighted by many different scientists, the fast growth of dangerous pathogens and their severe health effects is today challenge to modern science. Infections produced by pathogenic microorganisms are still a major problem in many fields such as surgery equipment, dental restoration, medical devices, healthcare products, hospital surfaces/furniture, and hygienic applications (e.g., water purification systems, textiles, food packaging and storage, major or domestic appliances). Antimicrobial polymers due to their capability to kill/inhibit the growth of microbes on surfaces and/or the environment have recently gained considerable interest. In effect, both academic research and industry have focused their attention in the use of polymers to improve several drawbacks found in the use of small antimicrobial molecules. In particular, polymers display enhanced efficacy, reduced toxicity, minimized environmental problems, do not produce antimicrobial resistance, and prolonged lifetime.

The use of antimicrobial polymers has increased steadily, during the last decades and is currently undergoing a fast expansion. As a result, a large variety of novel antimicrobial polymers have been reported and investigated in the past years. As has been depicted in this chapter, polymers have been employed for a broad range of application including implants, bone replacement and other prostheses, textile industry, healthcare products, wound healing, food packaging, and water treatment.

Today, many different approaches involving, for instance, the modification of polymers and fibrous surfaces, combined with variations in other parameters such as the porosity or surface wettability has led to biomedical devices with improved resistance to microbial adhesion and biofilm formation. However, some developments still needed in which the novel antimicrobial polymers must accomplish two important requirements. On the one hand, the actuation time required for novel biocidal polymers should be as short as possible. In this context, antimicrobial polymers that require contact times of hours in order to reduce the number of viable pathogens will have partial or limited practical value. Antimicrobials acting within minutes or seconds are interesting potential candidates for real purposes. On the other hand, the structural modification of the polymer as a result of the incorporation of the biocide must not adversely affect the use pattern. In addition, the development of novel polymer biocides that prevent microbial resistance is a requirement of current progressing medical technology. For this purpose, polymers alone or in combination with inorganic composites (bearing silver or other antimicrobial metals) have recently been a subject of intense study. This has led to search regarding antimicrobial composites, wherein polymers form the base for loading. These hybrid multifunctional antimicrobials have recently gained special attention for the development of biomaterials with enhanced antimicrobial properties.

Future advances require a multidisciplinary vision of this problem in which industry, research institutes, and regulatory agencies will work together for the fabrication of more performant antimicrobial polymeric materials.

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