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.

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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.

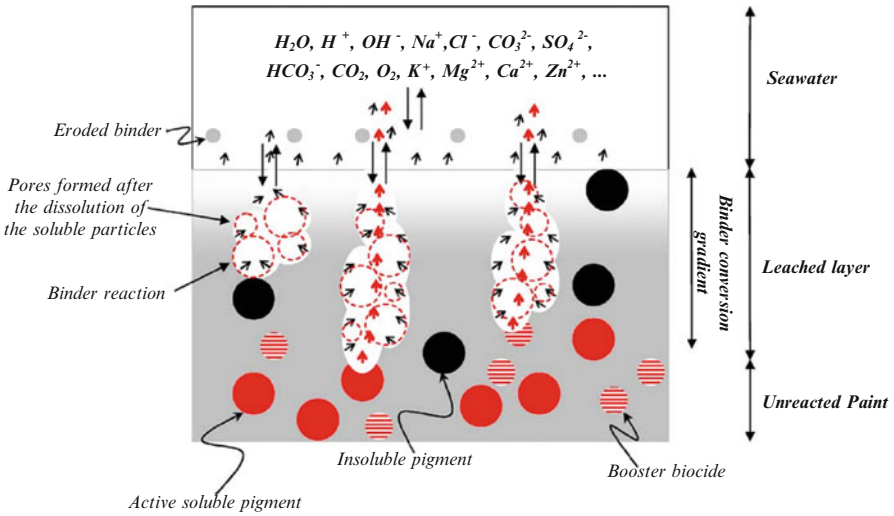

Fig. 10.1 Schematic illustration of the behavior of a biocide-based antifouling system exposed to sea water. Reproduced with permission from [7]

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

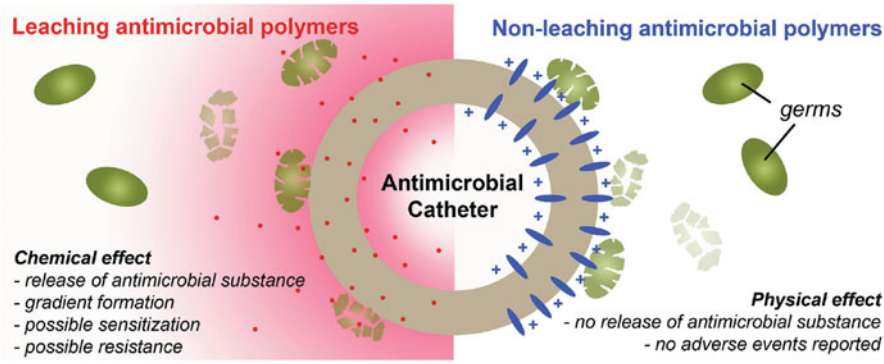

Fig. 10.2 Illustrative representation of the action mechanism of leaching versus nonleaching antimicrobial polymers. Leaching antimicrobial polymers (*red dots*) are released from the polymer to the environment to facilitate the antimicrobial effect by a chemical interaction with the germs (*green*). 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.

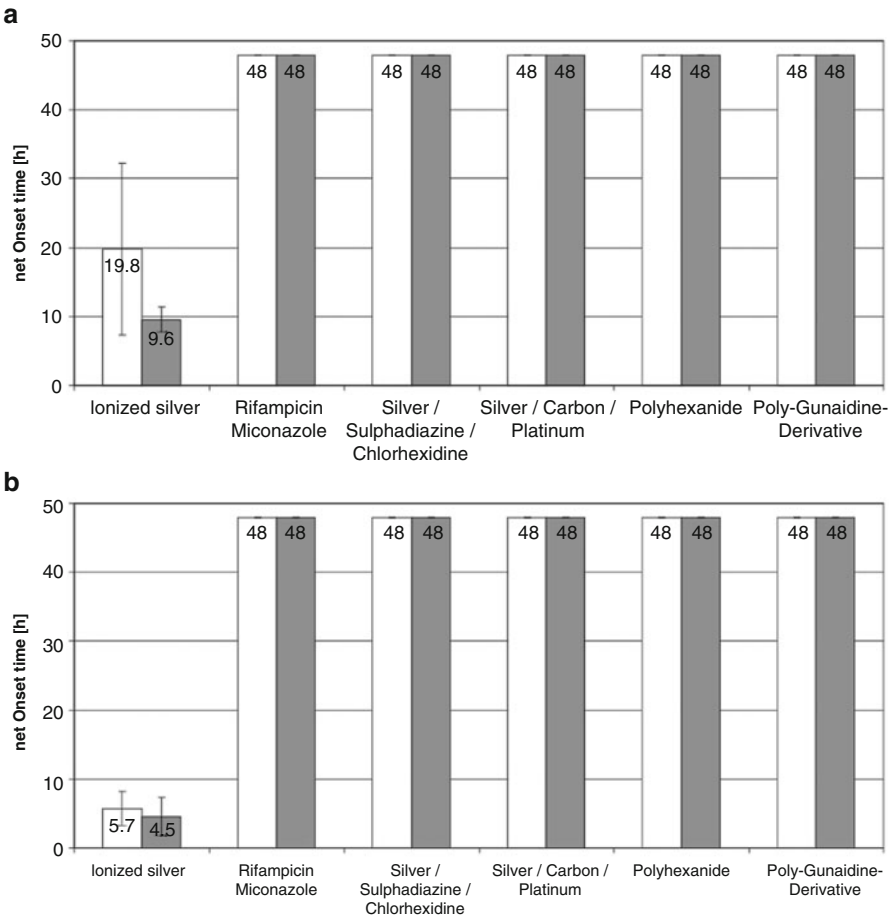

Fig. 10.3 Comparison of the antimicrobial efficacy of leaching and nonleaching before (left column) and after (right column) plasma preincubation. By using the Certika test, the antimicrobial efficacy was evaluated for (**a**) *S. epidermidis*, and (**b**) multiresistant *S. aureus* MRSA. The plasma 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

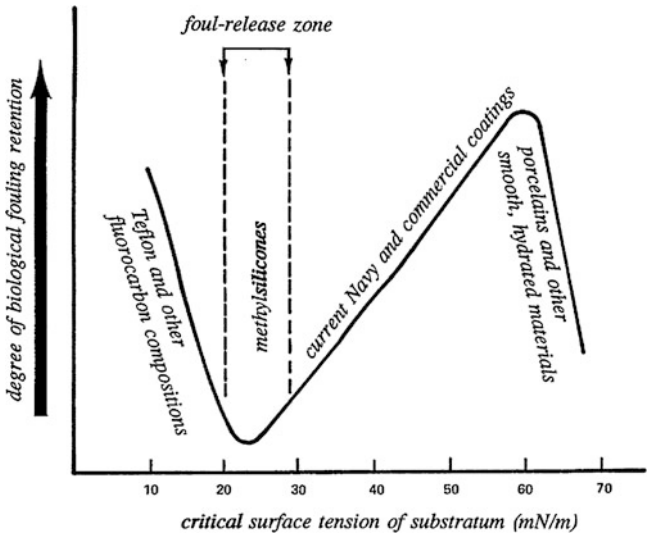

Fig. 10.4 Relationship between critical substratum surface tension and retention strength of attached biofouling organisms. This curve has been confirmed in different environments without significant changes. The minimum is always found in the zone between 20 and 30 mN/m although at different absolute 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

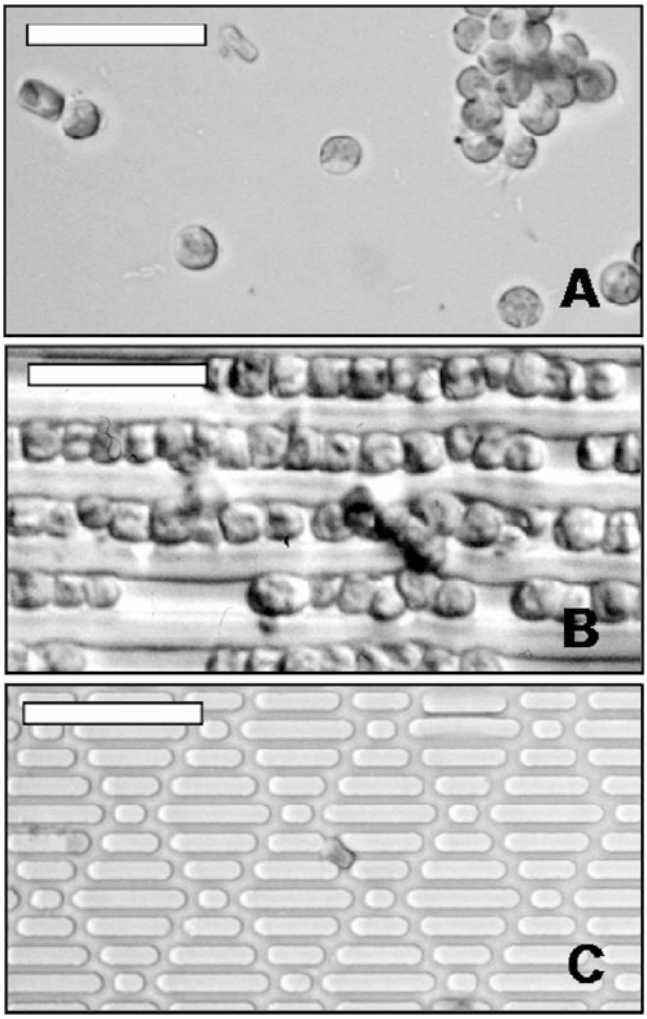

Fig. 10.5 Images of Ulva settlement on (**a**) a smooth surface; (**b**) 5 mm wide, 5 mm spaced, and 5 mm high channels; and (**c**) 4 mm high Sharklet AF TM in PDMS. Images were taken via light microscopy. Scale bars ¼ 25 mm. Reproduced with permission from [83]

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	

Reproduced with permission from [94]

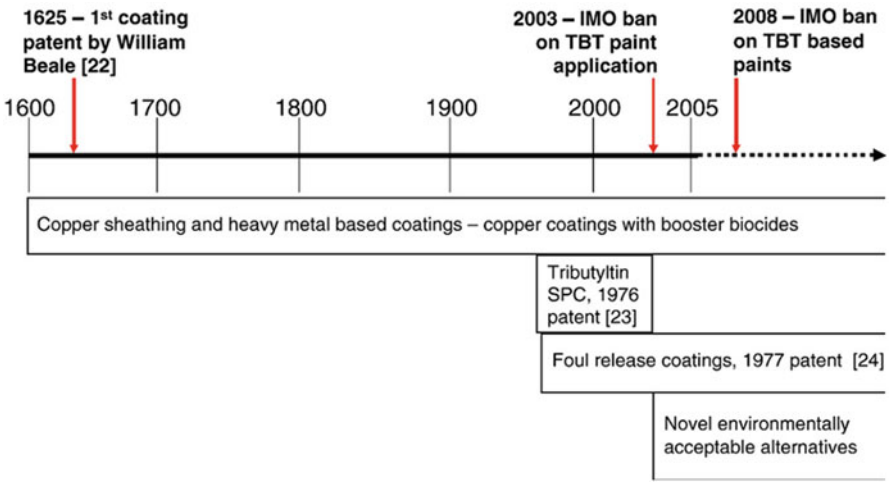

Fig. 10.6 Evolution of the antifouling generations. Reproduced with permission from [94]

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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