Biomaterial Functionalized Surfaces for Reducing Bacterial Adhesion and Infection

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Abstract

This chapter describes the current approaches to reduce bacterial adhesion to various biomaterial surfaces, focusing on nonfouling surfaces through patterning and hydrophobicity plasma-assisted surface treatment and deposition; incorporation of antimicrobials, antibiotics, antibiofilms, and natural extracts that are either immobilized or released; dual function antimicrobial surfaces; incorporation of nonpathogenic bacteria, bacteriophages, and biofilm dispersal agents but also reduced bacterial adhesion through tissue integration. To facilitate the design of new materials, the role of physical, chemical, and biological surface properties on bacterial adhesion is reviewed in each case, as an insight into the chemical and physical cues that affect bacterial adhesion and biofilm formation can provide ideas for creating successful antifouling or antimicrobial surfaces. The application of these surfaces is explored based on the clinical needs and the market gaps. How multidisciplinary research on surface design and engineering may have an impact on both fundamental understanding of bacterial adhesion to biomaterials and applied biomaterial science and technology is finally discussed.

Keywords

Bacterial adhesion • Biomaterials • Surface chemistry • Surface energy • Surface charge • Surface topography • Self-assembly • Plasma treatment • Plasma deposition • Antifouling • Antimicrobials • Antibiotics • Natural extracts • Surface analysis • Adhesion mechanism • Fluid shear

Introduction

Nowadays, irreparable damage to the human body does not necessarily imply functional loss or reduced quality of life. Millions of patients worldwide benefit from permanent implants such as prosthetic joints, dental implants, stents, vascular grafts, and pacemakers, or from temporary inserted devices such as intravascular and urinary catheters. Biomaterial implant and device applications, versatility, and performance represent in many cases a success story [1].

However, a non-negligible fraction of devices fail in practice due to device-associated infections (DAI), which are always connected with microbial contamination of an implant or device, either inferred during surgery or at a later stage [1–4]. Once microorganisms adhere to the biomaterial, they start proliferating

rapidly as biofilms, in which they are protected against both antibiotics and immune clearance [5]. Bacterial species living in a biofilm have great viability advantages requiring 500–5000 times higher doses of antibiotics to get eradicated compared to planktonic organisms [5]. DAI are therefore often resistant to many of the currently available antibiotics and have a substantial and largely unchanged clinical incidence, increased chances for revision surgeries, associated morbidity, and mortality [2–7].

Biomaterial compositions and applications may differ widely, but all attract microorganisms, representing niches for medical device-related infections in vivo. Continued microbial presence interferes with the intended function of an implant or device and adds risks to human use. DAI therefore constitutes one of the key reasons for clinical failure, impaired functionality, and reduced lifetime of medical devices, resulting in high distress for the patients and huge socioeconomic costs [6, 7].

In most cases antimicrobial strategies rely on the systemic administration of antibiotics. However, the extensive use of antibiotics worldwide during the last decades has led to a threatening situation where a large number of bacteria have developed resistance against conventional antibiotics [6–8]. This has therefore resulted in a number of infectious diseases, for which limited treatment exists.

Over the last few years there has been heightened international concern about the growing resistance of bacteria to antibiotics. An example is the methicillin-resistant *Staphylococcus aureus* (MRSA), which is one of the most widespread causes of hospital infections [6]. About a third of people carry some form of *S. aureus* on their skin, where the bacteria do no harm. However, if they enter the bloodstream, they can cause disease. And if the resulting illness cannot be treated because the bacteria are drug resistant, the infection can prove fatal.

Antimicrobial strategies supplemental to systemically administered antibiotics therefore often focus on modifying implant or device surfaces. Nowadays, three surface strategies are mostly employed toward the preparation of antimicrobial surfaces and these are surfaces that hinder bacterial adhesion and biofilm formation (nonfouling), antimicrobial surfaces that kill bacteria either by contact or release of antimicrobial agents and those that have dual function and both kill bacteria and resist bacterial adhesion or kill and release the attached bacteria (Fig. 1).

In this chapter, these strategies for designing antibacterial surfaces with single or dual functionality are introduced, while their inherent advantages and disadvantages are discussed.

The chapter is concluded with a presentation of future research directions for developing antimicrobial surfaces based on clinical needs and market gaps.

Nonfouling Surfaces

Plasma-Assisted Surface Treatment

A wide range of surface treatments have been used to prevent bacterial adhesion to polymers (Fig. 1a). Amongst these plasma processing of the material surface presents many advantages and some of these are (a) its ability to change the substrate

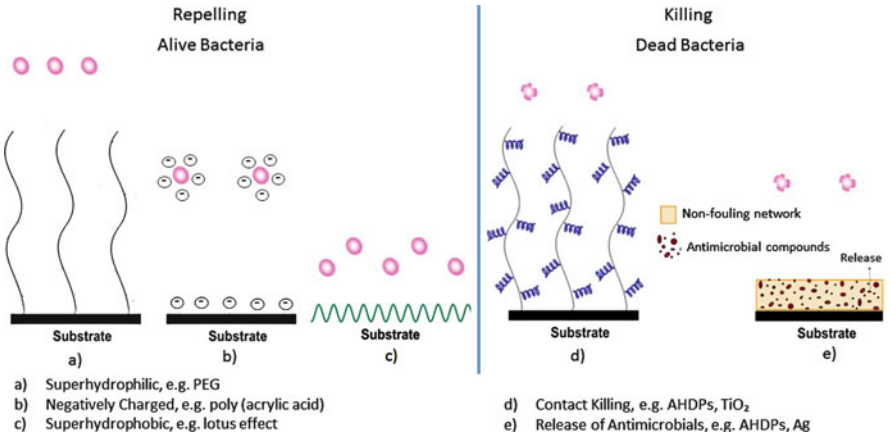

Fig. 1 Types of antimicrobial coatings

surface chemistry without altering its bulk properties; (b) the sterilizing effect of the plasma; (c) ease of process scale-up to industrial scale and shapes. For example, companies such as PlasmaTreat system currently have commercially available atmospheric plasma systems for the activation and coating of surfaces at processing speeds of 25 m/min. Therefore, not only the vacuum but mostly the atmospheric plasma surface modification technologies are readily scalable and raw material costs are relatively low compared with the potential added value that can be obtained using these surface treatments.

Increased material surface energy has been suggested as one way to reduce bacterial adhesion to material substrates and in this direction Katsikogianni et al. examined the effect of He and He/O_2 treatment of PET on the adhesion of *S. epidermidis* and the results showed that the adhesion was reduced on the treated materials in comparison to PET, whereas the aging effect and the consequent decrease in the surface free energy and polar component favored bacterial adhesion [9]. Therefore, the plasma parameters should be chosen in such a way so that the aging effect and the subsequent hydrophobic recovery are minimized. Similarly, Balazs et al. observed that O_2 plasma-treated PVC reduced *Pseudomonas aeruginosa* adhesion as much as 70 % [10]. However, in a recent study Rochford et al. showed that there are controversies concerning the effect of the material surface free energy on bacterial adhesion and this should be taken into account [11]. These controversies may be due to differences in the bacterial strains used or in the experimental conditions, and lead to questions about the applicability of this method for the preparation of antimicrobial substrates.

In the same direction the plasma deposition of PEO-like coatings has been proposed as an effective method for the preparation of surfaces resistant to bacterial adhesion [12, 13]. The long-term stability and performance of protective antifouling layers is, however, questionable. Kingshott et al. showed that physisorbed PEO polymers did not provide lasting reduction in bacterial adhesion, whereas PEO

chains covalently attached to a bulk material showed stable effectiveness [14]. An explanation is that bacteria can displace physisorbed polymer chains from the bulk material surface, whereas covalently surface-grafted polymer chains resist such displacement presenting longer-lasting effectiveness. Toward enhanced coating stability, a number of studies have used plasma polymer coatings as interlayers for the covalent grafting of fouling-resistant polymers or for the deposition of the antifouling polymers [12, 13, 15].

Moreover, plasma deposition of diamond-like carbon [16] and superhydrophobic coatings [17] has been proven to significantly reduce bacterial adhesion in comparison to untreated surfaces.

Surface Charge

The surface charge of the substratum surfaces is another parameter that significantly influences bacterial adhesion and toward material strategies that reduce bacterial adhesion negatively charged substrates may be a way forward (Fig. 1b). Katsikogianni and Missirlis observed that bacterial adhesion was lowest onto the OH-terminated glass which was negatively charged and this because the two tested bacterial strains appeared negatively charged, when bacteria were suspended in 0.01 and 0.1 M PBS [18].

Moreover, Kiremitci and Pesmen showed that bacterial adhesion was reduced on the negatively charged PMMA/AA (acrylic acid), while it was increased on the positively charged PMMA/DMAEMA (dimethylamino ethyl methacrylate) [19]. In the same direction, Tang et al. showed the bacterial antiadhesive properties of polysulfone (PSU) microfiltration membranes modified with poly(allylaminehydrochloride) (PAH)/poly(acrylicacid) (PAA) polyelectrolytemultilayers (PEMs) and this was partially attributed to their negative charge [20].

Surface Topography Modification

In addition to surface chemistry and charge, another factor influencing microbial adhesion is surface roughness and configuration. Surface modifications usually introduce numerous functional groups and chemical cross-links changing not only the surface energy but the roughness and the configuration as well [16, 21, 22]. While the effect of roughness on bacterial adhesion has not been systematically studied, amongst the results that have been reported is that plasma roughened LDPE exhibited higher initial microbial deposition rate than the smooth surface, whereas an inverse effect was found after long-term adhesion [21]. Katsikogianni et al. showed that surface modification by means of plasma for the deposition of CF_4, silver, amorphous carbon, hydrogenated and not, and combination of the latest with silver, changed not only the water contact angle and therefore the surface free energy of the substrates but the configuration and roughness as well [16]. In particular, it was observed that the amorphous carbon coating, which was the material with the highest

surface roughness, prevented bacterial detachment due to enhanced shear rate [16]. Moreover, Katsikogianni et al. showed plasma activation of PET substrate by He plasma changed not only the surface free energy γ_S^{tot} and its dispersive (γ_S^d) and polar components (γ_S^p) but significantly increased the roughness as well, by enhancing the already existing granular structures of PET [21]. It can be concluded therefore that surfaces with similar surface free energy but higher average surface roughness (Ra) values, as these were measured by means of AFM, favored bacterial adhesion in comparison to the ones with lower Ra values [16, 21, 22]. Furthermore, according to Truong et al., roughness variation of titanium substrates at the nanoscale, as this was observed by AFM but not by profilometry, influenced bacterial adhesion, with more bacteria adhering to the rougher substrate (Ra of 1.12 $\pm$ 0.30 nm, in comparison to 0.59 $\pm$ 0.27 nm) [23]. It has been therefore shown that the irregularities of a surface promote bacterial adhesion and biofilm deposition. This may happen since a rough surface has a greater surface area and the crevices in the roughened surfaces provide more favorable sites for colonization [16].

Moreover, the presence of grooves alters bacterial adhesion patterns, depending on the groove size. The synthetic shark skin pattern, for example, has been shown to reduce *E. coli* biofilm formation over a number of days in comparison to flat substrates [24] The widths of the grooves ranging between 10 and 40 μm displayed no effect on bacterial adhesion in some other studies, meaning possibly that bacteria preferentially adhere to irregularities that conform to their size (Fig. 2) since this maximizes the bacteria-surface contact area [25, 26]. Grooves or scratches too small, for the bacterium to fit them, reduce the contact area of the bacterium and hence binding [27].

Toward the preparation of nonfouling surfaces nature represents a source of inspiration particularly in the field of biomimetics, where biological systems are fundamentally studied for their biotechnological applications. Some of the low-adhesive, superhydrophobic, and self-cleaning surfaces found in nature have been investigated for their potentially antibiofouling characteristics [24, 28]. Indeed,

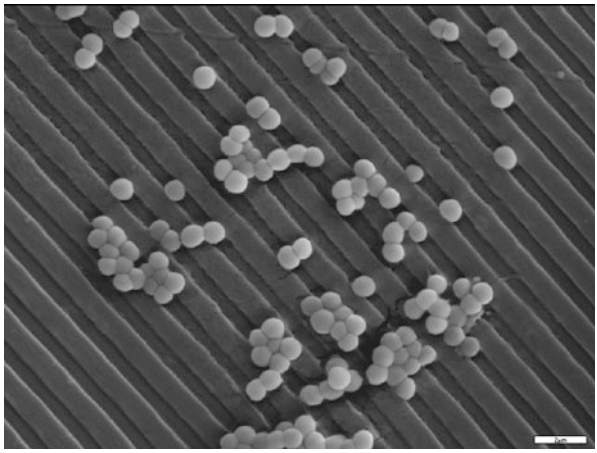

Fig. 2 *Scanning electron microscopy image of Staphylococcus epidermidis* adhering to grooved PCL (Katsikogianni MG and Missirlis YF, Unpublished data)

natural and biomimicked surfaces of insect wings [28], gecko skin [29], and shark skin [24] exhibit antibiofouling through their nanotopology – e.g., nanopillars or grooves – and chemical properties by preventing contaminating particles, algal spores, and bacterial cells from attaching to their surface.

Toward the use of nanotopology for the preparation of antifouling surfaces, a range of different nanofabrication techniques have been used to control surface roughness and configuration at the nm level. The main approaches are based on nanoimprint lithography [30], orientation of thin phase separated block copolymer films, which could subsequently be used as polymer masks/templates for nanolithography [31], self-organization of well-defined, nanosized gold islands onto a substrate [32], and microinjection molding [33]. Although these concepts generally lead to well-ordered surface nanostructures, up to date a high degree of patterning can only be obtained through proper manipulation of thin polymer layers. Moreover, uniform surface patterning is often only observed over a relatively small surface area as the costs associated with using these nanofabrication techniques preclude broader commercial applications outside the semiconductor industries.

Furthermore, it should be taken into consideration that bacterial strains – even within the same species – can vary significantly in size and shape. For a given material surface, different bacterial species and strains adhere differently since different species and strains have different physicochemical characteristics. Therefore, the relationship between roughness/configuration and attachment can be quite complicated and generalization should be avoided.

Additionally, to the best of our knowledge, no material has been developed so far that completely resists bacteria adhesion and the nonfouling surfaces do not actively kill bacteria. Therefore, these surfaces may eventually become contaminated especially due to their deterioration under physiological conditions. For these reasons, surfaces that kill bacteria have been suggested and are introduced in the following section.

Antimicrobial Surfaces

Contact-Killing Antimicrobial Surfaces

Surface Polymerization and Functionalization Toward the Incorporation of Antimicrobial Compounds

In order to kill adherent bacteria, contact-based bactericidal surfaces are coated with antimicrobial agents by either covalent conjugation or physical adsorption. The antimicrobials used in this respect range from synthetic chemicals such as quaternary ammonium compounds (QACs), polycations, and various antibiotics to natural biomolecules such as chitosan and antimicrobial peptides (AMPs).

Toward the immobilization of the antimicrobial compounds a number of methods have been developed and are briefly described below.

Surface polymerization takes place by the polymerization of an antimicrobial compound on the surface via different means such as covalent bonding or atom

radical transfer [34–37]. Surfaces possessing chemically bonded hydrophobic polycations of quaternary ammonium salts have been found to possess bactericidal properties [34]. Lee et al. have used an atom transfer radical polymerization (ATRP) approach to modify surfaces with quaternized ammonium groups or host (antimicrobial) defence peptides [35, 36]. This method shows a permanent antibacterial effect because the surfaces can be reused without loss of activity [35, 37]. Nevertheless, the commercial applications of this manufacturing method are still in development and require more investigation before they can be applied to wide-scale industrial implementation as it is time consuming and requires a number of steps.

Plasma polymerization is well suited toward the deposition of adhesive interlayers for the covalent surface immobilization of antimicrobial organic molecules. In contrast to ATRP, plasma polymerization is easy to be used and scaled up toward the deposition of coatings with good adherence on most substrate materials it provides reactive chemical surface groups, for covalent grafting, that are not available on the underlying bulk material/device [38]. Toward surface functionalization, surface treatments that involve the use and immobilization of antimicrobial compounds such as antibiotics, cationic compounds, and natural antimicrobials have been used effectively to prevent bacterial adhesion. In this direction, antimicrobial molecules that contain chemically reactive groups such as hydroxyl, carboxyl, amino, etc. can be covalently immobilized onto plasma polymer surfaces or ATRP, using well-known facile chemical interfacial reactions as described below.

Antibiotics

The application of commercial antibiotics onto the material surface is one way against bacterial adhesion. In order though to enhance the long-term stability and the effectiveness of the products, it has been suggested that the antibiotics should be covalently grafted on the surface and in this direction plasma pretreatment of the substrate has been used in order to enable the grafting of commercially available antibiotics [38]. Although effective, the ongoing presence of antibiotics promotes the development of resistant microbial strains [39].

The issue of selecting resistant bacterial strains through an excessive use of antibiotics is one of the main driving forces behind research into new antibacterial substances such as cationic compounds, synthetic and natural, but also natural antimicrobials.

Cationic Compounds

A number of cationic surfaces have been found to possess antibacterial activity in vitro. While the mechanism of action is not fully understood, the leading hypothesis is that the cationic chains attract the negatively charged bacterial cells, as described in the section about the effect of the surface charge on bacterial adhesion, and penetrate the cell membrane causing loss of the membrane integrity.

For the covalent grafting of various cationic compounds such as quaternary ammonium compounds (QACs) [34], cationic peptides such as melittin [40], host defence (antimicrobial) peptides (HDPs) [41] or chitosan [42], and cationic proteins

such as lysozyme [43] a number of studies have used plasma polymer coatings as interlayers or the ATRP method.

The difficulty, however, is to develop a readily scalable process to apply these chemical functionalities, as adherent coatings, while the main concern remains the cytotoxicity of many of these compounds, especially of QACs, or the effectiveness of others such as chitosan. For this reason the use of natural antimicrobials is further explored.

Natural Antimicrobials

Antimicrobial peptides (AMPs) and HDPs represent natural alternatives to traditional synthetic biocidal compounds. In particular, HDPs not only deactivate bacteria in biofilms at low concentrations [44] but also modulate the innate and adaptive immune responses, promote wound healing, inhibit proinflammatory responses to bacterial lipopolysaccharide, prevent biofilm formation through multiple mechanisms, or specifically kill bacteria within a biofilm [41].

These biomolecules can be immobilized on supporting surfaces either physically [45] or chemically [41] to fabricate bactericidal coatings with a broad spectrum of antimicrobial activity, high efficacy even at low concentrations, and a lack of susceptibility to bacterial resistance.

Apart from the AMPs and HDPs, the use of extracts from plants and herbs as well as of honey as a traditional remedy for bacterial infections has been known since ancient times. The antimicrobial compounds in plant materials are commonly found in the essential oil fraction of leaves, flowers or buds, seeds, and fruits [46]. The bioactive compounds found in plant extracts can be divided into several categories. Various phenols and phenolic acids, quinones, flavonoids, flavones, flavonols, tannins, coumarins, terpenoids, alkanoids, lectins, and polypeptides have been found to exert a broad spectrum of biological activities, including antimicrobial properties [47]. The mechanism of the antibacterial action of these substances remains largely unknown. However, recent studies suggested that inhibition of nucleic acid synthesis, binding to cell wall, disruption of the microbial membrane, interference with the two bacterial cell communication strategies of quorum sensing, and swarming or inactivation of bacterial adhesins, enzyme, and cell envelope transport proteins may be the primary causes of the antibacterial character of at least some of these compounds [47].

However, most of the active compounds found in the natural antimicrobials, such as furanones, do not possess convenient chemical groups for interfacial covalent bonding and they should therefore be linked using less common chemical strategies.

In agreement with the results observed using the self-assembled monolayers (SAMs) [18], hydroxyl groups are essential for the antimicrobial function. Therefore the furanone ring structure and the phenolic hydroxyl group in the case of serrulatanes should remain away from the substrate and undisturbed when covalent immobilization is attempted. For this reason copious pathways have been suggested that are slow and the antibacterial activity is less upon functionalization [48].

The difficulty therefore for the preparation of contact-active antimicrobial surfaces is to develop a readily scalable process to apply these chemical functionalities in a continuous process onto a wide range of materials. The surfaces that release antimicrobial agents appear as an alternative and are briefly described below.

Surfaces that Release Antimicrobial Agents

Incorporation of Antimicrobial Compounds

As in the case of contact-killing antimicrobial surfaces, these surfaces incorporate antimicrobial compounds such as antibiotics, natural antimicrobials, and metal ions, which are progressively released as described below.

Antibiotics

There are several types of release-based bactericidal surfaces that use antibiotics such as tobramycin incorporated into biomimetic hydroxyapatite coatings on titanium [49], gentamicin-loaded bone cements, and gentamicin sandwiched between titanium and PLGA overcoat [50]. Although effective, the release of antibiotics is quite difficult to be controlled over time and the ongoing release of antibiotics promotes the development of resistant microbial strains, which is one of the most important problems facing modern medicine [39]. The issue of selecting resistant bacterial strains through an excessive use of antibiotics is perhaps the main driving force behind research into new antibacterial substances.

Natural Antimicrobials

As described in the contact-killing antimicrobial surfaces section, there are a number of natural antimicrobial compounds such as AMPs, HDPs, and compounds found in plant extracts that can be released from various surfaces providing antimicrobial properties [45].

As observed in our recent study the release of HDPs such as the IDR-1018 or E6 at low concentrations (16 μg/ml) was sufficient to kill *Staphylococcus epidermidis* in an overnight formed biofilm (Fig. 3a, b), while nisin, a commercially available AMP, was not as effective (Fig. 3c) [44].

In addition to plant extracts, honey has been widely reported to exhibit antibacterial activity and a honey-infused bandage called Medihoney was granted FDA approval. Several studies have shown that certain honey types possess an antibacterial activity which persists even after removal of hydrogen peroxide by catalase. In particular it has been reported that Manuka honey, derived from the Manuka tree, has a very high level of antibacterial activity based on the 1,2-dicarbonyl compound Methylglyoxal, and its antibacterial action is due to its effect on the DNA, RNA, and protein synthesis in bacterial cells, while the common bacterial strains *Escherichia coli* and *Staphylococcus aureus* do not develop noticeable resistance against these surfaces [51].

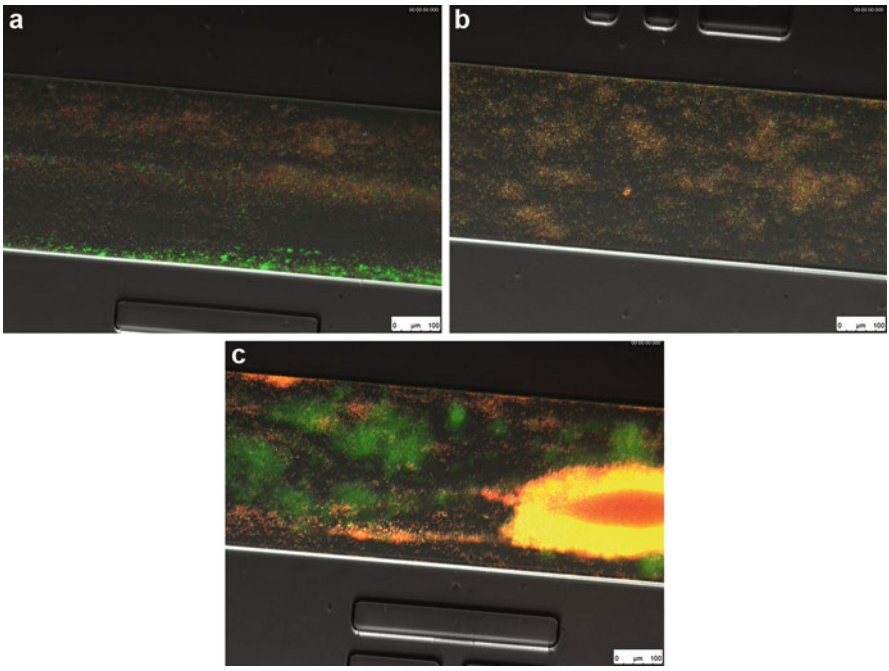

Fig. 3 Effect of 16 μg/ml of (**a**) E6, (**b**) IDR-1018, (**c**) Nisin on *S. epidermidis* overnight formed biofilms, after 3 h of exposure to the antimicrobials at 50 s^{-1} (Katsikogianni MG, Hancock REW, Devine DD, Wood DJ, presented in [44] and unpublished data)

However, honey has not been applied as a thin coating, reducing the cost and enhancing the mechanical properties and the stability of the coating, while, as in the case of antibiotics, the difficulty is the controlled release over a period of time and the fact that the surfaces lose their antimicrobial capacity after the completion of the antimicrobial compound release.

Metal Ions

Due to its antimicrobial properties silver has been widely applied on a number of commercially available products ranging from wound dressings to clothing, vascular and urinary catheters, and other medical devices. The problem, however, is the toxicity of the released silver into the environment and there have therefore been calls to severely limit its application [52]. Moreover, silver is an expensive element that compromises the optical properties of the final material.

A number of other metal ions are also known to possess antibacterial activity and their release from polymeric coatings can analogously be used to achieve short-term prevention of bacterial adhesion to materials. Copper [53] and ZnO [54] have been deposited by sputtering, ion implantation, plasma-enhanced chemical vapor deposition, or through microwave-plasma synthesis. As in the case of silver though, toxicity effects need to be considered.

Dual-Function Antimicrobial Surfaces

Dual-Contact and Release-Based Antimicrobial Surfaces

Nowadays, antimicrobial surfaces that rely on the usage of two different antimicrobial compounds incorporated into one system have been designed so that they operate through both contact-based and release-based mechanisms. These surfaces are of particular interest because they can provide long-term antimicrobial efficiency by killing both planktonic and attached bacteria. Such an example is the development of a coating that is composed of two layered functional regions; a polyelectrolyte multilayer reservoir for the loading and release of bactericidal silver nanoparticles (AgNPs) and a silica surface with immobilized QACs [55] Dual-function coatings of this type show high initial bacteria-killing efficiency due to the release of silver ions while they retain significant antimicrobial activity after the depletion of the embedded AgNPs due to the immobilized QACs.

As in the case of surfaces that release the associated active ingredients (silver, chlorohexidine, etc.) this results in environmental contamination and a growing microbial resistance.

Dual-Contact Killing and Nonfouling Surfaces

This type of dual-function antibacterial surfaces is based on the combination of antimicrobial and nonfouling properties. These surfaces can be divided into four categories based on the method used to incorporate the antimicrobials into nonfouling materials. The antimicrobials can be tethered to nonfouling hydrophilic polymers, embedded in a superhydrophobic coating, deposited as layers in between nonfouling layers, or stored in a nonfouling matrixes and progressively released.

Tethering of Antimicrobial Compounds to Nonfouling Hydrophilic Polymers

Hydrophilic polymers are widely used as spacers for the immobilization of bioactive molecules such as antimicrobial compounds, to create biofunctional surfaces because they are capable of resisting nonspecific protein adsorption as well as bacteria and cell adhesion, reducing unwanted biological responses, they can maintain the bioactivity of the antimicrobial compounds, and they can enhance the accessibility of the antimicrobial compounds to targets through their long chains [56]. In recent years, several hydrophilic polymers have been used for the attachment of antibiotic molecules to create antibacterial surfaces with both bactericidal and nonfouling properties. PEG is such a polymer and several antibiotics, including penicillin and gentamicin, can be immobilized on this, along with two different antibiotics such as penicillin and gentamicin simultaneously, using two different types of conjugation chemistry and aiming at simultaneously resisting the growth of their target bacteria strains, Gram-positive and Gram-negative [57]. One main drawback of antimicrobial surfaces based on PEG is that the concentration of

antimicrobial molecules that can be attached is quite limited as each PEG chain has only one functional group at its free end. To increase the density of binding sites for active antimicrobials, such as peptides, more attention has been paid to the application of comb-like polymers with side chains and the ATRP method (as shown in Fig. 1d) [36].

Layer-by-Layer Deposition of Nonfouling Layer and Antimicrobial Layer

The layer-by-layer (LbL) deposition of polyelectrolytes on charged surfaces offers another approach to simultaneously reducing bacterial adhesion and killing adhered bacteria onto a surface. In this method, antimicrobial compounds and nonfouling agents with opposite charges are physically adsorbed onto substrates alternately to form a multilayer film. This method presents advantages such as simplicity, low operating costs, and control over film thickness. A typical dual-function antibacterial surface prepared by the LBL method is based on chitosan, a cationic antibacterial molecule, and heparin, an anionic antiadhesive molecule. The results showed that the chitosan/heparin multilayer modified surface significantly reduced bacterial *E. coli* adhesion and killed the adherent bacteria [58].

Combination of Super Hydrophobic Surface and Antimicrobial Compounds

Super hydrophobic surfaces provide a biomimetic approach to significantly reducing bacterial attachment [17, 28, 29]. Although super hydrophobic films or coatings are effective in inhibiting short-term bacterial adhesion, it should be noted that their nonfouling properties may gradually deteriorate after exposure to complex environments. The combination of a super hydrophobic surface as a protection layer and the presence of antimicrobial compounds may provide a solution to this drawback. The combination therefore of super hydrophobic surfaces and antimicrobial compounds is an effective strategy toward antimicrobial surfaces that both resist and kill bacteria. Such an example is a novel lotus-leaf-like antimicrobial film which was produced by loading mesoporous silica microcapsule-supported AgNPs on a fluorosilicone resin film, followed by hydrophobic surface modification [59]. The combination of the hierarchical micro-/nanoscale structure of the mesoporous silica microcapsules and a surface coating with low surface energy reduced bacterial adhesion, while the silver ions reduced bacterial viability for both planktonic and attached bacteria [59].

Nonfouling Polymers that Release Antimicrobial Compounds

Most antimicrobial surfaces based on the contact-killing mechanism can effectively kill bacteria attached to surfaces, but they have limited antimicrobial activity against planktonic bacteria. On the other hand, the controlled release of antimicrobial agents from surfaces can be used to reduce microbial colonization on surfaces and inhibit the proliferation of planktonic bacteria. To achieve both bulk antimicrobial and surface nonfouling properties, we recently examined the incorporation of silver in organic coatings that were plasma deposited (as shown in Fig. 1e) and the results showed that such a system can significantly decrease the bacterial adhesion but also viability with time, in comparison to the organic coating alone [60].

Dual-Function Contact-Killing and Bacterial Released Surfaces

The third type of dual-function antimicrobial surface is based on the combination of antimicrobial compounds and bacteria-release properties into one system. Common contact-killing antimicrobial surfaces suffer a serious problem associated with the accumulation of dead bacteria and other debris, which not only degrades the antimicrobial activity but also provides nutrients and sites for other bacteria to attach. Toward maintaining the surface antimicrobial properties over long periods of time, it would be beneficial to be able to remove or release the killed bacteria from surfaces.

In this direction, Yu et al. developed a new platform exhibiting switchable bioactivity based on nanopatterned PNIPAAm brushes. The temperature-induced conformational changes of the nanopatterned PNIPAAm brushes provide the capacity to spatially control the conformation of biomolecules between brushes, leading to an ON/OFF switch for surface bioactivity [61–63]. Two antimicrobial compounds, QACs [61] and lysozyme [63], have been immobilized in the polymer-free regions between nanopatterned PNIPAAm brushes. Above the lower critical solution temperature (LCST) collapsed PNIPAAm chains facilitated the attachment of bacteria and exposed the antimicrobial compounds that kill adhered bacteria. Upon decreasing the temperature below the LCST, swollen PNIPAAm chains promote the release of dead bacteria.

Bacterial Interference

Improving materials antimicrobial performance and cost-effectiveness, while meeting environmental and toxicity requirements, is nowadays also being explored through alternative approaches to traditional antimicrobial agents and in particular through "bacterial interference." The concept that bacteria can actively inhibit one another while competing for resources in the same environment could be explored toward the preparation of materials that either kill or resist the attachment of pathogenic bacteria. This approach may be explored to prevent infections related to the use of devices implanted in areas normally occupied with microflora, such as urinary catheters or dental implants.

Trautner et al. employed the concept of "bacterial interference" to prevent catheter-associated infection, using a strain of *Escherichia* (*E.*) *coli* that lacks the virulence factors for infection but colonize the catheter material [64]. This *E. coli* strain, which was grown in a biofilm on catheters before implantation, prevented the adherence of pathogenic gram-positive, gram-negative, and fungal organisms in vitro, and was successful in patient studies [64].

In the same direction, *Lactobacillus acidophilus* was encapsulated into nanofibers of various polymers, toward the development of biohybrid nanowebs that could potentially treat bacterial vaginosis, through the delivery of the probiotics, however its effectiveness against pathogens has not been tested [65].

An oral probiotic organism *Streptococcus* (*S.*) *salivarius* may be used toward the prevention of pathogenic colonization of dental implants, as it has been shown to

inhibit the growth of oral pathogens such as *Streptococcus mutans* [66]. Its potential though has not been as yet explored for the purpose of precoating dental implants to prevent pathogenic colonization, while the effect of probiotics on tissue integration is unknown. Utilizing therefore probiotic bacteria and bacterial interference appears as a new and exciting approach for protecting biomaterials from pathogenic infection, for at least some particular applications that the biomaterial does not need to integrate, e.g., catheters.

Biofilm Dispersal Agents

Biofilm dispersal agents are molecules that either prevent the formation of biofilms by inhibiting their growth or inducing biofilm bacteria to detach and return to the planktonic state. A number of studies have shown that bacteria naturally produce biofilm dispersal agents when their quorum sense signals biofilm bacteria to detach toward colonizing new sites, and these agents include D-amino acids and naturally occurring peptides and enzymes amongst others [67, 68]. D-Amino acids, particularly D-tyrosine, D-tryptophan, D-leucine, and D-alanine, are particularly attractive as dispersal agents, as they are inexpensive and potent in combination [67]. Apart from dispersal agents' immobilization, current research aims at developing vehicles, such as polymer sponges and biodegradable microspheres, for their controlled and sustained release.

Bacteriophage Releasing Materials

Bacteriophages, and especially the ones that have been engineered, are viruses that can directly lyse bacteria as well as penetrate and destruct a biofilm, by cleaving the polysaccharide components, while maintaining their efficacy [69]. Materials science approaches have been employed toward the delivery of bacteriophages to destruct biofilms. Bacteriophages have successfully been immobilized on modified silicon surfaces [70] and once incorporated or delivered, the ability of bacteriophages to lyse both bacteria and biofilm components can be used to treat or prevent biofilm formation. Bacteriophages could therefore have great potential in preventing or treating infections, but due to their specificity, it may be difficult to use them as broad-range antimicrobials and translate this technology for biomedical applications.

Tissue Integration

Another approach that appears promising against medical device-associated infections is that of tissue integration. Clinically, oral wounds heal faster in comparison to epidermal ones [71, 72] with saliva promoting healing by containing an abundance of growth factors such as epidermal, fibroblast, nerve, and transforming growth factor (TGF)-α, maintaining host cell viability and proliferation [73]. The fast

healing of oral tissue in the continuous presence of commensal bacteria and opportunistic pathogens enables the formation of a soft tissue seal around the implant and this seems to offer protection of the osseointegrated part against invasion by periodontopathogens [74]. In particular, the presence of *Streptococcus mutans* seems to increase β1 integrin expression in periodontal ligament fibroblasts [75], leading to thoughts that bacterial presence and stimulation, without the development of infection, may improve healing that reduces infection risk and associated failure over time.

In this direction, surfaces with multiple functionalities that reliably select host cells and therefore tissue integration over microbes have been suggested and an example is the simultaneous incorporation of RGD, that enhances cell adhesion, and a HDP that reduces bacterial adhesion and viability [44]. Our preliminary results show promising signs for the use of RGD in combination with HDPs toward the preparation of antimicrobial materials that allow tissue integration [44]. Orthopedic and dental implants would therefore be a great application for this kind of combined HDPs/RGD systems, as the soft tissue seal represents an important barrier that provides implant with protection from pathogens and therefore its restoration seems to prevent infection.

Examples of Orthopedic and Dental Implants Used at Present

Clinically available orthopedic implants encompass a variety of materials: titanium, stainless steel, and poly(methyl methacrylate) (PMMA), and are used for a range of applications, such as fixation devices and osseointegrated implants, bone cements for arthroplasty, and antibiotic carriers [76]. In the past their properties were varied, toward enhanced osseointegration, by mainly varying the size, shape, topography, roughness and configuration, or the material itself.

While these materials have been successful in many cases and meet important medical needs, infection remains a major impediment to their long-term use. A recent study showed that PMMA is the most susceptible to bacterial colonization, followed by stainless steel, then titanium [76]. Moreover, there are studies that show that PMMA and antibiotic-loaded PMMA beads, a common clinical method for local antibiotic delivery that treats or prevents infections after orthopedic implantation, can host bacteria that cause acute, chronic, and delayed-onset infections [77, 78]. Furthermore, there are studies suggesting that bioactive substances such as hydroxyapatite may be more prone to bacterial adhesion than bioinert metals, such as titanium alloys and stainless steel [79]. Therefore, the choice of biomaterial in the case of orthopedic implants is not an easy-made decision and nowadays there are other vehicles for the controlled local delivery of antibiotics as described below.

In parallel, dental implants are one of the most common types of implanted devices that restore both function and aesthetics. These implants have three basic components: the implant screw, the abutment, and the crown. The implant screw interfaces with the craniofacial bone, the abutment is at the junction of the bone and

soft tissue, and the crown is eventually placed over the abutment. In the two-piece implantation system, the abutment is connected to the implant screw after some months, whereas the one-piece system has the abutment and screw fused together and implanted during the first procedure. While there are studies showing no histological differences in lesions between two-piece and one-piece systems in canine, there are other studies showing more inflammation and greater bone loss in the two-piece system [80].

The fact that dental implants are in contact with the oral flora, a microbiome consisting of more than 700 different bacterial species, makes them more vulnerable to infection [81]. Chronic bacterial infection associated to the use of dental implants is known as peri-implantitis, which is defined as an inflammatory reaction in the oral cavity with loss of supporting bone in the tissues surrounding an implant. Dental implant failures therefore refer to the disruption between mineralized bone and an implant, and recent data show that peri-implantitis affects 20 % of patients and 10 % of implant sites, making it a serious challenge in long-term implant dentistry [82]. Following peri-implantitis, bone resorption, and soft tissue damage at the implant site makes the replacement of the implant a real challenge, while the replacement has low survival rates. Designing therefore implants that are less prone to infection is a clinical need.

Over the last two decades, as in the case of orthopedic implants, the properties of dental implants that mainly varied was the roughness and configuration, along with the shape and size. As discussed in the "Surface Topography Modification" section, the studies that investigate the effect of roughness on bacterial adhesion present results that in some cases appear controversial [23]. In particular, it was observed that supragingival biofilm accumulation was increased on the rougher surface, but no difference was observed subgingivally [83]. In a canine model, surface roughness had no effect on plaque formation, inflammatory lesions, soft tissue, or microbial attachment and species [84]. However, in another canine model that peri-implantitis is allowed to continue spontaneously developing after ligature-induced inflammation [85], greater plaque formation, and bone loss was observed in the case of a rough porous titanium oxide surface [86].

Apart from metals, bioceramics have been of particular interest to researchers. Hydroxapatite (HA) has long been investigated and as a result is the most widely used bioceramic in medicine and dentistry due to the strong affinity to bone tissue. This property improves the implant-bone interface and thus favors early osseointegration [87]. HA though does not present antimicrobial properties and its use has declined due to reports of HA coating delamination from oral implants, resulting in poor performance and uncertain long-term success [88]. Likewise, comparing materials of different surface coatings, such as hydroxyapatite, sprayed titanium, and titanium alloy, has shown little difference on bacterial load [89].

The literature therefore suggests that more complex strategies and highly controlled studies are necessary for the prevention of peri-implantitis and orthopedic implants associated infections. In this direction, the following antibiotic delivery systems have been suggested and are currently used in the clinics.

Bone Graft-Based Delivery Vehicles

Bone graft materials have been suggested as antibiotic carriers. Autologous bone graft, demineralized bone matrix (DBM), but also Calcium sulfate can be mixed with antibiotics during surgery as an easy and efficient way to deliver antibiotics locally [90]. Incorporation of antibiotics into autologous bone graft is convenient, but release is not sustained. Approximately 70 % of incorporated antibiotic is released after 24 h with negligible release after 7 days [95]. Similarly, DBM releases approximately 45 % of its drug load after 24 h with negligible release after 7 days [91]. Calcium sulfate appears as a better option since it is resorbable and osteoconductive [90]. In particular, it has been shown that bactericidal levels of tobramycin elute from calcium sulfate over 14 days, but levels are sub-bactericidal by day 28, pointing this as a drawback due to the increased chances of antibiotic resistance development [90]. While therefore mixing bone graft materials with antibiotics is a simple process that takes place during the surgery, the release kinetics from these materials point the need for more sophisticated systems. In this direction, the use of synthetic and protein-based systems is suggested and discussed below.

Synthetic/Protein-Based Delivery Vehicles

As discussed earlier, nondegradable PMMA has been a popular carrier of antibiotics due to the ease of antibiotic incorporation, and the FDA has approved products that use PMMA as an antibiotic carrier. However, release of antibiotic from PMMA occurs in a nondesirable early "burst" followed by negligible release after the first few days [92]. To overcome the issue of "burst" release, degradable polymer matrices have been suggested as antibiotic carriers due to their ability to provide controlled release, preserve the bioactivity of the drug, and release almost all the incorporated drug. Some examples of biodegradable polymer carriers include poly (lactic-*co*-glycolic acid) (PLGA), poly(ε-caprolactone) (PCL), poly(DL-lactic acid) (PLA), and combinations of the above. These polymers are most commonly used to entrap antibiotics in microcapsules, microspheres, or electrospun fibers [93]. In contrast to antibiotic-loaded PMMA, antibiotic release from these constructs is on the timescale of weeks; a coelectrospun collagen and PLA carrier for gentamicin was capable of releasing antibiotics over at least 2 weeks [94], while PLGA microspheres have been able to maintain antibiotic release up to 35 days [95]. However, degradable polymers for antibiotic delivery are still in the investigational stage, and have been mostly used for the delivery of other types of drugs.

Protein-based materials for antibiotic delivery include collagen, gelatin, and fibrin glue [96, 97]. For the purposes of orthopedic drug delivery, collagen sponges are the most commonly used vehicle clinically, as they are flexible, can cover infected areas, and have excellent biocompatibility [98]. In a rat osteomyelitis model, gentamicin-loaded collagen sponges performed better than gentamicin-loaded PMMA [96]. However, even these sponges typically release most of the drug load within the first few hours, resulting in a large amount of drug initially with very little

sustained release after 7 days [99], requiring the design of systems that will release the antibiotic in a more controlled way.

Concluding Remarks and Future Perspectives

General Healthcare Market and Medical Devices

An overwhelming need to reduce the hospital-acquired infection rates is a serious concern in the health care industry which suggests the use of antimicrobial coatings. In most of the cases the hospital-acquired infections are associated to the use of medical devices and in the USA 40 % of all hospital-acquired infections are related to urinary catheters, while about 3 % of those ultimately result in mortality. According to the CDC report, urinary catheter infections affect 10–50 % of patients undergoing short-term catheterization (7 days) and virtually all undergoing long-term catheterization (28 days) [100].

According to the BCC Report (2012) the global medical device coating market reached $4.8 billion in 2010 and $5.4 billion in 2011 and is expected to grow to nearly $8 billion in 2017, a 5-year compound annual growth rate (CAGR) of 6.7 %, with North America and the European Union accounting for the vast majority of this market [101].

In particular the United States market for medical device coatings reached $2.7 billion in 2011, $3 billion in 2012, and should total nearly $4.4 billion by 2017, a 5-year CAGR of 8.1 %. The European Union market for medical device coatings reached $1.3 billion in 2011, $1.4 billion in 2012, and should surpass $2 billion in 2017, a 5-year CAGR of 7.0 %.

Therefore, the antimicrobial coating market is expected to grow with the increasing need to address microbial growth in end-application markets like the health care facilities. In this direction the antimicrobial coating suppliers, such as Smith and Nephew, SurModics Inc., Nanophase Technologies, and AcryMed Inc., are expected to focus on research and development activities to create competitive products, and offer extended product lines that provide a broad-spectrum application reach in health care facilities.

Regarding the medical devices, their compositions and applications may therefore differ widely, but all attract microorganisms. The antibacterial surface modification technologies could therefore have potential application for a wide range of implantable and non-implantable medical devices. The implantable devices include catheters (intravenous, urethral), stents (ureteral, prostatic, biliary, coronary), shunts, endotracheal tubes, lenses (intraocular), and the like. The nonimplantable devices category includes syringes, forceps, clamps, dressings, device packaging, etc. In addition to antibacterial applications of the surface modification technology the adhesion of cells or proteins onto biomaterials could also be enhanced or reduced by tailoring surface structure and chemistry.

Selecting some applications to help demonstrate the scale of the medical device market, the case of catheter-associated urinary-tract infections is highlighted

[102]. In 2002 there were 424,060 urinary-tract infections in the USA alone and it is reported that the costs associated with this type of infection are between $589 and $758 per infection [103]. In the case of permanent, totally internal devices, these face two challenges with respect to their extended use in vivo: medical device-associated infections and lack of native tissue integration.

In the case of antimicrobial wound dressings, Frost & Sullivan predict that the market will continue to grow at a rate in the USA of over 15 % annually up to 2017. The total Advanced Wound Care Market is expected to reach $3650 M in 2017 with a CAGR of 9.6 % [104].

In the case of bone and dental implants the infection incidence has been reported as 1–10 %, depending on the application. Although hip and knee joint replacements have a relatively low infection rate (2–4 %), the open fractures and especially those of the tibia that are more common than in any other long bone can be infected at a rate of up to 55 % [105]. Rate of tibial diaphysis fractures reported from 2 per 1000 population to 2 per 10,000 and of these approximately one fourth are open tibia fractures [106]. Health economic studies showed that in the UK £4.3 billion was spent on orthopedic and dental disease and trauma between 2011 and 2012 [107]. Nowadays, 230,000 fractures are recorded per year in the UK, many of these related to osteoporosis, and by 2016 are expected to be over 384,000 fractures per year. As our life expectations are increasing [108], these numbers are expected to further increase. If we also take into consideration the fact that the average costs of combined medical and surgical treatment of bony infections are as high as $25,000 per case [109], it becomes obvious that there is a great clinical need, commercial potential, and market gap that demands antimicrobial strategies.

Antimicrobial Strategies

Toward the fabrication of antimicrobial surfaces a large amount of research work has been done utilizing various materials such as polymers, metals, ceramics, glass, and composites, various bacterial strains-species and concentrations, but also experimental procedures; static and dynamic conditions amount of time and environmental parameters such as temperature and humidity.

From the results obtained using a number of surfaces it seemed that bacteria preferentially colonize surfaces that have lower surface energy and polar character. Taking the topography into consideration, it appears that increased roughness at the nano and microscale and especially irregularities that conform bacterial shape increase bacterial adhesion.

Therefore, surfaces that present OH- groups appear more resistant to colonization and therefore surface modification in this direction using either chemical or natural extracts seems a promising way to prevent biofilm formation. The use of natural extracts is not based on a single pharmaceutical agent or biocidal activity and therefore common bacterial strains have not developed noticeable resistance against these surfaces.

In the case of antimicrobial surfaces, an increase in roughness and positive charge would possibly enhance the antibacterial properties of the surface, by killing the more attached bacteria to their increased surface area.

Apart from the surfaces that present one function against bacteria either nonfouling or bactericidal, over the past few years, a significant number of dual-function antimicrobial surfaces have been suggested for the prevention of initial bacterial attachment and biofilm formation. These surfaces combine the antimicrobial activity through the presence of one or more antimicrobial compounds and the bacteria-resistant or bacteria-release capability accompanied by the use of certain materials, usually functional polymers. The dual-function surfaces present advanced properties compared with conventional antimicrobial surfaces with a single functionality.

Although a large amount of work has been done and considerable progress has been made in this area with promising experimental results, many challenges in both fundamental science and applied technology remain, and further efforts are required toward the optimization of the design and the fabrication process.

Regarding the dual-function antimicrobial surface multifunctionality, kill and resist, or kill and release, is achieved by two or more functional components. However, antimicrobial activity, through the use of various compounds, and bacteria-resistance/bacteria-release properties usually compromise each other. Therefore, the composition of the surfaces must be optimized to obtain the highest performance.

Apart from the surface chemistry, the integration of surface micro- and nanotopography has been suggested and it was briefly described above. Mimicking nature to provide engineering solutions offers a model for the development of functional surfaces with special antimicrobial properties. For example, bio-inspired structures that mimic shark skin and lotus leaves endow synthetic surfaces with effective nonfouling – bacteria-resistance properties. It will be interesting therefore to explore whether the integration of surface topography, especially on the nanoscale, into existing multifunctional antibacterial surfaces yields novel properties and the impact it has on other biological responses [110].

Depending on the application, apart from the antimicrobial properties, addition of other functionalities may be required so that synthetic surfaces exhibit specific properties. To date, a few research groups have incorporated additional functional groups into antibacterial surfaces to improve other specific properties and achieve better performance. For blood-contacting devices, hemocompatibility is a property that has to be present [111]. For orthopedic and dental implants, surfaces should inhibit bacterial colonization but promote osteoblast adhesion and this may be realized by the addition of both host defence (antimicrobial) peptides and RGD [44].

Parameters to be Taken Into Consideration

As mentioned earlier, many challenges in both fundamental science and applied technology remain, and further efforts are required toward the optimization of the

design and the fabrication process of antimicrobial surfaces especially if these need to present additional functionalities.

Since bacterial adhesion is a very complicated process affected by many factors, such as bacterial-material properties and environment, more investigations are still needed to advance our understanding of the mechanisms of bacterial adhesion and to attain appropriate methods to prevent them from happening.

To complicate matters even more, it needs to be mentioned that many bacteria are able to sense and respond to surfaces and environmental signals using mechanisms that remain poorly understood. Most of the research so far has been conducted to investigate bacterial responses to soluble biochemical factors, such as growth factors and bacterial density [112], salts, ethanol, iron, nutrient-limited factors and heat [113, 114], and to low-energy pulsed ultrasonic simulation [115]. The results have shown that there is an increase in biofim formation when bacteria are under stress due to various environmental factors that were altered in order to examine how bacteria-material interactions are influenced by their surroundings.

In our recent studies it was observed that not only bacterial adhesion but PIA, slime production, and biofilm formation were much higher on the CH_3-teminated glass than on the OH-terminated one, for four *Staphylococcus epidermidis* strains, and this was in agreement with the *icaA* and *icaD* gene expression results that showed increased expression for the bacteria adhering to the CH_3-terminated substrate, especially under the higher shear rate [116]. In addition, it was observed that *Staphylococcus epidermidis* strains *icaA* and *icaD* gene expression and slime production were enhanced by silver ions that were released at sub-bactericidal concentrations, under high shear rate conditions [60]. This shows that the release of the antimicrobial compound has to be designed very carefully so that it kills remaining bacteria and it prevents this kind of bacterial responses.

Moreover, surface chemical modifications often lead to surface heterogeneity and increased surface roughness. Trace impurities in many of the polymers used and coating defects result in uncertainties.

In the area of applied technology, the main difficulty in applying these antimicrobial strategies along with other functionalities, depending on the application, is to develop a readily scalable process to apply these functionalities, as adherent coatings, in a continuous process onto a wide range of polymers. Moreover, the main concern remains the toxicity of many of these compounds. It should be noted that, for in vivo biomedical applications, the toxicological effects of antibacterial surfaces should be determined first and that the biocompatibility of these surfaces must be improved. The fabrication of these surfaces should be low cost and reproducible.

Therefore, a rigorous study of the effects of surface chemistry/topography on bacterial adhesion and protein adsorption under conditions relevant in vivo remains a prerequisite for the understanding of the bacterial adhesion mechanism and toward the design of both nonfouling and antimicrobial materials, pointing the importance of the detailed surface analysis to ensure reliable interpretation of biointerfacial interactions.

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