The Importance of Antibacterial Surfaces in Biomedical Applications

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Abstract

Infections by pathogenic microorganisms present a serious concern in health care sector, as such infections kill more people than any other cause. The bacterial infections are commonly treated by antibiotics, but serious problems occur because of biofilm formation, which highly reduce the effectiveness of antibiotic therapy. Moreover, the overuse of antibiotics with excessively broad spectrum has increased the number of antibiotic-resistant bacterial strains, which are life threatening for patients. The bacterial infections due to implantable materials are connected with a high number of postsurgical complications, which cause immense health care costs because of prolonged antimicrobial therapy, multiple surgical intervention, and even implant removal. Thus there is an increasing demand to develop novel superior-quality medical implants that would prevent bacterial adhesion, biofilm formation and at the same time provide appropriate cell support for specific application. The rapid growth of nanotechnology and its immense potential in medicine has offered numerous opportunities for designing surfaces with superior properties, which will be used in new-generation medical devices. In this chapter, some of the antibacterial implantable devices are summarized, with emphasis on vascular implants. Bacterial adhesion and biofilm formation on implantable materials is discussed. Furthermore, the bacterial infections associated with vascular implants and the biological responses on these surfaces are presented in more detail. Research focuses on the major antibacterial mechanisms, reviewing antifouling and bactericidal approaches for the benefit of antibacterial surfaces.

1. INTRODUCTION

Biomedical devices have become essential in the human health care system. The usage of orthopedic implants, stents, heart valves, vascular grafts, and nonimplanted devices, such as orthopedic fixation screws, sutures, and catheters, saves lives and restores quality of living. However, device-related
infections (DRIs) in implant surgery, as well as short-term biomedical devices, present a huge problem that cannot be overlooked. In the United States, nearly 2 million health care-associated infections occur each year and almost 100,000 of them result in mortality [1−3]. The health care system tends to minimize DRIs due to short-term biomedical devices, meaning they are being constantly replaced with new ones. With implants, however, the problem cannot be solved so easily. Biofilm formation on implantable surfaces presents a serious threat, as in such cases the effectiveness of antibiotic therapy is also highly reduced, and according to Gbejuade et al. [4], more than 65% of all human infections are associated with biofilm. Before the biofilm develops, bacteria need to adhere to the chosen surface. The adhesion is performed through the contact of their membrane and different membrane appendages. Both gram-positive and gram-negative bacteria, which differ in structural properties of the lipid membrane, are found to cause implant-related infections [5,6]. For a better understanding of the differences in gram-positive and gram-negative bacteria, both membranes are schematically represented in Fig. 1.

The patients undergoing surgical procedure have already compromised health and lower immune system that in turn makes them more prone to bacterial infections. The highest risk of infections is during the first 48 h after implementation. Moreover, although sterilization can prevent early-stage infections, delayed infections can occur months after the surgery, as all individuals with implantable materials in the body and compromised health are more prone to biofilm infections. The patient abolition remains

![Figure 1](image.png)  
**Figure 1** Schematic representation of gram-negative and gram-positive bacterial membranes.
a problem to implant-related industries and health care institutions. Usually in case of bacterial infections, antibiotic therapy is prescribed; however, its success is mainly governed by the type of bacterial strains because of the increase in antibiotic-resistant bacterial strains. According to statistics, 25,000 patients in Europe die each year because of antibiotic-resistant infections [7]. It was estimated that global material damages in the European Union are around €1.5 billion per year [7].

Because of the fact that antibiotic-resistant microorganisms have become a serious health issue, research into new and effective antibacterial agents areas has emerged [8]. In order to minimize biofilm formation and bacterial colonization, development of a biocompatible implant surface that can withstand bacterial adherence is urgently needed, in addition to promoting bacterial evasion and osseointegration [9].

2. METAL IMPLANTS IN BIOMEDICAL APPLICATIONS

2.1 Dental Implants

Dental implantology implies fixing a device in the maxillary or mandibular bone aiming to replace a missing root and secondly, to support a prosthetic element; the endosseous implant is in contact with at least three different tissues: the buccal epithelium, the gingival connective tissue, and the alveolar bone [10].

In dentistry, nanotechnology has been used mostly in the development of restorative materials and many methods are being developed to treat diseases, although clinical use must be carefully considered. Nevertheless, nanoparticles promise better aesthetic traits (as they are more translucent) and improved wear properties. With the understanding of their physical and chemical characteristics, such as surface charge, the possibilities are plenty [11].

An indispensable part of a dental implant is its implant–bone interface, which allows the bone to grow and consequently the implant to connect with the tissue. For successful dental implants, enhancement of protein adsorption for cell adhesion must be achieved, as well as inhibition of bacterial colonization and hence biofilm formation. Biomolecules that enhance such integration include adhesion molecule (RGD)50 peptide, alkaline phosphatase, extracellular matrix (ECM) proteins, and calcium phosphate (CaP) coating. Regarding the surface properties of dental implants, it is important to carefully calculate the desired forms so as to maintain a successful implant–bone integration. Osseointegration is achieved first by
mechanical anchorage and then by biological anchorage. In nano terms the dimensions and spaces between surface patterns are designed as to alter the cell behavior. Changing the diameter of nanotubes from 30 to 70—100 nm has induced the transition from adhesion to differentiation of cells to nanostructured surfaces. A spacing of 58 nm between the nanotubes incited the formation of a stable adhesion onto the bone and therefore large cell growth, whereas a spacing of 108 nm slowed the process of cell proliferation. Regarding the bone—implant contact, higher density and a curvature of 60 nm demonstrated higher bone—implant contact than a curvature of 120 nm [11].

2.2 Orthopedic Implants

The earliest known example of an implantation with osseointegration reaches far back in history. Back then, iron was used as the only suitable metal for this purpose. With the development of metal engineering in the 20th, but mostly in the 21st century, the popularity of such procedures increased. Implants with primarily a mechanical function, e.g., mostly dental and orthopedic implants, have achieved considerable success for the reason that allows aging and injured individuals to retain their abilities [9].

The major reasons for failure of dental and orthopedic devices are aseptic loosening and infection. As of 2015, about 7 million individuals in the United States have received joint replacements. Within this group, infections occur in approximately 1.5% of total knee replacements and 1.6% of total hip replacements. Treatments involve extended antibiotic therapy, replacement of the infected implant, and at least one additional surgery. Reinfection rates can be considerably higher, with estimates in the literature ranging between 26% and 49% [9]. Because implants are designed to integrate with the bone, which proceeds by adhesion of proteins to the implant surface, osseointegration is interrupted by bacterial protein binding. Thin bacterial alloys are formed on the surface of implants and cause infection [9]. This leads to reoperation, replacement of the infected device, and incurred costs for health care system [2].

Knee and hip replacements are the most routine orthopedic surgeries of the 21st century. Arthroplasty surgeries (a surgery to replace a joint) enables injured and aging individuals who suffer from osteoarthritis to retain their physical functions and mobility and regain their independence on a daily basis [2]. Orthopedic implants are mostly used in fracture fixation, tissue reconstruction, spine fixation, and joint arthroplasties [12]. They can be roughly separated into two groups: joint replacements and fixation screws/discs (Fig. 2).
Prosthetic joint infection and surgical site infection remain the most problematic, which both require an additional operation to remove the infected implant. According to the data published in the literature, orthopedic implant-associated infection rate is up to 9%, nearly doubling for revision surgeries. According to Montanaro et al. [13] a rate of infections in revision surgery is reported between 3.2% and 5.6%. It is considered that 0.5%–2% of patients develop a biofilm orthopedic implant–associated infection in the first 2 years after surgery.

The most common pathogens of orthopedic implants that cause up to two-thirds of infections are staphylococci (Staphylococcus epidermidis, methicillin-sensitive Staphylococcus aureus, methicillin-resistant S. aureus [MRSA], coagulase-negative staphylococci). Other bacteria associated with implant infections are Escherichia coli and Pseudomonas aeruginosa [14–16].

2.3 Vascular Implants

Coronary artery disease is the main cause of death and represents approximately 31% (17.7 million people) of all global deaths in 2015, as reported by the World Health Organization. A coronary stent is the most prevalent procedure for this disease and a bare-metal stent is used in most cases (Fig. 3). However, such stents may lead to an in-stent restenosis in approximately one-third of cases [17]. If reendothelialization is delayed after stent implantation, platelets gradually adhere to the stent walls and/or the smooth muscle cells in the tunica media excessively proliferate inside stents. Blood
flow through the supported vessel is therefore impaired because of thrombosis and/or intimal thickening. Consequently, in situ reendothelialization has attracted attention as a strategy to solve in-stent restenosis and late thrombosis [18]. Furthermore, infections are a universal problem, affecting the success of a vascular stent. 

*S. aureus* is a highly adaptable microbial pathogen that is considered to be the leading cause of various community- and hospital-acquired infections, which have risen in accordance with the number of performed implantations of vascular stents. MRSA is considered to be amongst the most problematic strains [19]. Endovascular therapies are increasingly being applied in the management of infrainguinal ischemia. Stents are used primarily or selectively after a suboptimal technical result following angioplasty. Most common stents used in femoropopliteal arteries are balloon expandable stents; however, few published series document the superiority of nickel—titanium alloy (Nitinol) stents because of their self-expandable form and ability for crush recovery [20]. A more detailed description of the pathogens binding to the vascular implants is presented in Section 4.

**Figure 3** Self-expandable vascular stent made of nickel—titanium alloy (Nitinol). *Kindly donated by Rontis AG; photo courtesy of Ita Junkar.*
2.4 Common Materials Used in Implantable Biomedical Devices

2.4.1 Titanium and Its Alloys
Titanium is known for several beneficial properties in medical applications: good corrosion resistance, which is a consequence of a stable oxide layer formation; absence of tissue toxicity and allergic reactions; good strength, a factor for the device safety; and low elastic modulus [21]. In contrast to cobalt–chromium–molybdenum (CoCrMo), titanium has almost no allergic or immunogenic reactions. Moreover, because of the rapidly forming titanium dioxide, its implant-to-bone connection is stronger than that obtained using stainless steel. Titanium’s high affinity to oxygen contributes to rapid reparation of disruptions and damages on the implant. The biocompatibility of titanium can be increased by coating with bioactive titanium dioxide in nanoscales [22].

Titanium is mostly used in implant industry in two forms: as a pure titanium for low mechanical loads or as Ti-6Al-4V alloy for high mechanical loads [21]. Titanium is currently the prevalent material used in dental implantology; however, it is being replaced with zirconia and its alloys.

2.4.2 Stainless Steels
Stainless steels can be specified by their chemical composition, as well as microstructure. In implant industry, austenitic steels are used for their sustainability. They are also widely used for nonimplantable medical equipment and devices because of their good corrosion resistance [23].

Corrosion resistance of steels depends on the chromium content. Corrosion-resistant steels contain more than 12% of chromium, resulting in the formation of a thin and chemically stable oxide layer that leads to better antibacterial activity [24]. However, chromium is not the only substance that contributes to corrosion resistance. Nickel (Ni) and molybdenum (Mo) are also known for these properties [24].

Because of the content of nickel (Ni), which is toxic to human body, as well as for mechanical reasons such as crevice corrosion, its usage in temporary devices is limited. However, because of its low price, in comparison to other metallic biomaterials, it is still used in combination with cobalt–chromium and titanium alloys [24].

2.4.3 Cobalt–Chromium Alloys
There are two types of CoCrMo alloys that have been developed specifically for joint replacements. These are CoCrMo (ATSM F75 and F76) and
CoNiCrMo (ATSM F562). “Co-based alloys usually contain 26–30 wt.% of Cr, which favorably influences their biocompatibility and corrosion resistance via a strong tendency to create a thin Cr$_2$O$_3$ surface oxide film” [23].

Nevertheless, once implanted in vivo, CoCrMo implants perform under tribological contacts and loads while being lubricated by corrosive body environment, i.e., biological fluids. This leads to the release of metal particles (caused by wear) and metal ions (Co and Cr) from CoCrMo alloy, which results in implant loosening, cytotoxicity, and immunological reactions, overall leading to implant failure [25]. To reduce that, CoCrMo implants are manufactured via additive manufacturing methods, which improve their tribochemical performance. In addition, these methods empower manufacturing of customized (patient-unique) devices, which are highly needed among patients [26].

2.4.4 Tantalum Alloys
Tantalum is known for its high affinity to oxygen, resulting in the development of a thick, stable, and microbe-protective Ta$_2$O$_5$ layer. Because of this it is corrosion resistant, which has led to its massive usage in orthopedic fields. However, primarily because of its high price, as well as high density and high/low elasticity modulus properties, it is not considered to be suitable for fabrication of large implants [23,27]. Because of its beneficial attributes, it is used in joint and bone components exposed to high loads and components requiring bone ingrowth [23,27].

2.4.5 Silver and Its Alloys
Silver is known for its beneficial antimicrobial properties since the first great civilizations (Egyptians, Romans, Greeks). Back then, it was used for wound dressings and burn bandages [28].

In the implant industry, silver coatings are used in the form of nanoparticles. During the ion dissociation, Ag$^+$ ions from pure silver or even better, AgCl$_2$ interface with bacteria, leading to bacteria’s death. The problem arises when silver ions interact with human cells [28]. Silver molecules disrupt cell membranes; inhibit the metabolism of sugar and oxidative enzymes in bacterial cells, specifically Streptococcus mutans and Lactobacillus acidophilus; and induce proton leakage from membranes, resulting in cell degeneration [11,29]. Silver is also in use in other osseo implants for its impact on the E. coli respiratory chain and interference with its DNA replication [29].
2.4.6 Zirconia and Its Alloys

Research of zirconia shows promising features for biomedicine, as it is durable, osseoconductive, and resistant to corrosion. Several studies conducted with ZrO₂ have presented data of noncytotoxicity and noninflammatory effects of stated zirconia powder [29]. It is a bioinert material; therefore, encapsulation by connective tissue is faint and the release of residues is almost undetectable. Moreover, zirconia is known to be osseoconductive, which means that this ceramic facilitates bone formation when in contact with it [10].

2.4.7 Diamondlike Carbon

Diamondlike carbon (DLC) has been proposed for use in blood-contacting devices such as electrosurgical devices, artificial hearts, mechanical heart valves, and artery stents. Various reports have shown that the anticoagulation property of DLC is related to the bonding structure, hydrophobicity, and smooth surface. A DLC film is suitable to prevent blood clotting and is therefore appropriate to battle restenosis. DLC has excellent mechanical and biological properties, and its traits can be augmented by the addition of other substances. Nano–Cu particles, for example, diffuse into the film and successfully kill bacteria by destroying their cell membranes. The a–C:H films with Cu content have antibacterial properties against both E. coli and S. aureus. Thus the a-C:H/Cu film can be considered a promising antibacterial coating for applications in biomedical science and for minimally invasive surgery devices [30].

3. BACTERIAL ADHESION AND THE FORMATION OF BIOFILM ON METAL SURFACES

The perseverance of microorganisms in some of the most rigid environments, and their endurance despite unfavorable changes in the surroundings, is the testament to their adaptivity [31]. A very efficient form of adaptation to harsh conditions is biofilm formation. This is a colony of microorganisms (bacteria, algae, fungi, and protozoa) that are attached and layered over almost any substrate where the cells are nested in self-excreted ECM [32–35]. Interestingly, this kind of microorganism cell accumulation in the human body has been reported already in 17th century by observation of dental plaque under the predecessor of the microscope [36]. Among microorganisms, bacteria are the prevalent biofilm-forming species, as over 95% of bacterial populations are able to form films [37,38]. The
detrimental effect and obtrusive nature of biofilm formation can be found in several fields, such as biomedical implants and tools [39], food-processing facilities [40], marine-related industry [41], and common households [42]. However, one must also take into consideration the fields where biofilm occurrence is indispensable and that offer a significant advantage, e.g., in environmental remediation [43] and agriculture [44]. However, for the benefit of this chapter, the development of such a film is detrimental; it is to blame for a significant amount of infections, greatly decreases the longevity of implanted materials, and represents a serious health threat to the patients [45–47].

The biofilm community forms a multicellular body, where the differentiation of cells taking over different roles increases the adaptation, eases the intercellular communication, and separation of tasks, which all insures better efficiency and effective use of scarce resources [48,49]. Thus even though cells in the biofilm proliferate from a single cell, they are not mere duplicates of the original cell but show distinctly different phenotypical features, owing to different gene expression, which is dictated by the environment-specific assignment of the cell, i.e., depending on the amount of nutrients, oxygen, or fluids that are available in the gradient of the biofilm [34,50,51]. This confirms the evidence that almost all bacteria are proficient in film formation, but there is prominent heterogeneity in the formation of the ECMs [52]. Complexity and role differentiation of the biofilm ensures its resistance. Heterogeneity of the biofilm is also derived from its establishment; a biofilm formed from several spatially divided bacterial microcolonies as its basic units can be established by different species of bacteria. Through the growth of the colonies the biofilm spreads over the surface, and other bacterial species and even fungi can adjoin onto this growing mass [36,53,54]. Therefore we not only have specific gene expression within the species but also have the heterogeneity of specific genes across different species [54,55]. Cells enclosed in the biofilm can persevere by establishing a defense system secreting extracellular material, also known as slime or slimy secretion, which consists mainly of insoluble polysaccharides and proteins, as well as lipids, nucleic acids [56–59], flagella, and pili [60]. The composition, biochemical pathway expression, and shape largely depend on the bacterial species present. More and more evidence is revealed that speaks of complex preconceived protein localization with specific interaction between components to ensure the survival of the colony [61]. Moreover, the cells that fail to produce exopolysaccharides are not able to form complex architectures and are easily detached from the surface [62].
As mentioned earlier the polymeric building blocks partake different
shapes and sizes, which are largely dependent on the species of bacteria
that secret them. We have summarized the components and roles of the
biofilm ECM, also known as the glycocalyx coating or extracellular poly-
meric substance (EPS), in the scheme in Fig. 4A. The ECM represents
over 70% of the biofilm volume and thus has several important roles to
enable the prosperity of the bacterial microcolonies nestled inside [63].
ECM is essential for biofilm adhesion and thus for the adherence between
the cells, as well as for the adherence of the mature biofilm to the substrate.
In this manner, it offers mechanical stability to keep the growing community
of cells grounded [64]. It also provides the architectural support to the cell
community to grow into three-dimensional expansions of several small
microcolonies. Furthermore, the three-dimensional architecture is impor-
tant for the development of the sustenance system throughout the biofilm.
The ECM has the ability to harvest the nutrients from the biofilm surround-
ings via charged polysaccharide groups on its surface and to transport them
throughout the matrix for additional cell alimentation [65]. It has been
shown that the pronounced diversified topography, which is visibly
protruding from the surface of a mature biofilm, conceals a rudimentary
“circulatory system” [63,66]. The biofilm-resident bacteria are dependent
on the flow of nutrients, oxygen, and biofluids (or water) from the biofilm
surroundings [52], because as the film progresses, it can expand into several

![Figure 4](image)

Figure 4 (A) Schematic representation of different extracellular matrix (ECM)
component macromolecules together with the tasks attributed to the ECM. (B) Repre-
sentation of different layers of a mature bacterial biofilm. Adapted from H. Rohde,
S. Frankenberger, U. Zähringer, D. Mack, Structure, function and contribution of poly-
saccharide intercellular adhesin (PIA) to Staphylococcus epidermidis biofilm formation
layers of cells. For this purpose, ECM channels are formed to supply nutrients to the cells inside the biofilm. The cells near the substrate (base film) and the cells that do not reside near such channels still have the distinct disadvantage of living in straitened circumstances for nutrition, water, and oxygen availability [55,66]. The specific conditions of the biofilm thus dictate the direction of bacterial microcolony development, such as anaerobic or aerobic adaptation due to oxygen gradient formation. Regarding both lack of oxygen and nutrients the biofilm can also host dormant bacteria [67].

In addition to these roles the protective encapsulation allows the preservation of microorganisms in the biofilm against outside environmental stress and desiccation. Therefore unlike in the planktonic or suspended state, biofilm-based bacteria can build not only tolerance to innate and adaptive immune responses but also tolerance and resistance to several antibiotics or biocidal agents because the biofilm formation offers them protection against unfavorable chemical and physical conditions [65,68]. The ECM harbors specific mechanisms to battle the onset of antibiotics or other disinfectants and antibacterial agents. For example, not all bacterial species are resistant to antibiotics, but the formed biofilm architecture can delay the protrusion of drugs into the ECM through its ion-exchanging capabilities [55]. Additionally, even if the antibiotic penetration is successful, the biofilm architecture establishes an oxygen gradient formation (spatial limitation), which consequently subdues the metabolic activity; therefore, cells disregard the antibiotic presence, do not metabolize the drug, and are thus unaffected by it [69]. Also, specific antibiotic-inactivating enzymes (β-lactamase, formaldehyde lyase, formaldehyde dehydrogenase) can be embedded and concentrated in the ECM [55]. Even cation-chelating extracellular DNA is reported to contribute to the antibiotic inefficiency over the biofilm layer [70]. Adding to the antibiotic resistance is also an elaborate ECM-mediated cell-to-cell communication termed “quorum sensing”. This mechanism holds control over gene expression. The precondition for this mechanism to manifest itself is the required density of cells in the biofilm. This does not mean the cells can sense each other’s presence, but that they are susceptible to the increased levels of signaling molecules in their surroundings. Once the threshold is passed, bacteria in the biofilm communicate by horizontal transfer of genes (as opposed to vertically parent/offspring) [71–73]. In this respect the horizontal gene transfer allows for the spread of antibiotic resistance genes throughout the colony [55].

Fig. 4B is a schematic representation of a mature biofilm over a substrate consisting of three layers: conditioning film, biofilm base, and surface film
The biofilm base is denser, whereas the surface film is characterized by a looser layer of matrix-based bacteria, which can extend into the surrounding medium [55]. The protrusion of colonies depends on the shear forces present in the environment; when shear forces are low, the growth of the biofilm towers in the form of a mushroom into the surroundings. On the other hand, when the shear forces are dominant, the colonies grow in an elongated form that is able to withstand pressure [63].

3.1 Dynamics of Biofilm Formation

In order to produce a biofilm, bacteria first need to adhere to the substrate. By many researchers, this step is regarded as decisive in biofilm formation. The source of first colonizers is a liquid with resident planktonic bacteria. The adhesion of these cells is not final; in the first stage, bacteria can be detached from the surface. The first colonizers’ adhesion dynamics is influenced by several factors, which will be discussed later. The adsorption in the first stage is weak and does not persist for long; if the adhesion conditions are unfavorable, this stage is referred to as reversible adsorption [54]. The second stage of biofilm formation is the nonreversible stage, where microcolonies start to form and the bacteria intensely secrete EPSs. In the third and fourth stages, microcolonies or multilayered clusters are shaped and the film is in the process of maturation across the several microcolonies that had independently arisen on the surface, constructing its three-dimensional structure. Here, other species of bacteria from the environment join the growing biofilm [53,54]. In the final fifth stage, the biofilm reaches maturation; this leads to the release of planktonic bacteria, which allow the cycle to continue. In Fig. 5, the biofilm progression on a substrate is schematically depicted. We can see the progression of growth of the biofilm through the several stages. The first stage, the stage of adsorption, is very fast and takes place within seconds [36,75,76]. In the second, nonreversible stage and the third stage, the microcolonies grow exponentially and the process takes minutes to hours. In the final maturation phase, the growth is not as pronounced and becomes stationary. Within days, the mature biofilm life force can start to decline, but the shed cells warrant bacterial persistence [36].

The first interactions between the bacterial cells and the adhesion surface can be either specific or nonspecific. When bacteria are in the planktonic form and freely moving in the medium (e.g., water or blood), they first interact with cells by long-range interactions, which are nonspecific and take effect in distances over 50 nm. These interactions convey the bacteria closer to the material surface (e.g., electrostatic attraction/repulsion,
Brownian motion, van der Waals attraction forces). Specific interactions (hydrogen or other chemical bonding and ionic, dipole, and hydrophobic interactions) are distinctly localized, located in the radius less than 5 mm from the surface, and prevail once the cells are within the required distance [77,78]. Here, it is necessary to emphasize that both specific and nonspecific interactions have the same physio-chemical origin, the differentiation actually lies in the recognition of the specific highly localized molecular group interactions or specific events between parts of the entities in contact, whereas nonspecific ones refer to the interactions of whole entities, without setting out particular points of interaction or contact [79].

Regarding the surface—bacteria interactions, there are two factors to be taken into consideration: the material surface characteristics and the characteristics of bacteria [80]. Additionally, the medium or serum where these interactions take place also influences the course of the adhesion [79]. All these influence the physiochemical interactions and determine the path of bacterial adhesion. Knowing the parameters relevant to cell attachment promotion could lead to the prevention of the resilient biofilm formation. Typically, the general environmental factors influencing bacterial behavior are pH and the concentration of certain electrolytes, temperature, flow
conditions, and the possible antibiotic occurrence in the environment [37,77]. When we consider the implant’s final residence in the human body (with the exception of dental implants), the environment is considered sterile, e.g., blood, cerebrospinal fluid, bone, bone marrow, joint fluid, internal organs, and thus the infection-inducing bacteria have to enter this environment at the time of insertion during the operating procedure [81]. Excluding the postoperative antibiotic treatment, the newly implanted material environment is relatively beneficial to pathogen growth. Furthermore, rarely is the material used for different applications not affected by its surroundings. Thus when any kind of substrate is subjected to a nonsterile environment, (macro)molecules that are found near the implanted material adsorb onto the surface, forming the so-called conditioning film [82]. Conditioning film is composed of organic (mainly proteins) and inorganic components, which are brought into the vicinity of the film by, for example, flow movement [83,84]. Adhesion parameters in such conditions are of course different from those in sterile surfaces, as better adhesion and nutrient supply are provided [37].

Material characteristics in the absence of conditioning film represent a particular aspect of influence on bacterial adhesion. Among the relevant factors are hydrophilicity, surface topographical features, chemistry, and charge [85–88]. Surface roughness is generally accepted as an important factor with respect to bacterial adhesion. On initial consideration, ultra-smooth surfaces are regarded as less favorable for adhesion and consequent biofilm formation in comparison to surfaces consisting of imperfections (cracks, pores, dislocations) or grain boundaries [89,90]. Increased roughness offers better adhesion opportunities because of enhanced surface area, additional adhesion sites, and the protection from the environment, mainly from shear forces [53,89]. Nevertheless, the roughness issue is not that straightforward. While it can promote bacterial adhesion, the factors must be considered more closely. There have been reports that consider pristine surfaces, which lack any biological substrate, to be less favorable for adhesion, and it is on the pioneer cells to set the conditions for the biofilm to develop [91]. However, certain bacteria such as *P. aeruginosa*, *S. aureus*, and *S. epidermidis* are known to easily bind also to stainless steel and titanium orthopedic screws [47]. On the other side, the distinction between micro- and nanosized surface roughness has to be made. Prokaryotic cell size ranges from 100 nm to 5 μm. If the surface disturbances are in the range that can accommodate the bacteria between them, the bacteria will take advantage of this and maximize the contact area between themselves and the surface to have
the best possible grip [92–95]. The relationship between the bacterial size and the surface roughness is depicted in Fig. 6A. As opposed to results considering micrometer roughness, the reports on experimental results considering the nanoscale-textured surfaces are diverse. Some authors indicated that nanosized roughness is advantageous because it decreases bacterial adhesion. The two predominant reasons supporting this argument were that a decrease in contact points between bacterial cells takes place (Fig. 6B) and that the contact forces from the large area of the nanostructured surface repel bacterial cells [53,85,87]. Other authors, however, reported that nanosized surface roughness is beneficial for bacterial adhesion [90,96,97]. The ambiguity regarding the roughness of surfaces is not surprising, as it depends on nanosized surface [98], physical configuration [77,99], different bacterial strains [53] and their contrasting cell characteristics, membrane rigidity, metabolic activity, and production of EPSs [90]. Bacterial characteristics are actually of great importance in terms of adhesion, as bacteria are known for their adaptive features.

Friedlander et al. [100] have initially observed a decrease in bacterial adhesion to nanostructured surface, in comparison to flat controls. However, after prolonged exposure of the surfaces to bacterial cells, the adhesion on nanostructured topographies was substantially promoted. Bacterial cells have adapted by attaching themselves through flagella, the protein appendage, which can be produced through specific gene expression by the offspring of first colonizers as a response to dire adherence conditions, i.e., the time lapse between reduced and promoted adhesion, in this particular case. The irreversible phase is actually marked by the molecular and cellular interaction of specific bacterial surface structures or proteinous cell appendages.

Figure 6 The comparison of bacterial spatial configuration on surfaces with (A) micro- and (B) nanostructured topography [85].
with the chosen surface \[80,101\]. This testifies to the incredible ability of bacteria to adapt to new environments, because they can use different surface appendages to protrude the nanosized crevices. Besides flagella, bacteria can aid their adhesion also with pili or fimbriae. These protein bacterial membrane protrusions are able to gain access into the nanosized spaces and achieve a better grip; thus, some of the nanostructured surfaces actually offer good relief and numerous contact points for first colonizers to adhere to. Actually, it was shown that bacteria are able to produce distinct adhesion strategies depending on the topography they are adhering to \[53,101\]. Lorenzetti et al. \[85\] have concluded their observations with remarks that topography had prevailed over the effect of surface charge and hydrophilicity in terms of bacterial adhesion. Wettability, in general, is cited as a factor affecting adhesion, where hydrophobic surfaces are less advantageous owing to the repulsion; bacteria in aqueous media have a net negative surface potential \[80,86,102\]. On the other hand, there is evidence of hydrophilic cells adhering strongly to hydrophilic surfaces and, by analogy, hydrophobic cells to hydrophobic surfaces with the important consideration of the adaptivity of bacteria and alteration in their surface charge, according to the environmental conditions \[103–106\]. Yuan et al. \[86\] performed experiments considering wettability conditions affecting the growth of \textit{E. coli}. Fig. 7 schematically presents different wettability conditions influencing bacterial adhesion. In Fig. 7A the surface is superhydrophobic (very high water contact angle [WCA] of 105 degrees), the water in which bacteria reside, is repelled from the surface and bacteria are left to make contact with the limited surface protrusions, which were wetted. The highest bacterial adhesion was observed with moderate hydrophobicity (Fig. 7B), where there was a higher wettability of the rough surface. Here, the protein

Figure 7 Different interactions between the bacteria \textit{Escherichia coli} (black elements in A–C) and the rough surface in the context of wettability \[86\]. LB CA: Contact angles of Luria–Bertani medium. \textit{Published by The Royal Society of Chemistry}. 
appendages of *E. coli* have an important role in adhesion by providing adequate attachment of the cells to the surface. In contrast, in Fig. 7C, complete wettability can be observed but bacterial adhesion is lowest. This is due to the repulsive interactions between bacteria and the surface, despite the increased wettability.

Hitherto, no rule has yet been found with regard to the promotion or prevention of the adhesion of bacteria to nanorough surfaces. Several factors were found to influence the adhesion of bacteria onto nanostructured surfaces. Finding an appropriate set of surface conditions that decreases adherence of a certain strain does not necessarily mean the same conditions would also repel other strains. Further research is definitely needed to help address the answers to these complex problems.

### 4. PATHOGENS BINDING TO VASCULAR IMPLANTS

Infections involving vascular graft prostheses are infrequent but devastating complication of reconstructive vascular graft surgery and are associated with high morbidity and, in some situations, mortality [107]. Underlying comorbidities, such as diabetes mellitus or being immunocompromised, increase the risk of infection and serious infection-related complications. Vascular graft infections (VGIs) can be categorized broadly into those that occur in an extracavitary location, primarily in the groin or lower extremities, or in an intracavitary location, primarily within the abdomen or less commonly within the thorax. The incidence of VGI ranges from less than 1% for abdominal aortic grafts to 2.6% for lower extremity grafts to approximately 6% for infrainguinal vascular grafts [107–109]. Some authors suggest that the rate of infection may be increasing [110,111]. The rate of infection in prosthetic arteriovenous hemodialysis grafts is approximately 3.5% [112,113]. Medical as well as economic costs of VGI are extensive. The overall mortality related to VGI has been reported to be between 13% and 58%, and the amputation rate in survivors varies between 8% and 52% [109]. Similarly, estimates suggest that 20%—36% of all deaths in hemodialysis patients are related to infectious complications [113,114].

#### 4.1 Microbiology and Risk Factors

The microbiological cause of VGI has evolved over the years [5,107]. In early published studies, *S. aureus* was the predominant microorganism recovered [5,115]. Improvements in surgical techniques, administration of
prophylactic antistaphylococcal therapy, and other factors have resulted in a changing microbiological epidemiology. Surgeries performed in patients with complicated vascular anatomy and in patients with several underlying comorbidities, increased frequency of emergency procedures, and changes in hospital flora have all contributed to the changing spectrum of infection. A more diverse microbiological spectrum of infection includes multidrug-resistant strains, polymicrobial infection, and *Candida* species. Gram-positive cocci account for at least two-thirds of VGIs. Infections caused by coagulase-negative staphylococci are more common than those caused by *S. aureus*, with MRSA infections increasing in frequency. *P. aeruginosa* is now the most common cause of gram-negative infections and accounts for at least 10% of VGIs [5,6] (Fig. 8).

Assessing risk factors that predict perioperative mortality and graft patency is essential for selecting patients who would benefit from surgery [117]. Omitting surgical reconstruction and endovascular intervention may be preferable especially when multiple risk factors are present or in the absence of critical limb ischemia. Infrainguinal revascularization procedures are much more likely infected than aortic grafts [111]. The presence of a groin incision is an especially important risk factor for infection. Other commonly cited risk factors for VGI include diabetes mellitus, obesity, renal failure, revision surgery (particularly revision within 30 days), and use of

![Figure 8 Microbiology of prosthetic graft infections [116].](image)
Table 1 Risk Factors for Vascular Graft Infections

<table>
<thead>
<tr>
<th>Presence of Groin Incision</th>
</tr>
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<tbody>
<tr>
<td>Intrainguinal procedure</td>
</tr>
<tr>
<td>Wound-related complications (e.g., hematoma, poor wound healing)</td>
</tr>
<tr>
<td>Comorbid conditions (diabetes mellitus, obesity, chronic renal insufficiency)</td>
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<tr>
<td>Revision surgery, especially within 30 days</td>
</tr>
<tr>
<td>Emergent surgery</td>
</tr>
<tr>
<td>Use of prosthetic grafts</td>
</tr>
<tr>
<td>Prolonged operative time</td>
</tr>
<tr>
<td>Postoperative hyperglycemia</td>
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<tr>
<td>Perioperative infection at another site</td>
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prosthetic rather than native graft material [118] (Table 1). Prolonged operative time is also associated with infection. Other investigators have reported a link between VGI and perioperative infection at another site, postoperative hyperglycemia, immunosuppression, and infected central lines [115,119,120]. The lowest rates of infection following revascularization have been seen with anatomic, autologous reverse vein reconstructions [111]. For abdominal aortic grafts, endovascular repair does not appear to have a lower risk of infection than open repair; however, the risk of abdominal aortic graft infection is quite low with either approach [120].

4.2 Pathogenesis and Vascular Graft Infections

Part of the difficulty in treating bacterial infections is the fact that many involve the formation of a biofilm at some stage [121]. The host response to biofilm infection can inadvertently increase biofilm mass, as platelets and fibrin migrate and attach to the site of infection [122]. First, bacteria in a freely moving, planktonic state adhere to an artificial surface. Once they have adhered to a surface, the individual cells begin multiplication to form microcolonies. Development and maturation then occur (Fig. 9).

Bacteria produce an extracellular polymeric matrix that is the hallmark of a biofilm and may contain proteins, DNA, and polysaccharides [68]. Biofilms are a polymeric matrix produced by microorganisms that expand and mature as they adhere to surfaces, and they play an essential role in VGIs and other implant infections. When bacteria exist as a biofilm, they are significantly less susceptible to antibiotics. This is a result of metabolic changes in cells within the biofilm and structural features influencing drug permeability [123]. Also, within the biofilms, microorganisms display phenotypic phase variation between an embedded sessile form and a
free-floating planktonic state [124]. The sessile form is responsible for adhesion and biofilm formation, whereas the planktonic form is responsible for dissemination. Microorganisms in the sessile stage are comparatively phenotypically inert but exhibit complex metabolic changes and organized behavior much different to what would be expected normally.

The likelihood of an implant becoming colonized or infected will vary depending on the type and structure of the material involved, as well as the microbes involved [109]. Sessile microorganisms within biofilms are resistant to killing by both host immunity and antibacterial agents. The mechanisms of this resistance are not fully understood but appear to be due to multiple factors. Artificial implants stimulate a chronic low-grade inflammatory response that not only results in an immune-incompetent zone that impairs phagocyte bactericidal capacity but also leads to peri-implant tissue damage and increases the likelihood of implant failure and infection [124]. Microorganisms in biofilms have been shown to stimulate antibody production, but the humoral immune response is ineffective in killing biofilm-encased organisms [125].

![Figure 9](image_url) Biofilm contamination of catheters can lead to bacteremia. (A—D) The stages of colonization of a catheter where planktonic cells attach, form microcolonies, and then form a mature biofilm. *Reprinted with permission from G. Hughes, M.A. Webber, Novel approaches to the treatment of bacterial biofilm infections, Br. J. Pharmacol. 174 (2017) 2237–2246.*
Prosthetic grafts can become colonized via direct intraoperative bacterial contamination of the vascular graft, which is considered to be the most common cause. The second most common cause of contamination of vascular grafts is the spreading of the infection from the contiguous site, such as a surgical wound infection or an intra-abdominal or pelvic abscess, to other areas. Less commonly, VGI results from bacteremia via hematogenous seeding. In the 1980s, a surgeon A.G. Gristina published a paper on “A Race for the Surface” proposing that colonization of implants with host cells decreases the possibility for colonization with bacterial cells [126]. In concordance with this, the risk of hematogenous spread of VGI is highest in the early postoperative period (<2 months) and decreases over time because of partial endothelialization of the graft [107]. Different strategies/approaches were used to prevent bacterial colonization of implants, including antiadhesive/antiadsorptive approaches, antibacterial coatings (inorganic, organic), and multifunctional nanostructured surfaces and coatings [127].

4.3 Endothelial and Smooth Muscle Cells Covering Vascular Stents and Approaches to Limit Complications

Vascular stent implantation has been an important strategy to treat cardiovascular disease. However, in-stent restenosis and late thrombosis are serious complications affecting the success of treatment. There are several reasons attributing to these complications: proliferation of vascular smooth muscle cells (VSMCs) and slow endothelialization, whereby chronic inflammation along with delayed wound healing and adhesion/activation of platelets and fibrinogen also play prominent roles.

Bare-metal stents were first used to enable artery closure, but their efficacy was severely hampered by proliferating VSMCs and the resultant neointimal hyperplasia was the only mechanism responsible for restenosis after metal stent placement. Drug-eluting stents (DESs) proposed a solution by coating stents with polymers to elute drugs targeting VSMC proliferation. This approach used since 2002 resulted in a substantial attenuation of in-stent restenosis. But antiproliferative properties of DESs impair and/or delay also endothelialization, hence leading to late stent thrombosis. To prevent this, new approaches have lately been sought, including prevention of VSMC proliferation and acceleration of re-endothelialization.

4.3.1 Prevention of Smooth Muscle Cell Proliferation

While endothelial cells (ECs) compose a monolayer of intima, VSMCs are located in the media layer of the vessel. Increased neointimal proliferation
represents the main reason for in-stent restenosis, with increased growth and migration of VSMCs observed as the main reason for the changed vessel state. Changes in the endothelial layer (inflammatory and/or thrombotic activation) can trigger VSMC proliferation and migration and intimal hyperplasia.

DESs can elute antiproliferative drugs for prevention of VSMC growth. The first generation of DESs was generated from biostable polymeric drug carriers; however, antiproliferative effects proved to be problematic for re-endothelialization (restenosis and stent thrombosis) and hypersensitivity reactions. So the second generation of DESs from biodegradable polymers covering the stent offered a safer alternative to the uncoated bare-metal stents. Recently, completely biodegradable drug-eluting polymer stents were developed (biodegradable in 6–24 months), which reduce the probability of long-term complications [128]. The drugs certified for in-stent delivery compose of mTOR (mechanistic target of rapamycin) inhibitors (e.g., everolimus, sirolimus, zotarolimus, pimecrolimus, Biolimus) targeting phosphatidylinositol 3-kinases (PI3K)-Akt-dependent proliferation mechanisms, while Taxol derivatives (e.g., paclitaxel) are mitotic inhibitors stabilizing microtubules and thus blocking cell division in the G phase [129].

However, there are multiple issues to be considered when deciding which drug is appropriate. For example, sirolimus is used as an immunosuppressive drug for the treatment of cytopenias, associated with autoimmune lymphoproliferative syndrome [130], or after organ transplantation, especially following heart or kidney transplantation [131]. These patients have a high risk of developing symptomatic coronary artery disease and often require coronary stent implantation. A recent report investigated the effects of systemic sirolimus application on neointima formation in a mouse model of vascular injury [132]. This accepted mouse model for studies of postangioplasty restenosis closely resembles the angioplasty procedure that injures both the endothelium and vessel wall. The data showed that systemic application of sirolimus prevented postangioplasty, neointima formation, and vessel narrowing by inhibiting the inflammatory response to injury. However, and importantly, systemic sirolimus treatment also impaired endothelial regeneration after angioplasty. So the report warns that extreme caution should be paid to current approvals of new-generation DESs to reduce dual antiplatelet therapy time to 3 months only, as safety data for this approval have not been obtained in patients systemically treated with sirolimus or in organ transplant patients.

Although mice may significantly differ from humans, the data from this study clearly indicate the need for further clinical trials to determine the
optimal duration of antiplatelet therapy after coronary interventions in patients undergoing systemic sirolimus treatment [132].

As antiproliferative effects are not cell-type specific, the need for newer VSMC-specific antiproliferative drugs is currently leading the research. Recently, an interesting approach to search for enzymes released by VSMCs, but not ECs, was proposed to trigger drug release from stents, leading to the identification of matrix metalloproteinase-9 as a potential candidate [133].

4.3.2 Acceleration of Re-Endothelialization

In vessels, a healthy endothelial layer is responsible for antithrombotic and antiadhesive characteristics, barrier functions for leukocyte migration, and regulating inflammation and thrombosis/fibrinolysis. During the stenting procedure, the endothelial layer can be destroyed. Re-endothelialization consists of regrowth of the remaining endothelial layer, partly from circulatory endothelial progenitor cells (EPCs) and partly from bone marrow–derived cells circulating in low levels in the bloodstream.

Changing the surface of the vascular implant with single-molecule or multimolecule coating or layer by layer coating (with, e.g., heparin, fucoidan, chondroitin sulfate, hyaluronic acid, gallic acid, laminins, fibronectin, selenium catalyst nitric oxide release surface) has been used to mimic vessel architecture and promote endothelialization [18,134]. Alternative approaches include incorporation of adhesion molecule peptide sequences in a polymer base layer (such as laminin-derived RGD sequence or fibronectin-derived REDV sequence), with huge efforts in developing EC-type selective adhesion sequences [18,135].

Several approaches have been proposed for incorporating growth factors to increase endothelialization (e.g., vascular endothelial growth factor [VEGF]-eluting stents) [136], but as with antiproliferative agents, cell-type specificity is not high. Dual DESs have been developed by combining the mechanisms of one drug-preventing restenosis (e.g., antiproliferative substance) and another drug-preventing in-stent thrombosis with antithrombotic action (e.g., hirudin, triflusl) or as endothelialization promoters (e.g., estradiol, anti-CD34) [129].

Preseeding of EPCs to stents was proposed [137], with stents allowing for the capture of EPCs developed using anti-CD34 antibody [138] or VEGF receptor, VEGFR2. A few growth factors increasing adhesion of EPCs (namely, brain-derived neurotrophic factor, stromal cell–derived factor-1) were also proposed for use as stent material [139]. However, the question
of correct differentiation of the attached cells remains (as CD34+ cells could differentiate into other cell types, including VSMCs). Additional systemic treatments to increase EPCs in circulation have been applied (e.g., statins, erythropoetins) [139] in order to promote re-endothelialization.

Testing the biocompatibility and usefulness of the vascular stent material in vitro may prevent later complications. So in vitro seeding of ECs and VSMCs on stent materials in static or laminar flow conditions should be performed. The latter also enables the simulation of conditions for DESs and evaluation of the effects of inflammation and/or platelet/leukocyte adhesion. Cytotoxicity of stent material testing, rate of proliferation, and expression of molecules important for endothelial function (e.g., expression of thrombocytes, leukocyte cell adhesion molecules, nitric oxide production important for controlling thrombocyte/leukocyte activation, and VSMC proliferation) are all important parameters to study and predict the effectiveness that stents could show in vivo.

5. MECHANISMS OF ANTIBACTERIAL EFFECT

Bacteria can readily colonize surfaces of biomedical devices, implants, and vascular stents and can shield themselves with protective EPSs, i.e., biofilm. There is a strong need to reduce or prevent bacterial colonization and biofilm formation on such surfaces, either by chemical surface modifications or by topography adjustments. Antibacterial surface coatings exploit (1) antifouling effect or (2) bactericidal effect that kills bacteria before or after contact with the surface (Fig. 10) [140]. Antifouling strategy includes an antiadhesive principle that prevents the attachment of bacteria (fouling-resistant approach) and the reduction of the adhesion of bacteria (fouling-release approach). Fouling-resistant coatings include passive polymers, hydrogels, and superhydrophobic surfaces. Fouling-release coatings are usually made of polymers that enable reversible changes in the surface properties, e.g., wettability, charge, or topography [141]. Bactericidal strategies include the use of antibiotics and germicides, active cationic polymer chains (killing of the attached bacteria), peptides, ion-releasing metals, quaternary ammonium compounds, etc. Some coatings incorporate both these mechanisms, for instance, antibiotic release from mesoporous TiO2 films [142], which are both bacteria-repellent owing to the hydrophobic nature and bactericidal. In the following sections, the mechanisms involved in antibacterial performance of surfaces are discussed.
5.1 Antifouling Effect/Passive Surfaces

5.1.1 Antiadhesive Polymeric Surfaces

Passive polymer coatings can contain one of the uncharged polymers such as poly(ethylene glycol) (PEG), poly(2-methyl-2-oxazoline), polypeptoid, poly(n-vinyl-pyrrolidone), and poly(dimethyl acrylamide) [144]. Passive polymers prevent attachment of the microorganisms, especially adhesion of *S. aureus* and *S. epidermidis* [145,146], to surfaces by hydrophilic/hydrophobic repulsion, electrostatic repulsion, or low surface energy [144].

Owing to high chain mobility, large exclusion volume, and steric hindrance effect of a highly hydrated layer, PEG is one of the most extensively studied antifouling polymer coating, which can resist biomolecule adsorption and attachment of microorganisms due to formation of steric barrier. PEG is commonly grafted to the surface with a chemical anchoring group or by in situ polymerization of polymer from a surface-bound/grafted initiator [147]. Although the mechanisms of antibacterial repellence of surface-immobilized PEG are not fully understood, studies indicate that grafting density, chain length/thickness of the polymer layer, conformation, and wettability properties of the grafted polymer play an important role in resisting microorganism adhesion.

Despite excellent antiadhesive nature of PEG, development of antiadhesive coatings against bacteria remains a challenging task because the bacterial adhesion mechanism still surpasses the antirepulsion strategies and helps bacteria colonize the PEG-coated surfaces. A study by Zeng et al. [148] showed that bacteria can adhere to the PEG-coated titanium surfaces with polysaccharides and extracellular DNA; moreover, DNA could even cause desorption of the PEG coatings from the surfaces. They suggest that the
space between conventional polymer brushes enables the penetration of DNA and its interaction with the underlying surface. The authors found that this can be avoided by increasing the graft density, as shown in Fig. 11, by forming a tightly packed and extended conformation of the polymer that represents the steric barrier between the bacteria and the surface. Such coatings can inhibit the adhesion of nonproteinaceous bacterial adhesins such as polysaccharides and DNA.

5.1.2 Superhydrophilic/Superhydrophobic Surfaces
Structured surfaces that create unique surface characteristics such as superhydrophobicity and superhydrophilicity also exhibit microbial resistance through antifouling effect. Such surfaces form a barrier between bacteria and materials and prevent direct contact between them.

5.1.2.1 Superhydrophilic Surfaces
Superhydrophilicity of the surfaces can be achieved by various approaches, for instance, (1) nanostructurization or (2) coating of hydrophobic surfaces with hydrophilic counterparts.

Superhydrophilic layers of TiO$_2$ nanomaterials are widely studied for antibacterial applications. Younas et al. [149] developed an antibacterial superhydrophilic barrier consisting of TiO$_2$ nanoparticles on polyvinylidene...
fluoride (PVDF)-based membrane. Cai et al. [150] prepared antibacterial TiO$_2$ nanofilms on soda–lime glass. The excellent superhydrophilic behavior of TiO$_2$ nanomaterials can be attributed to physical and chemical factors. Junkar et al. [151] showed hydrophobic behavior of Ti foil in WCA measurements, whereas nanostructured Ti foil, on which TiO$_2$ nanotubes were fabricated, was superhydrophilic. In another work, authors demonstrated that the surface topography and roughness at the nano level substantially affect the TiO$_2$ surface–water droplet interaction, at least partially because of water penetration into the porous structure [152]. The reason for hydrophilic behavior of TiO$_2$ nanotubes could also lie in the reaction products, hydroxide compounds, on the surface of TiO$_2$ nanotubes [153]. The hydrophilicity of TiO$_2$ nanotubular surfaces can also be enhanced by annealing [154] and ultraviolet (UV) radiation [155]. It has been reported that the anatase crystal phase causes the formation of even more hydrophilic surface of TiO$_2$ nanotubes [156]. The effect of the crystal structure of the material on its wettability is especially important if the samples are irradiated with UV light before spell-out (WCA) measurements. It is known that crystalline materials facilitate the mobility of photogenerated charge carriers if exposed to light energy. However, the mechanism of the effect of photoinduced surface superhydrophilicity on the TiO$_2$ remains elusive [157]. It has been suggested that the reason is in photocatalytic decomposition of surface organic contaminants existing on the surface of metal oxides [158,159]. Another study proposed the hypothesis of desorption of weakly bonded water molecules from the external surface hydrolayers by the thermal action of light and increase of the surface hydrophilicity, which results from restoration of the original structure of hydrated layers [160].

Another approach to render surface superhydrophilicity is by coating the surface with polymers. One of the most common coatings used to contribute to surface hydrophilicity is PEG [161]. Often various polymers are coupled in order to create coating with multiple mechanisms of antibacterial action. Gao et al. [162] prepared a blocklike copolymer composed of fouling-resistant, fouling-release, and active bactericidal part (shown in Fig. 12) that significantly diminished adhesion and enhanced antibacterial activity against the gram-negative bacteria *E. coli* and the gram-positive bacteria *S. aureus*. Fouling-resistant poly(PEG methyl ether methacrylate) was responsible for improving hydrophilicity of the PVDF membrane matrix, thereby reducing hydrophobic interaction through the formation of a hydration shell. The poly(hexa-fluorobutyl methacrylate) segment created low surface energy and poly
[2-(methacryloyloxy)ethyl trimethylammonium chloride] quaternary ammonium salt served as an active antibacterial mechanism.

Similarly, Zhu et al. [163] developed antifouling and antibacterial poly-ethersulfone membranes by the addition of poly(2-dimethylaminoethyl methacrylate)-grafted silica (SiO$_2$-g-PDMAEMA) nanoparticles, which showed antibacterial activity against $E$. coli and $S$. aureus because of the hydrophilic surface. Pan et al. [164] developed an antifouling and antibacterial membrane consisting of Ag/SiO$_2$-PVDF. Yang et al. [165] prepared superhydrophilic salivary acquired pellicle (SAP) bioinspired tannic acid (SAP3-TA) coatings on hydroxyapatite, which showed antibacterial and antifouling properties against $S$. mutans.

5.1.2.2 Superhydrophobic Surfaces

Superhydrophobicity can reduce the adhesion force between bacteria and a material [166]. When the surface energy of bacterial cells is smaller than the surface energy of liquids in which cells are suspended, cells preferentially attach to hydrophobic materials [167]. The mode of action of superhydrophobic surfaces to reduce bacterial adhesion is, however, still not clear, because this is a relatively new topic [168].

Moreover, although superhydrophobicity is one of the factors affecting bacterial adhesion, it is generally not sufficient to repel organic matter.
Therefore the addition of TiO$_2$ nanomaterials to polymeric coatings is often required to make surfaces antibacterial [169]. Li et al. [170] prepared hydrophobic liquid-infused porous poly(butyl methacrylate-co-ethylene dimethacrylate) surface with $P$. aeruginosa PA49-resistance. Tang et al. [171] showed that adhesion of $S$. aureus is lower on superhydrophobic surfaces consisting of TiO$_2$ nanotubes treated with perfluoroctyl triethoxysilane than on hydrophobic and hydrophilic TiO$_2$ nanotubes. This is shown in Fig. 13.

5.1.3 Nanorough Surfaces
It was also shown that surface roughness plays an important role in bacterial attachment. Ohshima [172] suggests that bacteria can sense surface topography and adjust the attachment mechanisms. Lorenzetti et al. [85] showed that the bacterial attachment to TiO$_2$-anatase coatings is mainly regulated by surface topography, with only minor contributions from the wetting and surface charge. These authors demonstrated that despite its high hydrophilicity, nanorough surface of such materials significantly reduces bacterial adhesion because the voids in the surface do not offer full contact between bacteria and the material. Despite being chemically identical, surfaces with different topography differ in bacterial attachment.

5.2 Bactericidal Effect/Active Surfaces
According to the chemical composition, antibacterial surfaces with active bactericidal effect can be differentiated into two groups: organic and inorganic. Organic active surfaces generally consist of polymers and can be classified into at least three groups by the mode of action (Fig. 14): (1) biocidal polymers, (2) polymeric biocides, and (3) biocide-releasing polymers [173]. Inorganic surfaces include metals/metal oxides or nonmetals, such as selenium or graphene [174].

5.2.1 Organic Antibacterial Agents
5.2.1.1 Biocidal/Antibiotic Polymers
Biocidal polymers are antibacterial over the whole polymer chain. They mainly consist of quaternary ammonium groups, shown in Fig. 15, that generate quaternary ammonium compounds [175]. Other biocidal polymers contain cationic biocides, such as phosphonium, tertiary sulfonium, and guanidinium [144].

The mechanism of action of these polymers is via the positively charged polymeric surface that is destructive for mostly negatively charged microbial cells [175]. It is generally believed that the main antibacterial mechanism, by
which such biocidal polymers directly kill microbes, is disruption of cell membranes and complete loss of membrane function, with precipitation of intracellular constituents [176]. Li et al. [177] prepared a hydrogel based on dimethyldecylammonium chitosan (with high quaternization)-graft-PEG methacrylate (DMDC-Q-g-EM) and PEG diacrylate that is able to attract the sections of anionic microbial membrane into its internal nanopores, which

leads to microbial membrane disruption and microbial death. However, Chindera et al. [178] proposed a new model for the antibacterial action of polyhexamethylene biguanide, which enters microbes and causes the so-called chromosome condensation, suggesting that disruption of cell membrane is not the only mode of action of biocidal polymers.

Figure 14 Mode of action of (A) biocidal polymers, (B) polymeric biocides, and (C) biocide-releasing polymers [143].

Figure 15 Polymeric chains consisting of quaternary ammonium groups [143].
According to Siedenbiedel and Tiller [143], two other possible mechanisms of antibacterial action of biocidal polymers exist, namely, polymeric spacer effect and phospholipid sponge effect, shown in Fig. 16. The first mechanism suggests that the surface-grafted biocidal polymer might penetrate gram-positive bacterial cell wall, reach the membrane, and disrupt the phospholipid bilayer (Fig. 16A). The second mechanism involves the disruption of the phospholipid bilayer by adsorption of negatively charged phospholipids from the cell membranes of gram-positive bacteria (Fig. 16B).

5.2.1.2 Polymeric Biocides/Antibiotics
Unlike biocidal polymers, polymeric biocides are functional polymers that contain active specific segments only in the vicinity of their chains [179]. Attached biocide molecules can kill bacteria that adhere to the surface on contact. Killing of bacteria in active ways can be achieved through electrostatic or biocidal interaction [144].

5.2.1.3 Antibiotic-Releasing Polymers
In biocide-releasing polymeric systems, polymers do not act bactericidal, but serve as carriers for biocides/antibiotics. Polymers, such as poly(methyl methacrylate), poly-L-lysine, PEG, dextrans, and cyclodextrin, can be loaded with various inorganic or organic antibacterial active agents, such
as silver, gentamicin, tetraethylammonium bromide, and vancomycin [144,180]. Active agents are incorporated into the polymers either by chemical conjugation to premade polymers or by polymerizing an antibiotic-containing molecule [181].

Suhardi et al. [180] published a study in which they prepared implants made of ultrahigh-molecular-weight polyethylene embedded with antibiotic clusters. In such implants the drug is eluted over extended periods of time and the mechanical strength of the material remains unchanged, which do not require a two-stage surgery.

5.2.2 Inorganic Antibacterial Agents

The most studied metal nanoparticles that have shown antibacterial activity are silver (Ag), gold (Au), and zinc (Zn). A nanoparticle can be defined as any intentionally produced particle that has a characteristic dimension from 1 to 100 nm and has properties that are not shared by non-nanoscale particles with the same chemical composition [182]. The size and unique properties of nanoparticles, such as increased specific surface area with respect to particles of higher dimensions, make them good antibacterial agents and drug(antibiotic)-delivery systems. Their size enables them to penetrate through bacterial cell membrane and cause, among others, the leakage of cellular materials [183]. Choi et al. [184] showed that 20-nm Ag nanoparticles are able to penetrate a 40-μm E. coli biofilm within 1 h. The high surface area of nanomaterials enables them to serve as antibacterial delivery systems, which can improve the pharmacokinetics and therapeutic index of a drug in comparison to the free form of a drug [185]. Such an approach provides target drug delivery [186], sustained and controlled release of the drug [187], and prolonged circulation lifetime of the drug [188]. Besides, high surface area enables more bacteria-eliminating reactions and interactions with bacteria to occur.

Despite the fact that exact mechanisms of antibacterial performance of nanoparticles are still unclear, suggestions are as follows:

1. Accumulation and dissolution of nanoparticles inside or outside the bacterial cell can disrupt membrane permeability and cellular respiration [189,190].

Nanoparticles are able to attach to the bacterial cell wall through electrostatic interactions and disrupt the cell membrane [191,192]. Accumulation of nanoparticles on the bacterial cell membrane can block the
respiratory system of the cell, whereas accumulation of nanoparticles inside the cell causes the formation of aggregates that can damage the cell membrane [193]. This can change membrane permeability and release intracellular biomolecules into the cell surroundings [189].

2. Uptake of nanoparticles/dissolved ions in the cells can cause intracellular ATP depletion, disruption of DNA replication, and DNA damage [183,194].

Silver nanoparticles have a high affinity for binding with sulfur and phosphorus cell elements. Ag can therefore bind with the proteins in the cell membrane, as well as with the DNA [195]. Ramamurthy et al. [196] showed that silver nanoparticles cause DNA damage of *E. coli*. Also, ZnO particles can penetrate through the cell membrane and interact with the cell interior [197]. Mukha et al. [198] showed that the cell wall is destroyed by the penetration of Ag nanoparticles, while further on, also ROS are formed that inhibit ATP production and DNA replication.

3. Generation of reactive oxygen species (ROS) [199].

Zhang et al. [197] suggests that reasons for antibacterial performance of ZnO nanoparticles, among others, lies in chemical reactions on the surface and in the bacterial cell interior, with Zn\(^{2+}\) and reactive oxygen species (ROS), such as H\(_2\)O\(_2\), generated as a consequence of the presence of ZnO. ZnO can block the cell membrane transport channels and damage the membrane by abrasion. Similarly, dissolved silver cations interfere with the thiol and amino groups of proteins, with nucleic acids, and with cell membranes [200] and disrupt the bacterial cell membrane permeability and cellular metabolism. In addition, depending on the involved cell type, silver also contributes to the formation of ROS. Imani et al. [201] demonstrated that uptake of TiO\(_2\) microbeads with nanostructured surface leads to the generation of ROS inside the cancer cells after illumination. A similar mechanism is responsible for the destruction of bacterial cell wall after exposing the bacteria with attached nanoparticles to light [202], as presented in Fig. 17.

4. Binding of nanoparticles with enzymes can cause their inactivation and arrest of cellular respiration [203–205].

Nanoparticles can cause dysfunction of the respiratory electron transport chain by inhibiting respiratory chain enzymes [206]. ZnO nanoparticles can inhibit the activity of a typical enzyme, \(\beta\)-galactosidase, in *S. aureus* [207]. Similarly, nanoparticles such as SiO\(_2\), TiO\(_2\), ZnO, and Ag mediated the inhibition of urease and DNA polymerase in the same bacteria [208].
6. FUTURE OUTLOOKS FOR ANTIBACTERIAL IMPLANTABLE SURFACES

Surface coating techniques, such as ion implantation, electrochemical anodization, ion exchange, solgel techniques, plasma spraying, and incorporation of metal ions such as silver, copper, or zinc, have been vastly studied for the purpose of antibacterial material surface design [209,210]. With the growing nanotechnology field, much of the recent research has been devoted to the development of nanoparticles that are bound to the surface of materials. Many scientific efforts have been undertaken for the synthesis of ZnO, Cu, and Ag nanoparticles with biocidal properties, as already described. Although the antibacterial properties of the surfaces have gained more or less success by the mentioned procedures, the problem of cytotoxicity and thus the inability to promote adhesion and growth of the desired cell type (such as inhibited growth of osteoblast cells) is still not fully solved. Furthermore, the drawbacks of many coating procedures are that they are harmful to the environment, are expensive, and require long-lasting surface treatment procedures, while the coating usually does not provide the desired mechanical properties. Recently, also bioinspired approaches mimicking the natural surfaces, which have over the years developed its fascinating self-defense mechanisms to prevent bacterial colonization, have become...
popular. Thus another very prospective approach is to alter surface morphology and form a biomimetic surface that would prevent bacterial adhesion, as well as limit bacterial proliferation and biofilm formation. Although the bacterial cells are about 0.5–2 μm in size, they can interact with the surface on the nanometer scale with their filamentous appendages, as these appendages are about 10 nm in diameter and about 100 nm in length. Some reports already showed that surface nanofeatures can significantly influence bacterial adhesion. Additionally, it would be of great interest to also improve the proliferation of the desired cell type, for example, human osteoblast cells (bone-forming cells) in case of orthopedic implants or ECs in case of vascular implants. Studies done on TiO2 nanotubes with different diameters have already showed that nanotopography plays an important role in bacterial adhesion [211] as well as in osteoblast adhesion [212] and proliferation of ECs [213]. Similar results were also observed for other nanostructured materials, as alteration in surface features on the nanometer scale significantly influenced bacterial adhesion of gram-positive bacteria [214]. The appropriately nanostructured/biomimetic surfaces would present an intriguing way to prevent bacterial adhesion. Unfortunately, till now, not enough systematic studies have been conducted to facilitate reliable information on appropriate topographic features (nanotopography/microtopography) that would prevent bacterial adhesion. Probably, there is also synergistic influence of other surface features such as surface chemistry and wettability, which play vital roles in biological responses. For example, titanium alloys tend to passivate its surface in air by forming an amorphous nonstoichiometric oxide layer. This very thin film (2–6 nm thick) counts for the bioinertness of the titanium implants when used in the body; however, it is mechanically unstable and gets promptly covered by a contamination film, even after cleaning in organic solvents and after sterilization [215,216]. Massaro et al. [216] observed a large variation in the chemical composition of commercial titanium implants because of contaminants from the environment and/or fabrication processes. As surface contamination can influence the material bioperformances in vivo, rigorous quality controls and/or preparation protocols should be applied during implant production to deliver identical surfaces over time. Several surface strategies have been proposed to remove this thin layer of carbon contamination [215] and to mitigate the problem of bacterial attachment, among which different passive surface finishing/modifications (i.e., superhydrophilic or superhydrophobic surfaces, nanorough surfaces, etc.) were employed [174]. One of the interesting approaches for surface modification of biomaterials is
gaseous plasma treatment. Gaseous plasma not only easily removes contaminants from the surface but also modifies the topmost surface layer without influencing its bulk attributes. Treatment of titanium by oxygen plasma has already shown to be useful for improving cell differentiation and proliferation [212,213]. Plasma technologies are rapidly gaining importance in the medical field not only for surface treatment of biomaterials but also for direct treatment of wounds and skin diseases, for teeth bleaching, and in cancer therapy. Moreover, plasma modification is an environment-friendly process that enables the modification of surface chemistry, morphology (on the nanoscale), wettability, surface charge, and crystal formation of polymeric materials [217,218]. Plasma surface modification could be used for the preparation of antibacterial surfaces, not only for immobilization of antibacterial coatings (ion sputtering, ion planting, plasma spraying, chemical vapor deposition) but also to enable antibacterial properties on the surface of metal implants by altering the surface nanotopography and chemistry. Such changes should reduce the number of attached bacteria. For example, plasma pretreatment of polymer suture significantly reduced bacterial attachment [219]. Preliminary results already showed that Ti surfaces treated with radio-frequency (RF) oxygen plasma at high power (1000 W) have antibacterial influence against *E. coli* [220]. It was also shown that the surface of Ti alloy (Ti-6Al-4V) treated with RF oxygen plasma at high power (1000 W) reduces the adhesion of *S. aureus* [221]. In this case the surface morphology was altered by ion bombardment and formation of a thicker oxide layer on the surface. However, more systematic studies in this direction are needed in order to develop new-generation surfaces with improved biological characteristics not only for the prevention of bacterial adhesion and biofilm formation but also for improved proliferation of desired cell types needed for specific applications.

7. CONCLUDING REMARKS

In this chapter the importance of antibacterial surfaces has been examined. Common features in the surfaces of biomedical implantable devices have been reviewed. General biofilm formation mechanisms and pathogens related to vascular implants have been summarized. Biomedical devices including implants, usually made of metals, such as Ti and its alloys, stainless steel, cobalt-chromium etc. and polymeric materials like PEG, often do not offer full resistance to biofilm formation, which presents a serious problem to
public health. Millions of infections associated with biofilms are occurring each year, and moreover, there is rapid emergence of antibiotic-resistant bacterial strains. The infections most often involve coagulase-negative staphylococci and *P. aeruginosa*, but infections caused by *S. aureus* are increasing in frequency. Such infections are often associated with morbidity and also mortality. There are also some suggestions that the rate of infection may be increasing. Therefore implant-related infections present serious health care burden, and in the recent years, many different approaches have been proposed to lower the risk of infections from implantable devices or medical tools. Modifications to the surface topography by nanostructuring the surface or by using various coatings that inhibit adhesion of microorganisms or act bactericidal are therefore developing rapidly. However, antibacterial mechanisms of such surfaces are still poorly understood. Thus there is a huge demand to develop new-generation implantable materials with improved antibacterial or bacteriostatic surface properties.

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