Antibacterial surfaces: the quest for a new generation of biomaterials

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In this review we attempt to clarify the notion of what is meant by the term antibacterial surfaces and categorize the approaches that are commonly used in the design of antibacterial surfaces. Application of surface coatings and the modification of the surface chemistry of substrates are generally considered to be a chemical approach to surface modification (as are surface polymerisation, functionalisation, and derivatisation), whereas, modification of the surface architecture of a substrate can be considered a physical approach. Here, the antifouling and bactericidal effects of antibacterial surfaces are briefly discussed. Finally, several recent efforts to design a new generation of antibacterial surfaces, which are based on mimicking the surface nanotopography of natural surfaces, are considered.

Antibacterial surfaces

Despite considerable recent progress in the development of nanobiotechnology and nanofabrication techniques, the quest to design and fabricate new antibacterial surfaces (see Glossary) as an integral component of advanced biomaterials remains a high research priority [1–3]. Microorganisms are the oldest life form on our planet and over the millions of years of their existence they have developed versatile adaptive mechanisms for the colonisation of surfaces [4]. The colonisation of surfaces by bacteria is known to adversely affect the function of a variety of specific interfaces, such as those found in petroleum pipelines and aquatic flow systems, textiles, contact lenses, and medical implants [2,3].

In order to eliminate or substantially reduce the extent of bacterial attachment and biofilm formation on these surfaces, intensive efforts have been focused on the fabrication of new surfaces, or on the improvement of the performance of existing antibacterial surfaces by, for example, the application of surface coatings, or modification and/or alteration of the surface architecture [2,5]. In this review we attempt to clarify the definition of the term antibacterial surface and categorise the approaches that are commonly used in the design of antibacterial surfaces. It is suggested that antibacterial surfaces should be categorised as being either antifouling or bactericidal, depending on the effect that these surfaces have on biological systems with which they have contact. An array of antibacterial surfaces can be categorised according to the surface coating or surface chemistry modifications to which they have been subjected, for example, surface functionalisation, polymerisation, and derivatisation (i.e., chemical modification) or modification to the surface topography (physical modification). Several recent efforts to design a new generation of antibacterial surfaces, which are based on mimicking the surface nanotopography of natural surfaces, are also reported.

The concept of antibacterial surfaces

The paradigm of bacterial attachment and proliferation on surfaces was first recognised in the 1930s [6]. It was established that bacteria prefer to colonise a solid substrate that may be present rather than dwell in a planktonic state [7]. The formation of biofilms has been extensively studied over the past decades in an attempt to develop several surface modification approaches to prevent or reduce the extent of bacterial attachment using biocides, antibiotics, and surface treatment processes [1,2,8,9]. The rationale for these approaches was to design an antibacterial surface, which would prevent the initial attachment of bacteria, therefore preventing the subsequent formation of a biofilm.

Antibiofouling and bactericidal surfaces

Antibacterial surfaces may repel or resist the initial attachment of bacteria by either exhibiting an antibiofouling affect or by inactivating any cells coming into contact with the surface, causing cell death, therefore exhibiting a bactericidal effect. Antibacterial surfaces therefore can be broadly classified as either an antibiofouling [10] or bactericidal [1,11].

Antibiofouling surfaces may resist or prevent cellular attachment due to the presence of an unfavourable surface topography or surface chemistry with respect to the microorganisms [12,13]. Bacterial surfaces disrupt the cell on contact, causing cell death [1]. In some instances, an antibacterial surface may exhibit both antibiofouling
and bactericidal characteristics. For example, a polymer-coated surface that possesses the ability to switch reversibly between possessing both bactericidal and antibiofouling properties has recently been reported [14]. The surface is coated with a cationic N,N-dimethyl-2-morpholinone (CB ring) that is capable of inactivating bacteria in dry environments and a zwitterionic carboxybetaine (CB–OH ring) to resist bacterial attachment in wet environments [14].

**Natural or nature inspired antibacterial surfaces**

Nature represents an unexhausted source of inspiration for scientists and engineers, particularly in the field of biomimetics [15], where biological systems are fundamentally studied for their biotechnological applications [11,16]. Natural surfaces have been developed by nature through billions of years of evolution and it is thought that many of these surfaces might have developed the ability to resist or prevent bacterial colonisation [17]. Many of these surfaces are known to possess multiple integrated functions; some of the commonly studied surfaces include plant leaves, gecko foot, shark skin, insect wings, fish scale, and spider silk [16].

Some of the low-adhesive, superhydrophobic and self-cleaning surfaces found in nature have been investigated for their potentially antibiofouling characteristics [17]. Indeed, natural and biomimicked surfaces of insect wings, shark skin, and lotus leaves exhibit antibiofouling properties by preventing contaminating particles, algal spores, and bacterial cells from attaching to their surface [10,18]. The natural surface of taro (*Colocasia esculenta*) leaves immersed in water resists bacterial fouling [19]. Cicada (*Psaltoda claripennis*) wing surfaces appear to be bactericidal to *Pseudomonas aeruginosa* cells (Figure 1). The bactericidal effect of the cicada wings is exclusively due to the surface nanostructure of the wing rather than a surface chemical effect [11]. A 10 nm thin gold film coating changes the surface chemistry of a surface without changing the nanotopography of the surface, and does not adversely affect the bactericidal activity of the wing surface (Figure 1) [11]. Cicada wings are not actually antibiofouling (Figure 1c), because the cells attach onto the wings, but the attached cells are consequently mechanically ruptured by the action of the particular surface nanopattern within a short time after their attachment. A bacterial interaction mechanism with cicada wing nanostructures, which could be useful in the production of similar surfaces, has been recently proposed [20]. The adsorption behaviour of the bacterial outer layer depends on the geometry of the pillars. As bacterial cells adsorb onto the nanopillar structures on the surfaces of the wings, the cell membrane stretches in the regions suspended above the pillars, and if the degree of stretching is sufficient, this leads to cell rupture (Figure 1g) [20].

Antimicrobial peptides (AMPs) could also be used in the design of antibacterial surfaces [21]. For example, CM15, a well-known synthetic peptide that was developed to mimic cecropin A, the naturally occurring peptide in moths, has been studied for its antimicrobial activity [22]. AMPs are now sought as new antibiotics or coating agents on a range of medical devices due to their bactericidal activities against of a broad range of bacteria. It is believed that AMPs are effective at low concentrations and are effective against antibiotic-resistant bacteria [23]. Once a minimum concentration is reached, AMPs act by disrupting the membrane bilayer of the bacteria through various mechanisms, including the formation of pores, disintegration of the membrane and attacking the cytoplasm and metabolic functions of the cells (Figure 2) [21]. The initial interaction between the bacteria and the proteins is electrostatic because AMPs are cationic; this renders a strong interaction with the negatively charged bacterial membranes. There are few complexities associated with using AMP-coated surfaces to repel bacteria, such as the ability to control effectively the release of entrapped peptides and ensuring that conditions are such that a minimum inhibitory concentration is achieved to allow the antibacterial function to be attained [23].

**Artificial antibacterial surfaces**

Several traditional and advanced surface modification techniques have been widely used in construction of artificial antibacterial surfaces [24–26]. These surfaces comprise a range of polymer- and nanoparticle-based surfaces [26,27]. Some of these artificial surfaces have exhibited a bactericidal or antibiofouling effect (Table 1) [18,24–26]. Silver-based bactericidal surfaces typically comprise silver-doped, coated, silver-containing polymers, silver nanoparticles, or silver thin films [27–29].

**Surface modification techniques**

Over the past decade, a variety of surface modification or treatment techniques have emerged for the fabrication of antibacterial surfaces [1,26,30]. The surfaces have undergone either chemical or physical treatments. These surface treatments can be broadly categorised as being surface functionalisation, derivatisation, polymerisation, or mechanical or surface architecture modification [1,25,30–32]. Surface functionalisation, derivatisation, or polymerisation approaches dominantly involve the chemical modification of the surface [1]. Mechanical and surface structuring approaches are regarded as being a physico-mechanical modification of the surfaces [18,33].

**Surface polymerisation**

Surface polymerisation is where a surface is modified by the polymerisation of an antimicrobial agent on the surface. Although surface polymerisation may also be categorised as surface coating, the alterations in the surface chemistry that occur during the polymerisation mean that it is appropriate to consider these surfaces here. The polymerisation process can take place via different means, for example, by covalent bonding or atom radical transfer [1,34,35]. Surfaces possessing chemically bonded hydrophobic polycations of quaternary ammonium salts have been found to possess bactericidal properties [1]. In addition, polyethylene, polypropylene, nylon, poly(ethylene terephthalate), and glass surfaces containing covalently attached poly(vinyl-N-hexylpyridinium) (hexyl PVP), a hydrophobic quaternary ammonium cation, have shown antibacterial effects [1,26,36]. The range of antibacterial activity of this class of substrate has been tested on surfaces used in textile
applications, where \(N\)-alkylated poly(ethyleneimines) (PEIs) are covalently immobilised onto cotton, wool, nylon, and polyester surfaces (Figure 2). It has been shown that high molecular weight chains exhibit greater degrees of antibacterial activity compared to low molecular weight \(N\)-alkylated PEIs [36]. It has been shown, however, that it is difficult to control the molecular weights and proportion of polydispersities in these substrates reactions. In order to

**Figure 1.** Example of a natural antibacterial surface that arises as a result of the surface nanopattern present on the surface of cicada wings [11]. (a) Photograph of a cicada (Psaltoda claripennis). (b) Scanning micrograph of the hexagonal arrangement of nanopillars on the cicada wing surface; each nanopillar is approximately 200 nm in height, 70 nm in diameter, and the pillars are 170 nm apart from centre to centre, scale bar = 200 nm. (c) Interaction of the Pseudomonas aeruginosa cells with the wing surface, scale bar = 1 \(\mu\)m. (d) Viability experiments of bacterial cells stained with propidium iodide. The red colour indicates that the cells are nonviable, scale bar = 5 \(\mu\)m. (e) Atomic force microscopic image (area scan of approximately 5 \(\mu\)m \(\times\) 5 \(\mu\)m) showing the bacterial cells affected by the action of the surface (arrows). (f) Bacterial cell interactions with a cicada wing that has been sputter coated with gold, demonstrating that the bactericidal effect is retained with the modified surface chemistry, demonstrating that the surface chemistry of the cicada wing has little if any role in controlling the antibacterial nature of the surface. (g) Schematic representation of cellular attachment onto the cicada wing nanopillars. (h) Illustration of the apparent rupture of the cell wall in the region suspended between the nanopillars, consistent with the biophysical model of the bacterial mechanisms of the cicada wing nano-pattern proposed in [20]. Reproduced, with permission, from [11,20].
overcome these problems, Lee et al. have used an atom transfer radical polymerisation (ATRP) approach to modify surfaces with quaternised ammonium groups, which displayed antibacterial effects [34]. This method is highly controlled, tuneable, and shows a permanent antibacterial effect because the surfaces can be reused without loss of activity [34,35]. The ATRP approach has been successfully utilised, is well understood, and is being applied in antibacterial surface studies [35]. There are potential applications of this method in the food, medical, and military industries, along with everyday household items. 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 [37].

Other surface modification approaches include the immobilisation of antibacterial agents on substrate surfaces via the mechanism of physicochemical adsorption. Such agents can contain various antibacterial polymers, enzymes, and peptides [21,26,32]; each exhibiting a different type of antibacterial effect. Polymeric molecules such as poly(methacrylate) and poly(hexamethylene biguanidinium hydrochloride) are just two examples of commonly studied antibacterial agents that have been used for this
Table 1. Current approaches in the design of antibacterial surfaces

<table>
<thead>
<tr>
<th>Approach</th>
<th>Antibacterial effects</th>
<th>Antifouling</th>
<th>Comments</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface coating</td>
<td>Silver, QACs, fluoride ion, antibiotics, N,N-dimethyl-2-morpholimine, doped coatings</td>
<td>HA, titanium coatings, zwitterionic carboxy betaine coatings</td>
<td>Coatings are often nonuniform; mechanically weak, and lacking long-term stability. The leaching time decreases the optimum concentration level and affect the antibacterial efficacy</td>
<td>[46,48–50]</td>
</tr>
<tr>
<td>Surface modification</td>
<td>Covalent bonding, hydrophobic polycations of quaternary ammonium salts: hexyl PVP, PEIs</td>
<td>Mostly bactericidal. General problems arise with the regeneration of antibacterial agents, development of bacterial resistance against leaching and nonleaching agents, and the effect on durability of the target surface</td>
<td></td>
<td>[1,26,36,40,56,65]</td>
</tr>
<tr>
<td>Surface functionalisation</td>
<td>Quaternary ammonium; DDA bromide; phosphonium, sulfonium; single-walled carbon nanotubes; alkylated polyethyleneimines</td>
<td>Polymeric microarrays, cyclic hydrocarbon moieties, catalytic chain polymerisation, polyethylene glycol, zwitterionic polymers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surface structuring</td>
<td>Nanopatterned cicada wing structures</td>
<td>Microscale shark skin, lotus and taro leaves-like surfaces, hierarchical micro- and nanopatterned artificial surfaces</td>
<td>Require further comprehensive and systematic studies</td>
<td>[10,31,46]</td>
</tr>
</tbody>
</table>

purpos[26]. Antibacterial polycationic polymers are known to affect adversely bacteria by disrupting the net negative charge of the membrane of the bacteria, which causes cell lysis and death. In addition to antimicrobial polymers, there are several antimicrobial enzymes that control the formation of biofilms [38]. For instance, protein-hydrolyzing enzymes such as subtilins are able to hydrolise bacterial proteins, resulting in an antibacterial effect [38]. Polysaccharide-degrading enzymes such as amylases and lysozymes are effective against a variety of Gram-positive and Gram-negative bacteria [39]. Some other oxiditive enzymes that are involved in superoxide production have also been studied in the context of their antibacterial activity [38].

A newly emerging class of polymers such as polynorbornene or poly(phenylene ethylene) derived from antimicrobial peptides (such as magainin or defensins) have been shown to exhibit high degrees of antibacterial activity together with low levels of cytotoxicity [24,26,32]. Such molecules are called synthetic mimics of antimicrobial peptides (SMAPs). The polymeric SMAPs are amphiphilic and cause disruption of the cytoplasmic membrane of the cells [24]. Application of these surface modification strategies in the design of antibacterial surfaces could significantly improve and enhance the antibacterial performance of substrate surfaces.

**Surface functionalisation and derivatisation**

Surface functionalisation and derivatisation are surface modification methods that have been developed to enhance the effectiveness of antibacterial surfaces [1,36]. This approach involves the introduction of long chain, hydrophobic, positively charged coatings such as alkylated polyethylene amines [26,39] or the introduction of a functional group on the surface via polymerisation, covalent linkages, and/or plasma treatment [40,41]. The key step in this approach is the introduction of particular functionality, such as a quaternary ammonium-phosphonium- or sulphonium-containing group [41,42], which imparts antibacterial properties to the surface. Recently, the fabrication of surfaces functionalised with nanomaterials, such as single-walled carbon nanotubes, to impart antibacterial property to the surface has been reported [25].
Plasma-assisted surface treatment

Plasma-assisted surface treatment can be classified as either chemical or physical modification of the surface, because plasma-assisted surface treatments change both the surface chemistry and morphology [30,43]. Due to the combination of exceptional surface stability, controlled chemical functionality and topography, plasma-based surface modifications offer an excellent platform for the improvement of biocompatible and antibacterial materials [30,43].

The surface chemical modification approaches discussed in this section suffer from several complications; the complex chemical surface treatment processes need to be carefully designed and carried out because the resulting functionalised surfaces may undergo further reactions that may adversely affect their bactericidal properties. Other problems include the nonuniformity of the coating substrate, and the possibility that the attached polymer chains undergo cleavage [44,45]. Higher functional group concentration has been linked to the extent of antibacterial behaviour, therefore, more work is needed to determine the functional group density to achieve optimal antibacterial behaviour [24,45].

Antibiofouling and bactericidal coatings

The application of surface coatings is one of the most frequently used methods for the fabrication of antibacterial surfaces [27,46]. A surface coating can be defined as the build-up of an antibacterial material onto the substratum surface [47]. The antibacterial coatings act by either being toxic when coming into contact with the bacteria or by releasing an effective chemical or antibacterial agent from the surface [1,8]. Surface coatings are commonly used in biomedical applications when surfaces are required to sustain the complex environments, for example, for the integration of tissue [46]. Medical implant coatings can include antibiotics, silver, titanium, hydroxyapatite, and the fluoride ion [46,48–50].

Silver-based coatings are widely used in medical implants due to the silver ions released from the surface being bactericidal against both Gram-positive and Gram-negative bacteria [8,28].

Similarly, hydroxyapatite (HA) coatings provide implant surfaces such as titanium with antibacterial properties [51]. Unfortunately, HA coatings have the drawback that they are mechanically weak and can be nonuniform in density and thickness. They have also been shown to fail in long-term stability trials [52].

Quaternary ammonium compounds (QACs) are widely utilised as antibacterial agents [53]. Unlike the release-based antibacterial mechanism of silver ions [54], QAC coatings possess a long-lasting contact-based antibacterial mechanism [53]. Despite these properties, it has been reported that certain bacteria are able to develop resistance against these surfaces [55].

Recently, a combination of different antibacterial agents has been tested. For example, the combined release and contact-killing abilities of silver and quaternary ammonium salts has shown potential as an effective antibacterial agent [56]. Similar combinatorial approaches have been applied using silver-doped silica films, titanium-doped iron, silver-doped inorganic–organic hybrids, silver-doped phenyltriethoxysilane, silver-doped titanium, and silver-doped HA coatings [57,58].

The use of surface coatings as antibacterial agents has revealed several shortcomings. Bacteria can develop resistance against antibiotics and antibacterial agents [55,59,60]. The antibiotics or antibacterial agents can take a long time to be released from the surface, and the concentration of the released agents may not be sufficient to maintain effective antibacterial activity [9,61]. In addition, the durability of the target surface may not be sufficient to maintain long-term antibacterial behaviour [9,45].

Surface topography modification

The role that substrate micro- and nanoscale surface topographical features play in controlling bacterial attachment has not been comprehensively characterised [62], and this area of nanotechnology has only recently become an area of intense research focus [31,63]. The important role that the surface nanotopography and architecture play in bacterial attachment and biofilm formation has recently been recognised [10,31,63,64]. The notion that bacteria would not be able to attach effectively onto nanoscopically smooth surfaces has been shown to be incorrect, because it is currently well documented that bacteria can successfully colonise surfaces with an average surface roughness ($R_q$) of the order of only a few nanometres or sub-nanometres [63].

Bactericidal effect of antibacterial surfaces

Chemically modified surfaces act on bacterial cells through direct contact between the substrate antimicrobial agents and the bacterial cell walls [1,26]. For example, a polymerisation reaction has been used to functionalise a substrate with the antibacterial $N,N$-dimethyldodecylammonium (DDA) bromide functional group, along with an addition of hydrophilic cationic satellite group, ethylendiamine (EDA) [40]. The combination of these two groups results in a surface that both attracts and damages any bacterial cells coming into contact with the surface [65].

Surfaces containing ammonium salts or quaternary ammonium groups have been shown to damage both Gram-positive and Gram-negative cells by the disruption of their cellular membranes [1]. The positively charged nitrogen in the ammonium group interacts with the negatively charged head groups of acidic phospholipids in the bacterial cellular membrane, causing general perturbations in the lipid bilayers [66]. This causes the cells to release potassium ions, which in turn causes the cell to lose its ability to undergo osmoregulation and other physiological functions [24,42,66,67]. The bactericidal activity of quaternary ammonium cations has been found to be dependent on the alkyl chain length. For example, QACs containing an alkyl chain containing 14–16 carbons show resistance to Gram-positive bacteria, whereas alkyl chain lengths of 12–14 carbons are more effective against Gram-negative bacterial cells [66,67]. It is notable that the alkyl groups containing <4 and >18 carbons have been shown to be ineffective against bacteria [66]. The characteristics and functions of a polymer change according to the length of the alkyl chain length of the QAC. As a result, the effect of these QACs on bacteria will also be affected. For example,
an increase in the adsorption ability and lipophilicity would compromise the hydrophobic–hydrophilic balance of the polymer. This variation leads to different levels of bactericidal activity of the polymers against different bacterial groups (i.e., Gram-positive and Gram-negative). Moreover, these changes lead to different affinities being exhibited between the polymer and the outer membrane or cytoplasmic membrane of the bacterial cells. Hence, surface modification of a substrate using variable alkyl chain lengths would require a thorough understanding of the resulting antibacterial effect to be obtained [24]. Other cationic antibacterial agents, including QACs, polymers, photosensitisers conjugates, and polysaccharide chitosan, act by damaging the cell membrane, causing cell death [53]. Hydrophobic cationic polymers such as polyethyleneimine target the outer membrane and peptidoglycan layer of the bacterial cells by penetrating the periplasm and cytoplasm, which affords the substrates bactericidal activity [51]. Short or long highly dense polymer brushes created on the surface by means of ATRP have the ability to inactivate bacterial cells without penetrating their membrane. ATRP efficiency may result from the maximisation of surface charge rather than obtaining an optimal alkyl chain length [53].

Antibacterial agents such as nanoparticles, alkylated polymers, polymeric compounds with specific functionality of the end groups have also been reported to be bactericidal (Table 2) [24,26,32,40,45,65,67–69]. Silver, photocatalytic TiO₂, nitric oxide releasing nanoparticles and metal oxides such as magnesium and zinc nanoparticles have been shown to be bactericidal [69–71]. It is thought that the bactericidal behaviour of nanoparticles arises from their electrostatic forces, basic character, oxidising power of halogens, generation of reactive oxygen species, and accumulation of nanoparticles near the cytoplasm, which kills the cells [69,72].

Polymeric surfaces containing alkylated groups have also been shown to be bactericidal [32,68]. The surfaces of alkylated polymers have been shown to exhibit bactericidal activity due to the hydrophobicity of the polycation, or more precisely the length of the alkyl chain [32,68]. Alkylated polymers are known to affect the bacterial membrane of the cell rather than by affecting the metabolic processes of the cells [68]. Similarly, polymers containing bactericidal end groups such as DDA are attached to the nonbactericidal satellite groups at the opposite distal end [65]. The bactericidal activity has been linked to the nature of the satellite groups (methyl, decyl, hexadecyl groups) however no direct correlation has been found between bactericidal activity and the length of the alkyl chain [24,65].

Silver-containing surfaces have been reported to exhibit bactericidal activity against a wide variety of Gram-positive and Gram-negative bacteria, namely P. aeruginosa, Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, and Klebsiella pneumoniae [15,28]. The silver ions released from silver-containing surfaces cause damage to the bacterial membrane, causing a disruption to the function of the bacterial enzymes and/or nucleic acids [29,73]. It is known that silver ions react with the negatively charged nitrogen, oxygen, or sulfur atoms present in the bacteria as phosphate, amino, carboxyl, and thiol groups in the cellular proteins and DNA [27,73]. The interaction of silver ions with thiol groups (–SH groups) is solely responsible for the observed bactericidal activity [29]. The silver ions also inactivate the respiratory chain and tricarboxylic acid (TCA) cycle enzymes, and induce hydroxyl radical formation, causing subsequent damage of the cellular DNA [29]. The minimum concentration of silver ions that is required to induce the bactericidal effect has been shown to be 0.1 ppm [54], whereas eukaryotic cells have been shown to be able to withstand 10 ppm exposure [73]. There are, however, inconsistent data regarding the maximum silver ion concentration that eukaryotic cells can withstand without suffering cell death [28].

Recently the surface nanoarchitecture of the cicada (Ps. claripennis) wing surfaces was found to be bactericidal [11].

**Antibiofouling effect of antibacterial surfaces**

The antibiofouling effect of antibacterial surfaces is believed to be due to the surface chemistry and/or surface architecture and topography of the surface [2,18,31,33,63,74,75]. Silicone surfaces, reconstructed as a polymeric microarray coated with esters, fluoro-substituted alkanes, and linear and cyclic hydrocarbon moieties, have been found to be antibiofouling [75]. This type of chemically modified surface has been found to be 30 times more efficient in repelling bacteria compared to that obtained using commercially available silver hydrogel-coated surfaces. Metallic surfaces containing sub-nanometre and nanometre scale surface roughness have exhibited differential antibiofouling properties against rod-shaped and spherical bacteria. It has been reported that P. aeruginosa cells are unable to sustain their attachment on such surfaces. S. aureus cells, however, are able to attach successfully to the surface [12]. Recently, it has been shown that the superhydrophobic microstructure arrays named as slippery liquid-infused porous surfaces (SLIPS) fabricated on a silicon wafer prevented 99.6% of P. aeruginosa, S. aureus, and E. coli biofilm attachment in flow conditions (Figure 2) [74].

**Biocompatibility of antibacterial surfaces**

The biocompatibility of antibacterial QACs that are commonly used as disinfectants in hand solutions, cosmetics, and environmental treatment plants, has been recently reviewed [67]. QACs such as poly(4-vinylpyridine) (PVP) are known to be toxic to mammalian cells [76]. Although the copolymerisation of 4-vinylpyridine (VP) with a bio-compatible monomer, such as hydroxyethyl methacrylate and polyethylene glycol methyl ether methacrylate, have shown to exhibit excellent biocompatibility with red blood cells without compromising the antibacterial activity [24,77], further work is required to assess accurately and compare the biocompatibility of different cell lines and the antibacterial activity of such polymers [77]. The effect of different antibacterial polymers on mammalian cells is summarised in Table 2.

The toxicological implications of other antibacterial surfaces such as nanoparticles and AMP have also been investigated [72,73,78]. Concentrations of ions such as silver and TiO₂ play an important role in determining
Table 2. Different antibacterial materials and their effects

<table>
<thead>
<tr>
<th>Antibacterial agents</th>
<th>Materials</th>
<th>Properties/characteristics</th>
<th>Applications</th>
<th>Toxicology</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polymers</td>
<td>PEI-based polymers such as alkylated PEI</td>
<td>Polycationic bases. High molecular weight PEIs have higher antibacterial activity</td>
<td>Textiles, medicine, dental implants</td>
<td>No toxic effect on macrophage cell line and secreted levels of tumour necrosis factor α</td>
<td>[36,80]</td>
</tr>
<tr>
<td></td>
<td>Polyvinylpyridine (PVP) based polymers such as alkylated PVP, benzyl PVP</td>
<td>Polycationic quaternary ammonium or sulfonium or phosphonium salts. The bactericidal activity depends on the alkyl chain length</td>
<td>Environmental disinfectants, wastewater treatments, horticulture, pharmaceutical products, preservatives, ointments, cosmetics, healthcare devices</td>
<td>Quaternised PVP exhibits minimum biocompatibility with human red blood cells but it can be improved by copolymerisation with hydrophilic co-monomers, hydroxyethyl methacrylate, and polyethylene glycol methyl ether methacrylate</td>
<td>[24,67]</td>
</tr>
<tr>
<td></td>
<td>DDA bromide</td>
<td>Polymers contain the bactericidal DDA end-groups. The antibacterial activity depends on the nature of the satellite (methyl, decyl, hexadecyl) group</td>
<td>Medicine</td>
<td>Less haemotoxic polymer as tested on porcine erythrocytes</td>
<td>[40]</td>
</tr>
<tr>
<td></td>
<td>N-halamines such as polymers with oxidative halogens (Br⁻, Cl⁻)</td>
<td>Polymeric compounds with N-halamine groups impart bactericidal activity</td>
<td>Dental, medical, textile, paper, food packaging, and wastewater treatment</td>
<td>Used as drug delivery carriers along with covalently attached antibiotics</td>
<td>[24,42]</td>
</tr>
<tr>
<td>Nanoparticles (NPs)</td>
<td>Silver</td>
<td>Silver NPs possess significant bactericidal activity due to the release of silver ions</td>
<td>Medical, biosensors, footwear, paints, wound dressings, cosmetics, plastics, optical and conductive applications</td>
<td>In toxicological research, the ion release rate as function of nanoparticle size is a very important aspect that needs to be controlled. The NPs show in vitro and in vivo toxicity to mammalian and non-mammalian cells and organs such as skin, lung, liver, kidney, brain, structural malfunctions in mice, rats, Drosophila, and fish. The NPs affect the cell membranes, mitochondria and genetic material.</td>
<td>[54,69,78]</td>
</tr>
<tr>
<td></td>
<td>TiO₂</td>
<td>The NPs possess no activity in dark condition. The bactericidal activity is stimulated by photoactivation. Some bacteria such as Cupravidus metallidurans CH34 are resistant to TiO₂ nanoparticles</td>
<td>Medical, water purification systems, membranes.</td>
<td>Does not cause loss of cell viability of mouse neuroblastoma. Although they exhibit slight toxicity in high concentrations</td>
<td>[71,72]</td>
</tr>
<tr>
<td></td>
<td>Nature inspired or natural antibacterial agents</td>
<td>AMPs</td>
<td>Cationic and hydrophobic AMPs have a highly rigid backbone and amphipathic (opposite polar and apolar side groups) conformations, which helps in bactericidal activity</td>
<td>Antibiotics, biomaterials, pharmaceuticals</td>
<td>Nontoxic to mammalian cells as tested against human osteosarcoma and blood cells. Does not activate human platelets or initiate complement activation.</td>
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<td></td>
<td>Plant leaves, animal skin and insect wings.</td>
<td></td>
<td>Repel bacterial cells due to micro/nanoscale surface topography</td>
<td>Unable to kill cells but keep surfaces fouling resistant such as ship hulls. Could also be used in fermentation industry where directing bacterial cells towards a specified region is a key requirement.</td>
<td>[10,16,17]</td>
</tr>
<tr>
<td></td>
<td>Insect wings (cicada)</td>
<td>Nanopillar arrangement on cicada wings capable of killing bacterial cells due to specific surface geometry</td>
<td>Potential use in medical implants, wastewater treatment systems, ship hulls, windows, household objects, industrial vessels and pipes</td>
<td></td>
<td>[11]</td>
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the cytotoxicity effect on mammalian cells. Threshold values for different mammalian cells have been reported [72,73,78]. AMPs have been tested against mammalian cells and it has been shown that AMPs exhibited no toxicity towards the cells, and that platelet adhesion and activation is insignificant [79].

Concluding remarks and future perspectives

The number of antibacterial surfaces that has been identified is quickly expanding. This review provides an overview of existing antibacterial surfaces, including those that have recently been developed, and the current approaches to their design are discussed. Antibacterial surfaces are capable of repelling bacterial cells, preventing their attachment, or inactivating/killing cells that do come into contact with the surface. It is, therefore, important to understand the mechanisms responsible for the antibacterial action. Several questions have been raised throughout the exploration of the currently available antibacterial surfaces, and these are summarised in Box 1. Although chemically based bactericidal mechanisms are known to be effective, the duration and specificity of any particular chemical antibacterial mechanism needs to be thoroughly evaluated. The newly emerging range of bactericidal surfaces that have the capability of killing any bacteria that come into contact with them, the killing mechanism being based primarily on their surface structure, may prove to be a good starting point for a new and innovative direction in the design of biomaterials as an alternative to the traditional, chemical-based approaches. Greater consideration should be given to studying the role that surface topography plays in the creation of an antibacterial or antifouling surface, particularly at the nanoscale. Other factors such as determining the biocompatibility of the bactericidal surface and/or their toxicological effect also require comprehensive investigation (Table 2). To date, only a limited number of studies have been performed to address these issues. The recent developments in methods for modifying the nanotopography of surfaces may prove to be very useful techniques for the fabrication of a new generation of bactericidal biomaterials.

References


Box 1. Outstanding questions

- How do we define the limit at which chemical-based bactericidal mechanisms are effective, particularly for those that are efficient for only a limited period of time or to a specific group of cells?
- What is the optimum alkyl chain length for the generation of a bactericidal surface through various surface modification techniques?
- Is it appropriate to use a trial and error approach in the development of antibacterial surfaces through chemical surface modification mechanisms?
- Should the role that surface topography plays in creating an antibacterial or antifouling surfaces, particularly at the nanoscale, be given greater consideration than it currently receives?
- What represents the most effective design of a universally efficient antibacterial surface?
64 Ahamed, K. et al. (2010) The interaction of cells and bacteria with surfaces structured at the nanometre scale. Acta Biomater. 6, 3824–3846
65 Waschinski, C.J. et al. (2008) Insights in the antibacterial action of poly(2-methoxyethoxamine)s with a biocidal end group and varying satellite groups. Biomacromolecules 9, 1764–1771