Trends in Biotechnology

Bio-inspired soft robotics

Cell PRESS

Review

Cell
PRESS

Antibacterial surfaces: the quest for a new generation of biomaterials

Jafar Hasan, Russell J. Crawford, and Elena P. Ivanova

Faculty of Life and Social Sciences, Swinburne University of Technology, PO Box 218, Hawthorn, Victoria, 3122, Australia

In this review we attempt to clarify the notion of what is meant by the term antibacterial surfaces and categorise the approaches that are commonly used in the design of antibacterial surfaces. Application of surface coatings and the modification of the surface chemistry of substrata 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 antibiofouling 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]. Bactericidal surfaces disrupt the cell on contact, causing cell death [1]. In some instances, an antibacterial surface may exhibit both antibiofouling

---

CrossMark

Glossary

Antibacterial surfaces: Surfaces that are capable of reducing the extent of attachment and proliferation of bacteria. They could be classified as antibiofouling and bactericidal surfaces.

Antibiofouling: Antibiofouling generally implies repelling bacterial cells from attaching onto the surfaces; this is achieved through unfavourable conditions on the surfaces.

Bactericidal surfaces: Surfaces that inactivate the bacterial cells largely through chemical mechanisms or agents.

and bactericidal characteristics. For example, a polymer-coated surface that possesses the ability to switch reversibly between possessing both bactericidal and anti-biofouling 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 of 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 physicomechanical 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 teryphthalate), 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
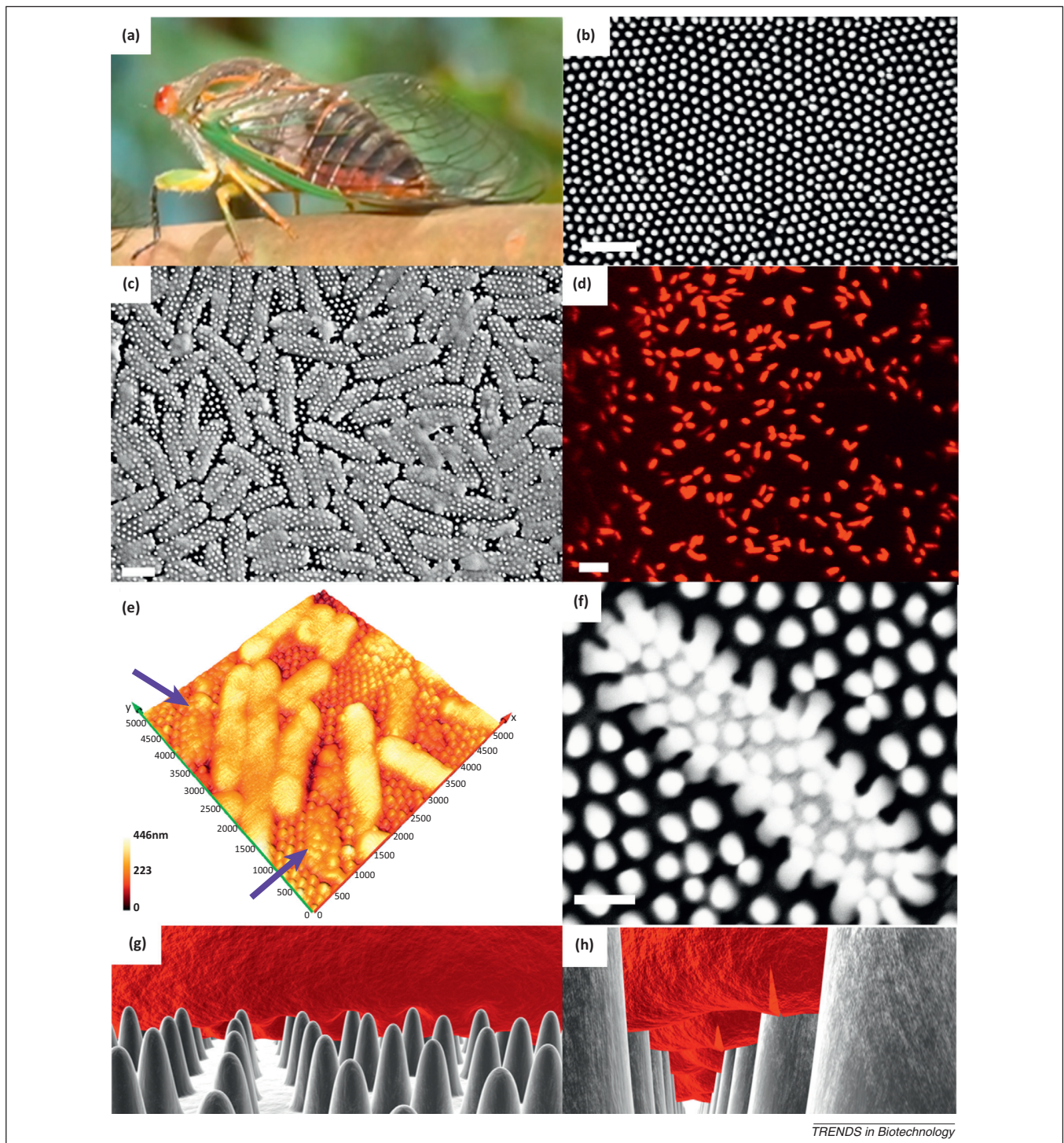
Figure 1. Example of a natural antibacterial surface that arises as a result of the surface nanotopography present on the surface of cicada wings [11]. **(a)** Photograph of a cicada (*Psaltoda claripennis*). **(b)** Scanning micrograph of the hexagonal arrangement of nanopillars on the cicada wing surfaces; 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 μm. **(d)** Viability experiments of bacterial cells stained with propidium iodide. The red colour indicates that the cells are nonviable, scale bar = 5 μm. **(e)** Atomic force microscopic image (area scan of approximately 5 μm × 5 μm) 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].

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
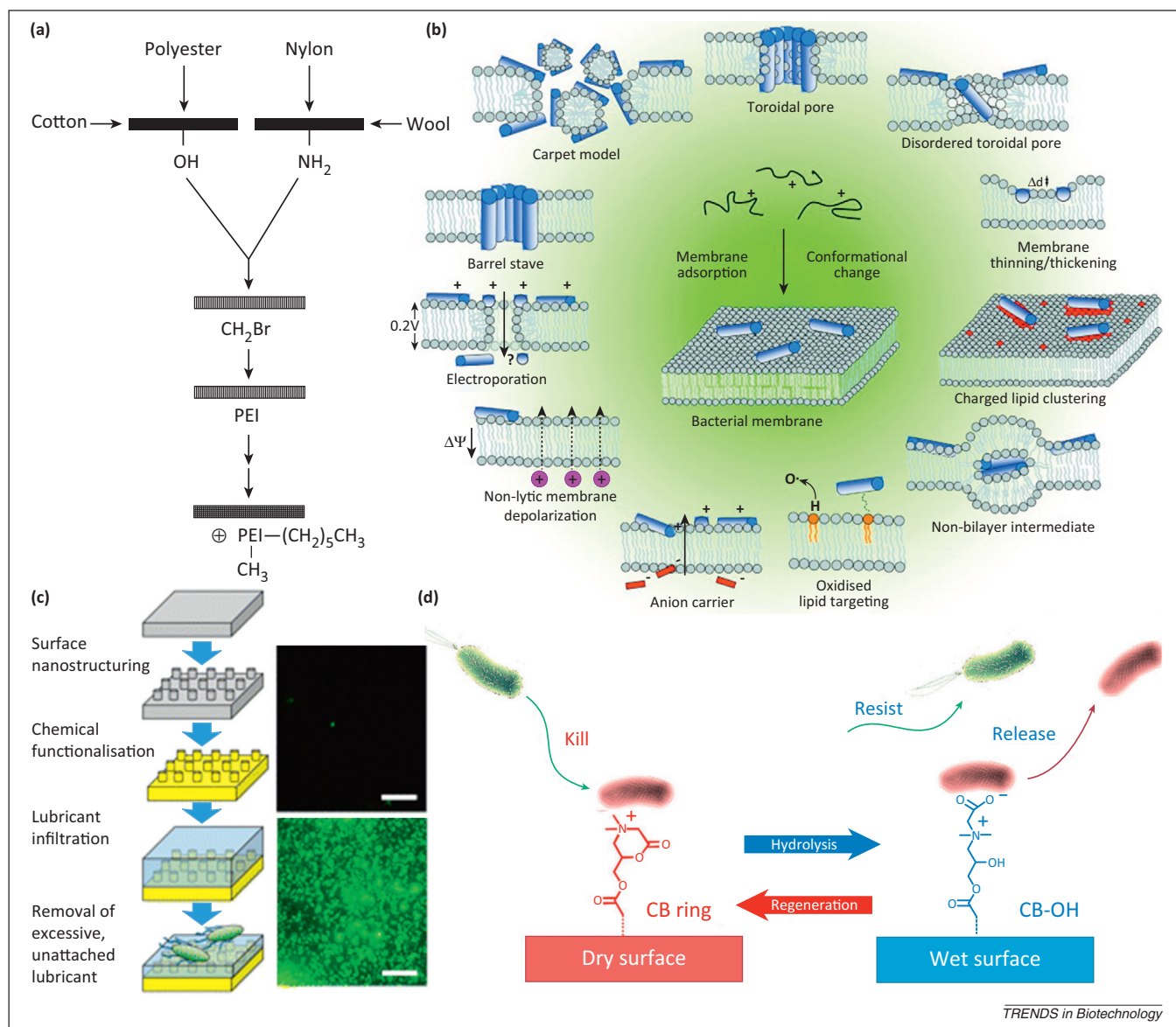
Figure 2. An array of strategies used in the design of antibacterial surfaces. **(a)** Example of a bactericidal surface used in the textile industry; the sequential synthesis of *N*-alkylated polyethylenimines (PEIs) performed by covalent immobilisation on cotton, wool, polyester, or nylon cloths. Reproduced, with permission, from [36]. **(b)** Modes of activity of antimicrobial peptides (AMPs) interacting with the bacterial cytoplasmic membrane. Different models of peptide adsorption demonstrate the disruption of the membrane. Reproduced, with permission, from [21]. **(c)** An example of an antibiofouling surface which was fabricated using nanostructuring and chemical functionalisation techniques. The surface (fluorescent image, top), which is termed slippery liquid-infused porous surfaces (SLIPS), prevented 99.6% of *Pseudomonas aeruginosa* biofilm attachment over a 7-day period compared to that obtained using the control surface (fluorescent micrograph, bottom). The control surface was a superhydrophobic nanostructured polytetrafluoroethylene (PTFE) substratum, which accumulated a biofilm within hours of contact with the bacteria, scale bars = 30 μm. Reproduced, with permission, from [74]. **(d)** A switchable polymer surface that shows antibiofouling as well as bactericidal properties in both dry and wet conditions. A cationic *N,N*-dimethyl-2-morpholinone (CB ring) was used to attack and kill the cells in dry conditions, whereas zwitterionic carboxy betaine (CB-OH) defended the bacteria in wet conditions. Reproduced, with permission, from [14].

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

Approach			Antibacterial effects		Comments	Refs
			Bactericidal	Antibiofouling		
Surface coating			Silver, QACs, fluoride ion, antibiotics, N,N-dimethyl-2-morpholinone, doped coatings	HA, titanium coatings, zwitterionic carboxy betaine coatings	Coatings are often nonuniform; mechanically weak, and lacking long-term stability. The leaching time decreases the optimum concentration level and affect the antibacterial efficacy	[46,48–50]
Surface modification	Surface chemistry	Surface polymerisation	Covalent bonding, hydrophobic polycations of quaternary ammonium salts: hexyl PVP, PEIs		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 Surface functionalisation is concentration dependant and there is a risk of further chemical reactions. Surface polymerisation cannot control molecular weight and low polydisperities. Atom free radical polymerisation can result in fabrication of long-lasting bactericidal surfaces	[1,26,36, 40,56,65]
		Surface functionalisation or derivatisation	Quaternary ammonium; DDA bromide; phosphonium, sulfonium; single-walled carbon nanotubes; alkylated polyethyleneimines	Polymeric microarrays, cyclic hydrocarbon moieties, catalytic chain polymerisation, polyethylene glycol, zwitterionic polymers		
	Surface structuring		Nanopatterned cicada wing structures	Microscale shark skin, lotus and taro leaves-like surfaces, hierarchical micro- and nanopatterned artificial surfaces	Require further comprehensive and systematic studies	[10,31,46]

purpose [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-hydrolysing enzymes such as subtilins are able to hydrolyse 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 oxidative 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 polynorbornenes or poly(phenylene ethylene) derived from antimicrobial peptides (such as magainin or defensin) 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 sulfonium-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 surfaces 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_a) 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, ethylenediamine (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, photosensitiser conjugates, and polysaccharide chitosan, act by damaging the cell membrane, causing cell death [53]. Hydrophobic cationic polymers such as polyethylenimine 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 TiO$_2$, 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 ppb [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-vinylpryidine (VP) with a biocompatible 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 TiO$_2$ play an important role in determining

Table 2. Different antibacterial materials and their effects

Antibacterial agents	Materials	Properties/characteristics	Applications	Toxicology	Refs
Polymers	PEI-based polymers such as alkylated PEI	Polycationic bases. High molecular weight PEIs have higher antibacterial activity	Textiles, medicine, dental implants	No toxic effect on macrophage cell line and secreted levels of tumour necrosis factor α	[36,80]
	Polyvinylpyridine (PVP) based polymers such as alkylated PVP, benzyl PVP	Polycationic quaternary ammonium or sulfonium or phosphonium salts. The bactericidal activity depends on the alkyl chain length	Environmental disinfectants, wastewater treatments, horticulture, pharmaceutical products, preservatives, ointments, cosmetics, healthcare devices	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	[24,67]
	DDA bromide	Polymers contain the bactericidal DDA end-groups. The antibacterial activity depends on the nature of the satellite (methyl, decyl, hexadecyl) group	Medicine	Less haemotoxic polymer as tested on pork blood cells and porcine erythrocytes	[40]
	N-halamines such as polymers with oxidative halogens (Br$^+$, Cl$^+$)	Polymeric compounds with N-halamine groups impart bactericidal activity	Dental, medical, textile, paper, food packaging, and wastewater treatment	Used as drug delivery carriers along with covalently attached antibiotics	[24,42]
Nanoparticles (NPs)	Silver	Silver NPs possess significant bactericidal activity due to the release of silver ions	Medical, biosensors, footwear, paints, wound dressings, cosmetics, plastics, optical and conductive applications	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.	[54,69,78]
	TiO$_2$	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 TiO$_2$ nanoparticles	Medical, water purification systems, membranes.	Does not cause loss of cell viability of mouse neuroblastoma. Although they exhibit slight toxicity in high concentrations	[71,72]
Nature inspired or natural antibacterial agents	AMPs	Cationic and hydrophobic AMPs have a highly rigid backbone and amphipathic (opposite polar and apolar side groups) conformations, which helps in bactericidal activity	Antibiotics, biomaterials, pharmaceuticals	Nontoxic to mammalian cells as tested against human osteosarcoma and blood cells. Does not activate human platelets or initiate complement activation.	[79]
	Plant leaves, animal skin and insect wings.	Repel bacterial cells due to micro/nanoscale surface topography	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.		[10,16,17]
	Insect wings (cicada)	Nanopillar arrangement on cicada wings capable of killing bacterial cells due to specific surface geometry	Potential use in medical implants, wastewater treatment systems, ship hulls, windows, household objects, industrial vessels and pipes		[11]

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 antibiofouling 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?

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 antibiofouling surface, particularly at the nanoscale. Other factors such as determining the biocompatibility of the antibacterial 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

1 Tiller, J.C. *et al.* (2001) Designing surfaces that kill bacteria on contact. *Proc. Natl. Acad. Sci. U.S.A.* 98, 5981–5985

2 Bazaka, K. *et al.* (2012) Efficient surface modification of biomaterial to prevent biofilm formation and the attachment of microorganisms. *Appl. Microbiol. Biotechnol.* 95, 299–311

3 Arciola, C.R. *et al.* (2012) Biofilm formation in *Staphylococcus* implant infections. A review of molecular mechanisms and implications for biofilm-resistant materials. *Biomaterials* 33, 5967–5982

4 Kraigsley, A.M. and Finkel, S.E. (2009) Adaptive evolution in single species bacterial biofilms. *FEMS Microbiol. Lett.* 293, 135–140

5 Variola, F. *et al.* (2011) Nanoscale surface modifications of medically relevant metals: state-of-the art and perspectives. *Nanoscale* 3, 335–353

6 ZoBell, C.E. and Allen, E.C. (1935) The significance of marine bacteria in the fouling of submerged surfaces. *J. Bacteriol.* 29, 239–251

7 ZoBell, C.E. (1943) The effect of solid surfaces upon bacterial activity. *J. Bacteriol.* 46, 39–56

8 Ivanova, E.P. *et al.* (2011) The influence of nanoscopically thin silver films on bacterial viability and attachment. *Appl. Microbiol. Biotechnol.* 91, 1149–1157

9 Warnes, S.L. and Keevil, C.W. (2011) Mechanism of copper surface toxicity in vancomycin-resistant Enterococci following wet or dry surface contact. *Appl. Environ. Microbiol.* 77, 6049–6059

10 Chung, K.K. *et al.* (2007) Impact of engineered surface microtopography on biofilm formation of *Staphylococcus aureus*. *Biointerphases* 2, 89–94

11 Ivanova, E.P. *et al.* (2012) Natural bactericidal surfaces: mechanical rupture of *Pseudomonas aeruginosa* by cicada wings. *Small* 8, 2489–2494

12 Ivanova, E.P. *et al.* (2011) Differential attraction and repulsion of *Staphylococcus aureus* and *Pseudomonas aeruginosa* on molecularly smooth titanium films. *Sci. Rep.* 1, 165–172

13 Mrabet, B. *et al.* (2009) Anti-fouling poly(2-hydoxyethyl methacrylate) surface coatings with specific bacteria recognition capabilities. *Surf. Sci.* 603, 2422–2429

14 Cao, Z. *et al.* (2012) Reversibly switching the function of a surface between attacking and defending against bacteria. *Angew. Chem.* 124, 2656–2659

15 Fullenkamp, D.E. *et al.* (2012) Mussel-inspired silver-releasing antibacterial hydrogels. *Biomaterials* 33, 3783–3791

16 Liu, K. and Jiang, L. (2011) Bio-inspired design of multiscale structures for function integration. *Nano Today* 6, 155–175

17 Barthlott, W. and Neinhuis, C. (1997) Purity of the sacred lotus, or escape from contamination in biological surfaces. *Planta* 202, 1–8

18 Fadeeva, E. *et al.* (2011) Bacterial retention on superhydrophobic titanium surfaces fabricated by femtosecond laser ablation. *Langmuir* 27, 3012–3019

19 Ma, J. *et al.* (2011) Nanostructure on taro leaves resists fouling by colloids and bacteria under submerged conditions. *Langmuir* 27, 10035–10040

20 Pogodin, S. *et al.* (2013) Biophysical model of bacterial cell interactions with nano-patterned cicada wing surfaces. *Biophys. J.* 104, 835–840

21 Nguyen, L.T. *et al.* (2011) The expanding scope of antimicrobial peptide structures and their modes of action. *Trends Biotechnol.* 29, 464–472

22 Fantner, G.E. *et al.* (2010) Kinetics of antimicrobial peptide activity measured on individual bacterial cells using high-speed atomic force microscopy. *Nat. Nanotechnol.* 5, 280–285

23 Onaizi, S.A. and Leong, S.S.J. (2011) Tethering antimicrobial peptides: current status and potential challenges. *Biotechnol. Adv.* 29, 67–74

24 Timofeeva, L. and Kleshcheva, N. (2011) Antimicrobial polymers: mechanism of action, factors of activity, and applications. *Appl. Microbiol. Biotechnol.* 89, 475–492

25 Tiraferri, A. *et al.* (2011) Covalent binding of single-walled carbon nanotubes to polyamide membranes for antimicrobial surface properties. *ACS Appl. Mater. Interfaces* 3, 2869–2877

26 Siedenbiedel, F. and Tiller, J.C. (2012) Antimicrobial polymers in solution and on surfaces: overview and functional principles. *Polymers* 4, 46–71

27 Knetsch, M.L.W. and Koole, L.H. (2011) New strategies in the development of antimicrobial coatings: the example of increasing usage of silver and silver nanoparticles. *Polymers* 3, 340–366

28 Kelly, P.J. *et al.* (2009) A study of the antimicrobial and tribological properties of TiN/Ag nanocomposite coatings. *Surf. Coat. Technol.* 204, 1137–1140

29 Gordon, O. *et al.* (2010) Silver coordination polymers for prevention of implant infection: thiol interaction, impact on respiratory chain enzymes, and hydroxyl radical induction. *Antimicrob. Agents Chemother.* 54, 4208–4218

30 Bazaka, K. *et al.* (2011) Plasma-assisted surface modification of organic biopolymers to prevent bacterial attachment. *Acta Biomater.* 7, 2015–2028

31 Bazaka, K. *et al.* (2011) Do bacteria differentiate between degrees of nanoscale surface roughness? *Biotechnol. J.* 6, 1103–1114

32 Muñoz-Bonilla, A. and Fernández-García, M. (2012) Polymeric materials with antimicrobial activity. *Prog. Polym. Sci.* 37, 281–339

33 Hochbaum, A.I. and Aizenberg, J. (2010) Bacteria pattern spontaneously on periodic nanostructure arrays. *Nano Lett.* 10, 3717–3721

34 Lee, S.B. *et al.* (2004) Permanent, nonleaching antibacterial surfaces. 1. Synthesis by atom transfer radical polymerization. *Biomacromolecules* 5, 877–882

35 Yang, W.J. *et al.* (2011) Biomimetic anchors for antifouling and antibacterial polymer brushes on stainless steel. *Langmuir* 27, 7065–7076

36 Lin, J. *et al.* (2003) Mechanism of bactericidal and fungicidal activities of textiles covalently modified with alkylated polyethylenimine. *Biotechnol. Bioeng.* 83, 168–172

37 He, D. *et al.* (2011) In situ-generated Ru(III)-mediated ATRP from the polymeric Ru(III) complex in the absence of activator generation agents. *J. Polym. Sci. Part A: Polym. Chem.* 49, 4594–4602

38 Thallinger, B. *et al.* (2013) Antimicrobial enzymes: an emerging strategy to fight microbes and microbial biofilms. *Biotechnol. J.* 8, 97–109

39 Kenawy, E-R. *et al.* (2011) Biocidal polymers: synthesis, antimicrobial activity, and possible toxicity of poly (hydroxystyrene-co-methylmethacrylate) derivatives. *J. Appl. Polym. Sci.* 120, 2734–2742

40 Fik, C.P. *et al.* (2011) Impact of functional satellite groups on the antimicrobial activity and hemocompatibility of telechelic poy(2-methyloxazoline)s. *Biomacromolecules* 13, 165–172

41 Al-Bataineh, S.A. *et al.* (2009) Covalent immobilization of antibacterial furanones via photochemical activation of perfluorophenylazide. *Langmuir* 25, 7432–7437

42 Kenawy, E-R. *et al.* (2007) The chemistry and applications of antimicrobial polymers: a state-of-the-art review. *Biomacromolecules* 8, 1359–1384

43 Bilek, M. and McKenzie, D. (2010) Plasma modified surfaces for covalent immobilization of functional biomolecules in the absence of chemical linkers: towards better biosensors and a new generation of medical implants. *Biophys. Rev.* 2, 55–65

44 Sambhy, V. *et al.* (2008) Multifunctional silane polymers for persistent surface derivatization and their antimicrobial properties. *Langmuir* 24, 7549–7558

45 Madkour, A.E. and Tew, G.N. (2008) Towards self-sterilizing medical devices: controlling infection. *Polym. Int.* 57, 6–10

46 Zhao, L. *et al.* (2009) Antibacterial coatings on titanium implants. *J. Biomed. Mater. Res. B: Appl. Biomater.* 91, 470–480

47 Geesink, R. *et al.* (1988) Bonding of bone to apatite-coated implants. *J. Bone Joint Surg. Br.* 70, 17–22

48 Hume, E.B.H. *et al.* (2004) The control of *Staphylococcus epidermidis* biofilm formation and in vivo infection rates by covalently bound furanones. *Biomaterials* 25, 5023–5030

49 Ding, S.J. *et al.* (2003) Environmental effect on bond strength of magnetron-sputtered hydroxyapatite/titanium coatings. *J. Mater. Sci. Lett.* 22, 479–482

50 Price, J.S. *et al.* (1996) Controlled release of antibiotics from coated orthopedic implants. *J. Biomed. Mater. Res.* 30, 281–286

51 Shah, N.J. *et al.* (2012) Osteophilic multilayer coatings for accelerated bone tissue growth. *Adv. Mater.* 24, 1445–1450

52 Lazarinis, S. *et al.* (2011) Effects of hydroxyapatite coating on survival of an uncemented femoral stem. *Acta Orthop.* 82, 399–404

53 Murata, H. *et al.* (2007) Permanent, non-leaching antibacterial surfaces – 2: how high density cationic surfaces kill bacterial cells. *Biomaterials* 28, 4870–4879

54 Kumar, R. and Münstedt, H. (2005) Silver ion release from antimicrobial polyamide/silver composites. *Biomaterials* 26, 2081–2088

55 Takenaka, S. *et al.* (2007) Adaptation of *Pseudomonas* sp. strain 7-6 to quaternary ammonium compounds and their degradation via dual pathways. *Appl. Environ. Microbiol.* 73, 1797–1802

56 Li, Z. *et al.* (2006) Two-level antibacterial coating with both release-killing and contact-killing capabilities. *Langmuir* 22, 9820–9823

57 Yin, Y. and Wang, C. (2011) Multifunctional performances of nanocomposite SiO_2/TiO_2 doped cationic EBODAC film coated on natural cellulose matrix. *J. Sol-Gel Sci. Technol.* 59, 36–42

58 Marini, M. *et al.* (2007) Antibacterial activity of plastics coated with silver-doped organic-inorganic hybrid coatings prepared by sol-gel processes. *Biomacromolecules* 8, 1246–1254

59 Bridier, A. *et al.* (2011) Resistance of bacterial biofilms to disinfectants: a review. *Biofouling* 27, 1017–1032

60 Hall Sedlak, R. *et al.* (2012) Engineered *Escherichia coli* silver-binding periplasmic protein that promotes silver tolerance. *Appl. Environ. Microbiol.* 78, 2289–2296

61 Wahlig, H. and Dingeldein, E. (1980) Antibiotics and bone cements: experimental and clinical long-term observations. *Acta Orthop.* 51, 49–56

62 Whitehead, K.A. *et al.* (2005) Retention of microbial cells in substratum surface features of micrometer and sub-micrometer dimensions. *Colloids Surf. B: Biointerfaces* 41, 129–138

63 Truong, V.K. *et al.* (2010) The influence of nano-scale surface roughness on bacterial adhesion to ultrafine-grained titanium. *Biomaterials* 31, 3674–3683

64 Anselme, 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(methyloxazoline)s with a biocidal end group and varying satellite groups. *Biomacromolecules* 9, 1764–1771

66 Gilbert, P. and Moore, L.E. (2005) Cationic antiseptics: diversity of action under a common epithet. *J. Appl. Microbiol.* 99, 703–715

67 Buffet-Bataillon, S. *et al.* (2012) Emergence of resistance to antibacterial agents: the role of quaternary ammonium compounds – a critical review. *Int. J. Antimicrob. Agents* 39, 381–389

68 Wong, S.Y. *et al.* (2010) Dual functional polyelectrolyte multilayer coatings for implants: Permanent microbicidal base with controlled release of therapeutic agents. *J. Am. Chem. Soc.* 132, 17840–17848

69 Upendra Kumar, P. *et al.* (2011) Study of mechanism of enhanced antibacterial activity by green synthesis of silver nanoparticles. *Nanotechnology* 22, 415104

70 Carpenter, A.W. *et al.* (2011) Influence of scaffold size on bactericidal activity of nitric oxide-releasing silica nanoparticles. *ACS Nano* 5, 7235–7244

71 Jeng, H.A. and Swanson, J. (2006) Toxicity of metal oxide nanoparticles in mammalian cells. *J. Environ. Sci. Health A: Tox. Hazard. Subst. Environ. Eng.* 41, 2699–2711

72 Hajipour, M.J. *et al.* (2012) Antibacterial properties of nanoparticles. *Trends Biotechnol.* 30, 499–511

73 Schierholz, J.M. *et al.* (1998) Efficacy of silver-coated medical devices. *J. Hosp. Infect.* 40, 257–262

74 Epstein, A.K. *et al.* (2012) Liquid-infused structured surfaces with exceptional anti-biofouling performance. *Proc. Natl. Acad. Sci. U.S.A.* 109, 13182–13187

75 Hook, A.L. *et al.* (2012) Combinatorial discovery of polymers resistant to bacterial attachment. *Nat. Biotechnol.* 30, 868–875

76 Li, G. *et al.* (1998) Study of pyridinium-type functional polymers. II. Antibacterial activity of soluble pyridinium-type polymers. *J. Appl. Polym. Sci.* 67, 1761–1768

77 Stratton, T.R. *et al.* (2009) In vitro biocompatibility studies of antibacterial quaternary polymers. *Biomacromolecules* 10, 2550–2555

78 Ahamed, M. *et al.* (2010) Silver nanoparticle applications and human health. *Clin. Chim. Acta* 411, 1841–1848

79 Gao, G. *et al.* (2011) The biocompatibility and biofilm resistance of implant coatings based on hydrophilic polymer brushes conjugated with antimicrobial peptides. *Biomaterials* 32, 3899–3909

80 Beyth, N. *et al.* (2008) Surface antimicrobial activity and biocompatibility of incorporated polyethylenimine nanoparticles. *Biomaterials* 29, 4157–4163