

Efficient surface modification of biomaterial to prevent biofilm formation and the attachment of microorganisms

Kateryna Bazaka · Mohan V. Jacob ·
Russell J. Crawford · Elena P. Ivanova

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Abstract Biomaterials play a fundamental role in disease management and the improvement of health care. In recent years, there has been a significant growth in the diversity, function, and number of biomaterials used worldwide. Yet, attachment of pathogenic microorganisms onto biomaterial surfaces remains a significant challenge that substantially undermines their clinical applicability, limiting the advancement of these systems. The emergence and escalating pervasiveness of antibiotic-resistant bacterial strains makes the management of biomaterial-associated nosocomial infections increasingly difficult. The conventional post-operative treatment of implant-caused infections using systemic antibiotics is often marginally effective, further accelerating the extent of antimicrobial resistance. Methods by which the initial stages of bacterial attachment and biofilm formation can be restricted or prevented are therefore sought. The surface modification of biomaterials has the potential to alleviate pathogenic biofouling, therefore preventing the need for conventional antibiotics to be applied.

Keywords Biomaterials · Surface treatment · Biofilm · Bacterial attachment

Introduction

Whether they are intended to sustain functions and physiological processes critical to life, to restore or preserve a level of activity, for diagnosis of disease, treatment delivery, tissue engineering, or to be used as a part of an elective aesthetic procedure to improve contour and visual appearance, biomaterials play an important role in disease management and in the advancement of health care (Tan et al. 2011; Van Vlierberghe et al. 2011; Ansari and Husain 2012). Their applications range from being coatings on tablets and capsules in pharmaceutical preparations, to being essential components of extracorporeal devices such as contact lenses or kidney dialyzers, and indwelling devices and implants (Dorozhkin 2011; Wagoner and Herschler 2011). Their chemical composition and physical and biological properties are equally diverse, with incessant advancements in technology being driven by the increasing need to care for an ageing population, with its associated need for extended medical care (Hoppe et al. 2011; Khan and Sefton 2011; Lewis 2011; Saito et al. 2011; Shadanbaz and Dias 2012). An increasing human life expectancy places a strong emphasis on the need for the long-term, stable performance of permanent devices and biomaterials used to replace host tissues and organs (Cardoso et al. 2011; Gioe et al. 2011; Kitao et al. 2011; Zhao et al. 2011). Concurrently, bioresorbable materials are gaining importance for the facilitation of host tissue restoration. This is done as an alternative to failed tissue replacement, hence overcoming any limitations that might be imposed by the use of foreign structures (Bendrea et al. 2011; Naderi et al. 2011). These limitations include poor biocompatibility, causing inflammation, inadequate tissue integration, and non-haemocompatibility, and time-dependent deterioration of properties (Donald 2011; Sun et al. 2011).

K. Bazaka
Electronic Materials Research Lab, School of Engineering,
James Cook University,
Townsville, Queensland 4811, Australia

M. V. Jacob · R. J. Crawford (✉) · E. P. Ivanova (✉)
Faculty of Life and Social Sciences,
Swinburne University of Technology,
PO Box 218, Hawthorn, Victoria 3122, Australia
e-mail: RCrawford@swin.edu.au
e-mail: EIVanova@swin.edu.au

The development of infections in or around the indwelling biomaterial devices, such as orthopaedic prostheses, urinary tract and cardiovascular catheters, intraocular lenses and dentures, continues to be a key issue that hinders the utilisation of these devices (Hanssen 2002; Montanaro et al. 2007). While evidently detrimental to the healing process and overall well-being of the patient, the attachment of bacterial cells, together with the ensuing formation of a biofilm, may also impede the performance of the device onto which they colonise (Liu et al. 2004; Stobie et al. 2008). Schematization of the four-stage universal growth cycle of a biofilm with common characteristics, including initiation, maturation, maintenance, and dissolution is presented in Fig. 1. The functions that the biofilm performs are vast, ranging from acting as a physical defence barrier against phagocytic predation and preventing cell detachment under normal flow conditions, to working as a

selective permeability barrier (Costerton et al. 1999; Subbiahdoss et al. 2009). Physical and chemical permeability ensures the effective transport of organic molecules and ions to cells at distance from the surface of the biofilm, binding and concentrating nutrients close to cells to ensure the survival of the cell population (Decho 2000). The very same mechanism limits the diffusion of agents that are damaging to the bacteria, including the systemic antibiotics routinely used to treat post-operative infections, and antibacterial agents leaching out from the biomaterial matrix. In some instances, the inability for conventional antibiotic therapies to make ready contact with the bacteria leads to only partial suppression of the infection, resulting in the development of chronic infections (Pavithra and Mukesh 2008). Elderly and immune-compromised patients are particularly susceptible to the development of sepsis, in some cases resulting in lethal

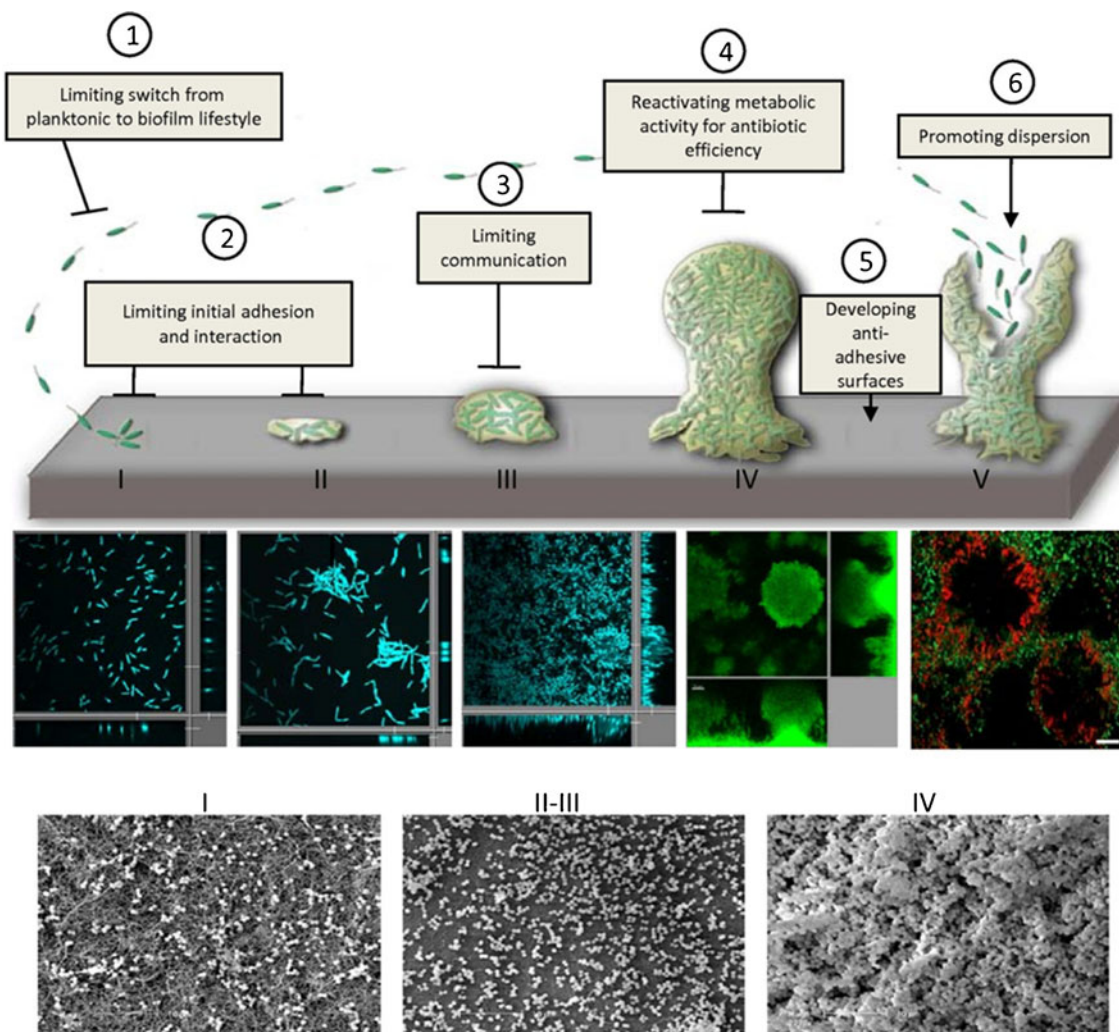


Fig. 1 The temporal evolution of a biofilm. Schematic representation of the characteristic steps associated with the formation of a biofilm, including initiation (I), maturation (II and III), maintenance (IV), and dissolution (V). *Pseudomonas aeruginosa* cells present during these steps are represented in the second row of images, the first three images

obtained using DAPI, followed by chromosomal GFP, then a LIVE/DEAD BacLight kit, as observed with confocal microscopy. The *S. aureus* attachment is presented in the third row, each obtained using scanning electron microscopy. (From Bordi and de Bentzmann 2011, used with permission)

consequences. Furthermore, continuous exposure of bacteria to high doses of conventional antimicrobials exerts a selective pressure on these microorganisms, positively affecting their ability to resist those broad-spectrum antibiotics (Andersson and Hughes 2011; Freire-Moran et al. 2011). It is not surprising then, that treating biomaterial-associated infections can be complicated, often demanding the physical (surgical) removal of the infected device.

Clearly, follow-up surgeries resulting in the requirement for extended hospital care are of no benefit to either the patient or the already overburdened healthcare system. Therefore, rather than addressing the consequence of bacterial colonisation, scientists are searching for methods by which the initial stages of bacterial attachment can be prevented. This can be achieved via a synthesis of novel materials and composites with advantageous characteristics integrated directly into the material matrix. A more economically attractive approach, however, is to enhance materials that are already being used by the medical industry (Raynor et al. 2009). Presently, numerous surface modification techniques are under intense investigation for their ability to effectively impart biocompatibility and biofunctionality onto a variety of biomaterials, while preserving their valuable bulk properties and physical dimensionality (Fu et al. 2011; Morent et al. 2011; Solouk et al. 2011; Vasilev et al. 2011; Wu et al. 2011a). In this review, we will concentrate on the methods currently being used to manipulate the nanoscale surface architecture with a view to imparting bactericidal activity onto the surface, and the effect of these modifications on bacterial attachment and biofilm formation. We will first give a brief overview of the current opinion regarding the factors that contribute to controlling cell–surface interactions, with particular regard to the influence of the surface micro- and nanotopographies on this process. We will then review several instances where the deterministic modification of a biomaterial surface has been successfully employed in order to mitigate the extent of bacterial attachment and biofilm formation. Finally, interesting developments in the area of bactericidal biomaterial coatings will be discussed.

Cell-surface dynamics

Although concentrated research efforts have been made in order to understand cell-surface dynamics, many of the facets of these mechanisms remain unclear. There is a particular scarcity of theoretical and experimental evidence on this topic on the nanometer and atomic length scales. Recently, the advancement of manufacturing and characterisation techniques has equipped researchers with the necessary tools for the effective investigation of the physico-chemical, mechanical, and biological cues that affect cell–surface interface dynamics (Desmet et al. 2009; Parreira et al. 2011).

The fundamental behaviour of living cells, including their adherence to biomaterials, has been found to be intimately linked to the surface properties of such a material and the characteristics of the medium that separates the cells and the substrate surface. The latter can either promote or impede cell attachment, in part by influencing the characteristics of the biomaterial surface, and by applying selective pressure on the bacterial cells (Fig. 2) (Döring et al. 2011; Lee et al. 2011). Phenotypic plasticity in response to environmental stimuli include turning regulatory genes on and off, thus regulating the size, shape, metabolic rate, and the substances that the cells excrete. In addition, these stimuli can cause the production of diverse progeny with distinct phenotypes via hypermutation and localized increased mutation rates (Dobrzyński et al. 2011; Lele et al. 2011). Chemical composition, temperature, nutrient availability, presence of other colonisers, the concentration of harmful substances (antimicrobials, metabolic by-products, and ions), and predation by host immune cells are some of the environmental factors that will directly impact the interface dynamics (Ardehali et al. 2002; Liu et al. 2011; Saldarriaga Fernández et al. 2011). For instance, the flow conditions around a substrate surface have a profound influence on the biofilm initiation, development, and subsequent stability, by affecting the rate at which bacterial cells are delivered to the surface, the time they reside in close proximity to the surface, and the probability for the cell detachment (Busscher et al. 2010). A significant decrease in desorption probabilities have been demonstrated for bacteria subjected to several minutes of direct cell–surface contact, owing to the exponential increase in the adhesion forces, namely, acid–base interactions, amongst cells and between cells and the abiotic surface (Andrews 2009; Busscher et al. 2010). Similarly, the acidity and ionic strength of the physiological solution have been shown to profoundly influence the degree of hydrophobicity of the respective bacterial cell and abiotic target surfaces, and as such on the strength of the electrostatic interactions within biofilm architecture (Pavithra and Mukesh 2008). Even though a precise understanding of the role that the medium plays is essential for adequate cell–surface interface characterisation and indeed, it can be effectively controlled for *in vitro* three-dimensional tissue formation, it is not the case for *in vivo* biomaterial utilisation.

Over the past few years, a variety of advanced nanotechnological tools have become available that allow for a high degree of manufacturing control over the surface characteristics of biomaterials. These have the potential to be used to effectively modulate the cell response (Fu et al. 2011; Morent et al. 2011; Solouk et al. 2011; Vasilev et al. 2011; Wu et al. 2011a). It is well established that both the stability of cell attachment and the dynamics of the colonisation process differ with surface architecture, chemical composition, and surface energy of the material, and how these compare to the bacterium-specific factors. The bacterium-specific factors

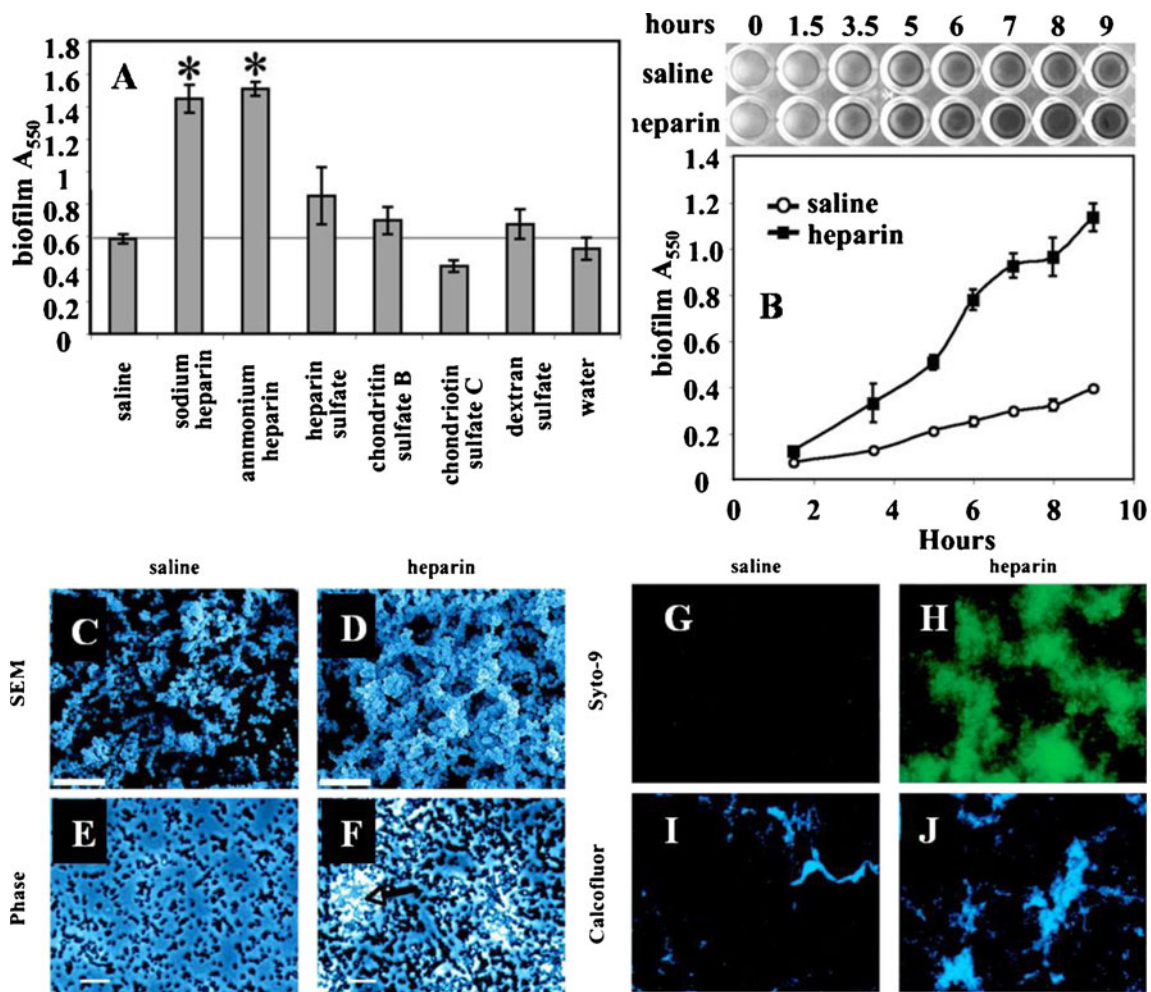


Fig. 2 **a** Formation of a biofilm by *Staphylococcus aureus* on polyvinylchloride in response to various charged molecules and glycosaminoglycans. The asterisk shows the statistical difference ($p < 0.01$) from a saline-only control. **b** Effect of heparin, which is frequently used as an anticoagulant coating for catheters, on the adherence kinetics of *S. aureus*. Sodium heparin (1,000 U/ml, 10 % vol/vol) stimulated biofilm formation of *S. aureus* on polystyrene and polyvinylchloride. Microscopic images of the *S. aureus* biofilms incubated with and

without heparin. Scanning electron micrographs of 12-h biofilm (e and d, $\times 12,500$ magnification, bar=10 μm). Phase-contrast microscopy of 4-h biofilms on polystyrene (e and f, bar=20 μm ; the arrow indicates a phase-bright microcolony), and epifluorescent microscopy (g and h, Syto-9 fluorescent staining, 250-ms exposure, $\times 400$ magnification). Epifluorescent microscopy (i and j, $\times 100$ magnification, calcofluor staining) of 5-h biofilms. (From Shanks et al. 2005, used with permission)

include the hydrophobicity, surface energy, and electrophoretic mobility of the microorganism, and the existence of extracellular appendages, such as pili and flagella (Donlan 2002; Dorobantu et al. 2009). The nature of extracellular polymeric substances (EPS), the level of secretion, and the manner in which these undergo changes in spatial conformation and structural arrangement in response to the nanoscopic features of the abiotic surface also influence cell–surface interactions (Cappella and Dietler 1999). Although obtaining a complete description of an EPS biochemical profile would be a challenge, most EPS is known to be comprised of a wide range of polysaccharides, proteins, glycoproteins, and glycolipids, and in some cases, substantial amounts of extracellular DNA (Flemming et al. 2007; Flemming and Wingender 2010). Recently, the structural and functional diversity of

bacterial extracellular polysaccharides, together with their effect on the colonisation, survival and pathogenicity of bacteria, have been reviewed (Bazaka et al. 2011b). The paper highlighted the difficulties associated with obtaining a detailed physico-chemical analysis of bacterial extracellular carbohydrates, owing to the diversity of sugar monomers, linkages, and unique structures present in the carbohydrate fraction of the EPS, and the complexities associated with the isolation of individual polysaccharides from environmental biofilms. The biochemical composition of the EPS is also dependent on the maturity of the microbial community, as well as the local physico-chemical environment (Shenga et al. 2010). Jiao et al. (2010) showed that the EPS obtained from a mid-developmental stage and a mature acidophilic microbial biofilm were both qualitatively and

quantitatively different, with more than twice as much EPS being present in the mature biofilm. The presence and relative concentrations of carbohydrates, metals, proteins, and minor quantities of DNA and lipids, were also different between the two biofilm stages.

Pseudomonas aeruginosa is a model biofilm-forming bacteria, with many extrapolations commonly being made from observations of *P. aeruginosa* biofilm behavior and properties to biofilms in general (Flemming et al. 2007). However, recent studies have shown that such an extrapolation may be highly misleading. Even within a single species of bacteria, there is a high degree of extracellular polysaccharide diversity. For instance, a polyanion polysaccharide, alginate, is the best-characterized component of *P. aeruginosa* biofilms. The other two biofilm-related polysaccharides produced by the microorganism are Psl and Pel. Early studies of mucoid *P. aeruginosa* biofilms positively correlated the surface-dependent induction of *algC* expression with attachment stability, with cells not exhibiting *algC* up-expression shown to be less capable of remaining at a surface under flow conditions (Davies and Geesey 1995). Subsequent studies demonstrated that non-mucoid *P. aeruginosa* strains, which are thought to be the first to colonize cystic fibrosis patients, were not reliant on alginate for biofilm development (Petrova and Sauer 2012). Similar specificity has been demonstrated for Pel, which is crucial for maintaining cell–cell interactions; it acts as a primary structural scaffold for the PA14 biofilm community (Colvin et al. 2011). Deletion of *pelB* in this strain resulted in a severe biofilm deficiency due to an impaired extent of monolayer formation, with little effect on the ability of PA14 cells for biofilm initiation being observed. On the other hand, in PAO1 strain, Psl was demonstrated to be the primary structural polysaccharide for biofilm maturity, with deletion of *pel* operon having no effect on PAO1 attachment or biofilm development (Colvin et al. 2011). Disruption of *pslA* and *pslB*, however, severely compromised the initiation of biofilm formation by a *P. aeruginosa* PAO1 strain, suggesting that expression of *psl* plays a notable role in cell–surface and intercellular interactions (Ma et al. 2006). Other polysaccharides produced by *Pseudomonas* spp., such as levan and other yet-to-be-identified exopolysaccharides, may have an equally significant role in biofilm formation (Laue et al. 2006). Extracellular DNA has been shown to contribute to biofilm formation by PAO1 *P. aeruginosa* isolates (Whitchurch et al. 2002; Yang et al. 2007), as well as by other bacterial species (Vilain et al. 2009); however, the role of eDNA changed as biofilms evolved. It is not surprising then, that genotypically distinct bacteria will have notably different attachment preferences towards a surface.

By reconfiguring the spatial distribution of chemical functionalities and nanoscale morphological features on the surface of the biomaterials, it is possible to attenuate

the attachment, propagation, and biofilm formation. Potential uses for such surfaces extend beyond preventing bacterial attachment to indwelling biomaterial devices and other clinically relevant surfaces. The effect of surface hydrophobicity as a function of both surface chemistry and topography on the attachment preferences of different bacterial species has been discussed by Bazaka et al. (2011d). This is due to focal adhesion sites of the microorganism being in the range comparable to that of the nanoscale features of the abiotic surface (Brunetti et al. 2010), and due to the fact that surface attachment is a fundamental stage in biofilm development and in the formation of chemical signalling pathways amongst bacterial cells (Hochbaum and Aizenberg 2010; Petrova and Sauer 2011). The environmental signals and signaling pathways that regulate biofilm formation, the components of the biofilm matrix, and the mechanisms and regulation of biofilm dispersal, have been reviewed by Karatan and Watnick (2009). A cell-to-cell communication system, quorum sensing, is believed to regulate motility, adhesion, cell-to-cell aggregation, and biofilm formation, as well as virulence and metabolic activity in several bacterial species (Bjellanda et al. 2012).

The surfaces of several animals and plants have been studied for their behaviour, since they have evolved an ability to prevent surface fouling even in environments that are highly populated by biofilm-forming microorganisms. The skin of whales, sharks, and dolphins remain clean of bacteria, owing to a favourable fusion of surface chemistry and micro- and nanoscopic topographies. Similarly, the hydrophobic chemistry of *Nelumbo nucifera* (lotus) leaf is augmented by a two-layer morphology, resulting in a superhydrophobic surface with low adhesion (Rios et al. 2006, 2007; Dodiuk et al. 2007; Bhushan et al. 2008). Materials that mimic these naturally occurring surfaces are proving equally useful in effectively averting bacterial fouling without the need to use bactericidal agents and by providing a research platform for investigating the cell–surface dynamics. The former is important since it provides the means of responsible management of disease, particularly in the context of rapidly evolving antibiotic resistant pathogenic strains (Baum et al. 2002; Chung et al. 2007; Fadeeva et al. 2011). The latter is essential for intelligent surface design, as it provides researchers with an understanding of how the individual surface characteristics of an abiotic material interact with the chemical and topographical particulars of surfaces of living cells (Malkin and Plomp 2011).

It is important to note that alteration of individual properties, such as only the nanoscopic topography or chemical functionality, is often difficult to achieve (Whitehead et al. 2010). First described by Wenzel (1949), there exists an intimate link between the surface chemistry and topography of a substrate, which becomes increasingly important as we approach nanometer and sub-nanometer scale regimes. The

relationship states that a chemically hydrophilic surface will become increasingly more hydrophilic as the surface roughness is increased. By the same reasoning, the wetting ability of a hydrophobic surface decreases with increasing surface roughness. Hence, the wetting behaviour and surface energy of an abiotic surface are likely to change in response to alterations in the chemistry and/or topography of a surface. As a result, decoupling the contribution of a single variable, such as surface roughness, from a bacterial response that is invoked by a complex of material surface characteristics is challenging. In part, this is due to the paucity of data existing for the fundamental behaviour of cells in a response to physical environment at nanoscopic length scale (Anselme et al. 2010; Bazaka et al. 2011a). Finally, most surface modifications are directed towards optimisation for a given application, frequently modulating both surface chemistry and topography.

Nanotopography and surface nanostructuring

As is the case with other external environmental stimuli, there is little doubt that micro and nanoscale topographies have an effect on the attachment behaviour and metabolic activity of microorganisms (Bos et al. 1999). Yet, the extent to which bacterial attachment and subsequent biofilm formation is affected by the surface nanotopography remains a subject of dispute (Anselme et al. 2010). Similarly, there are vast differences in opinion regarding the length scale at which the influence is most profound (An et al. 1995; Boulangé-Petermann et al. 1997; Medilanski et al. 2002; Whitehead et al. 2005, 2006). In the literature, a number of early studies determined surface topography to be a comparatively insignificant factor in bacterial adhesion, with microorganisms observed to have little predilection for topographical cues (An et al. 1995; Bos et al. 1999; Scheuerman et al. 1998). Subsequent investigations reported a feature–cell size correspondence, where features of the size comparable to the size of the bacterium allowed for maximisation of the bacteria–surface contact area, hence increasing the microorganism’s binding potential (Katsikogianni and Missirlis 2004). The same reckoning was applied to the interpretation of reduced bacterial attachment to surfaces with topographic attributes of smaller dimension than that of the cell (Edwards and Rutenberg 2001). Regularity of the nanoscopic features of the surface was also shown to alter bacterial attachment preferences (Díaz et al. 2007; Rowan et al. 2002; Rozhok et al. 2006; Whitehead et al. 2005). Surfaces with regularly distributed pits of 1 and 2 μm were demonstrated to enhance the extent of attachment of *P. aeruginosa* and *Staphylococcus aureus*, whereas topographies characterised by irregularly scattered 0.2 and 0.5 μm did not instil the same effect (Whitehead et al. 2005). The attachment patterns and

growth of pathogenic *P. fluorescens* and *S. aureus* were influenced by the presence of defined trenches, within which cells preferred to align and grow (Díaz et al. 2007; Harris et al. 2004). On the other hand, a random cell distribution was observed for smooth surfaces (Scheuerman et al. 1998; Díaz et al. 2007). Similarly, an aligned attachment of *Escherichia coli* cells was detected for surfaces with 1.3- μm -wide and 120-nm-deep microgrooves (Díaz et al. 2007) but not for topographies containing grooves of 50 nm in height and period of 1.6 μm (Ploux et al. 2009).

Our own investigations into the role that surface properties play in bacterial cell adhesion, proliferation, and biofilm development have shown that bacterial interactions with materials are influenced by the level of surface roughness and unique topographical peculiarities of that surface. A variety of topographically distinct glass and polymer substrates seeded with cells of taxonomically different bacteria were investigated, with results showing enhanced attachment and increased secretion of EPS for nanosmooth surfaces irrespective of the microorganism species being used in the study (Ivanova et al. 2008; Mitik-Dineva et al. 2008, 2009). In addition to being a major determinant of virulence for numerous pathogenic bacteria, EPS impede antibody opsonisation and phagocytosis, and induce inflammation and aberrant complement activation that can be damaging to host tissues (Bazaka et al. 2011b). Importantly, these promote the colonisation of tissues and surfaces by enabling cell adhesion and co-aggregation via dipole interactions, covalent or ionic bonding, steric interactions, and hydrophobic association. Components of free EPS can be released onto unfriendly surfaces, thus pre-conditioning the target for subsequent colonisation. Indeed, *Staleyia guttiformis* cells were observed to excrete EPS to facilitate attachment to poly(*tert*-butyl methacrylate) polymeric surfaces via the creation of biopolymer network (Ivanova et al. 2008). The size and distribution of topographical peculiarities were also demonstrated to be significant, with optical fibres etched with regular features attracting lesser levels of bacterial attachment compared to unmodified irregular topographies (Mitik-Dineva et al. 2010).

Significant research efforts have been devoted to exploring how metallic surface nanotopographies, such as those of a titanium biomaterial, influence bacterial cell–surface dynamics. Excellent mechanical properties, biocompatibility, and environmental stability make titanium and titanium alloys a biomaterial of choice for a broad range of medical applications, including orthopaedic and dental implants, cardiac valves and in the microsurgical restoration of middle ear function. Yet, similar to other implantable materials, titanium surfaces are susceptible to bacterial colonisation that is highly detrimental to the performance of the implant. Furthermore, a reduction in the average grain size of titanium, imparted in order to improve mechanical strength,

enhance the viability of mice fibroblast cells and accelerate the proliferation of mice preosteoblastic cells (Estrin et al. 2009; Valiev et al. 2008), was also found to be susceptible to a significant increase in levels of attachment of bacteria (Truong et al. 2010a). Despite being chemically similar to the pristine titanium, nanostructuring of the surface enhanced the hydrophilicity of the material. The complex morphology of the nanostructured titanium surface was designed to combine nanoscale smoothness with evenly spaced 100- to 200-nm micro features. Despite some differences, *S. aureus*, *P. aeruginosa*, and *E. coli* cell attachment was found to have increased appreciably on nanostructured titanium compared to that of the nano-rougher untreated sample, accompanied by contemporaneous increase in the production of EPS and changes in cell morphology (Truong et al. 2009). The result is contradictory to the finding of other researchers that showed that preferential cell attachment occurred on surfaces that were more hydrophobic (Gottenbos et al. 2001). These results suggested that surface nanotopography was indeed the major contributor to the observed cell–surface dynamics (Truong et al. 2010b). Subsequent experiments using magnetron sputter thin titanium films with corresponding surface roughness parameters of R_q 1.6, 1.2, and 0.7 nm confirmed an augmented attachment response by *S. aureus* and *P. aeruginosa*, with 2- to 3-fold increases in the number of retained cells and an elevated level of EPS secretion being observed (Ivanova et al. 2010; Truong et al. 2010c). Interestingly, the attachment of *S. epidermidis* to titanium surfaces with an average roughness between 0.43 and 1.25 nm was not influenced by the degree of roughness (An et al. 1995).

Given the importance of titanium as a biomaterial, there is a distinct need to design nanostructured metallic surfaces that effectively limit bacterial attachment and formation of complex biofilm communities, and hence reduce the potential for implant-associated infections and inflammations. Surface structuring, as a means to achieve antifouling surfaces, has been identified as an attractive solution for the long-term prevention of bacterial adhesion. The approach takes its inspiration from a number of naturally occurring superhydrophobic surfaces that possess water-repellent, self-cleaning and anti-icing properties due to a favourable combination of a low intrinsic surface free-energy and a hierarchical structural configuration (Webb et al. 2011). Such complex hierarchical nanotopography can minimize the contact area between an abiotic surface and the physiological fluid containing bacterial cells, extending the maximum water contact angles of 120° that can be achieved on a flat surface. A number of fabrication techniques have been developed to mimic these superhydrophobic surfaces, including vacuum-ultraviolet lithography, e-beam lithography, soft lithography, template lithography, templating from anodised alumina,

replica moulding, and microwave plasma-enhanced chemical vapour deposition micropatterning (Fadeeva et al. 2009). Recently, superhydrophobic ($\theta_w=166^\circ$) titanium surfaces mimicking the surface of the lotus leaf were successfully fabricated using a femtosecond laser-based micro- and nanostructuring (Fadeeva et al. 2011). The resulting surfaces were highly effective against *P. aeruginosa*, greatly reducing the colonization propensity of the microorganism, whereas attachment of *S. aureus* cells was enhanced by the presence of the superimposed nano- and microtopography. The morphological dissimilarity, with *P. aeruginosa* being a larger rod-shaped and *S. aureus* being a smaller spherical cell, led to differences in the surface contact area being achieved between the respective cell and the surface, further influenced by the ability of EPS to adequately anchor the cells to the surface. The vastly distinct attachment preferences of the two pathogens in response to the same surface suggest that there exists the possibility to design topographies that would selectively inhibit the attachment and growth of one cell type while encouraging the other. Structures that induce opposing response from human versus pathogenic cells, such as osteoblasts and *Staphylococcus epidermidis* (Colon et al. 2006) or *E. coli* K12 (Ploux et al. 2009), respectively, are of particular interest (Wu et al. 2011b). Since these have the capacity to favour the competitive colonization by eukaryotic over bacterial cells, such surfaces hold the potential to enhance biocompatibility and bacterial retardation of medical implants (Ploux et al. 2010).

Bactericidal coatings

Bioactive coatings are becoming an increasingly attractive solution for the prevention of biomaterial-associated infections, for they not only have the ability to inhibit bacterial adhesion, but also to eliminate the attached pathogenic cells that managed to overcome the antifouling property of the surface (Fu et al. 2005; Gabriel et al. 2007; Norowski and Bumgardner 2009; Zilberman and Elsner 2008). The latter is achieved by means of releasing antibacterial agents, such as antibiotics vancomycin, amoxicillin, and gentamicin, in a controlled time-resolved fashion, thus inhibiting bacterial growth at the implant site (Price et al. 1996; Stigter et al. 2004). However, with the quickly dwindling efficacy of many systemic antibiotics and antiseptics and the speedy rise of multi-resistant nosocomial bacteria (Madkour and Tew 2008), alternative antimicrobial agents are being investigated. These include silver ions (Kumar and Münsterd 2005; Zaporojtchenko et al. 2006), nitric oxide (Nablo et al. 2001), bioactive antibodies (Rojas et al. 2000), antimicrobial peptides (Shukla et al. 2010), and some naturally

occurring broad-spectrum biocidal compounds, such as essential oils (Bazaka et al. 2011c,d; Low et al. 2011).

The strong bactericidal effect of silver ions against *S. aureus*, *S. epidermidis*, *P. aeruginosa*, *E. coli* and *Klebsiella pneumonia* among others is well documented (Birla et al. 2009; Rai et al. 2009). The antimicrobial activity of Ag-based materials is typically associated with their Ag⁺ content, where Ag⁺ is believed to interact with the cytoplasmic components and nucleic acids, to inhibit respiratory chain enzymes (Holt and Bard 2005), and to interfere with membrane permeability (Lok et al. 2007). In *Vibrio cholera*, low concentrations of Ag⁺ were shown to induce a massive proton leakage through the membrane, leading to a collapse of proton motive force (Dibrov et al. 2002). Tile inactivation of microorganism enzymes takes place via silver complexes with electron donors containing sulfur, phosphorous, oxygen or nitrogen, such as thiols, carboxylates, phosphates, hydroxyl, amines, imidazoles, indoles (Ahearn et al. 1995). Encouraged by the minimal detrimental effects to eukaryotic cells (Silver 2003; Agarwal et al. 2010), silver-containing coatings, nanoparticles, silver-exchanged zeolites, and hybrid silver composites with dendrimers and polymers are being intensely investigated (Marambio-Jones and Hoek 2010; Shao and Zhao 2010b; Li et al. 2011).

Electroless plating, sputtering, ion beam-assisted deposition, and chemical deposition are among the techniques employed to introduce silver onto the surface of the biomaterial (Sardella et al. 2006). Sol–gel processing was used to fabricate silver-doped organic–inorganic hybrid coatings of tetraethoxysilane- and triethoxysilane-terminated poly(ethylene glycol)-*block*-polyethylene, which at a weight ratio of 80:20 and a 5 wt.% silver salt were found to have notable activity against *E. coli* and *S. aureus* (Marini et al. 2007). Similar antibacterial activity against *S. epidermidis* and *S. aureus* was observed for co-sputtered silver-containing hydroxyapatite (Chen et al. 2006) and silver-coated polymers, where deposition was achieved by combining magnetron sputtering with a neutral atom beam plasma source (Dowling et al. 2001). Physical vapour co-deposition of titanium/silver hard combined coatings provided significant antimicrobial potency against *S. epidermis* and *K. pneumonia* strains and cytocompatibility with osteoblast and epithelial cells (Ewald et al. 2006). With mechanical performance similar to that of pure titanium, the application of TiAg coatings can impart antimicrobial activity on load-bearing endoprosthetic surfaces. Sol–gels containing titanium dioxide in combinations with silver were found to attenuate *P. aeruginosa* growth; however, the effect on *S. aureus* colonization was limited (Tarquinio et al. 2010).

The photocatalytic activity of titanium dioxide can be utilised to impart antiviral, antibacterial, and fungicidal properties, and effectively inhibit biofilm formation on the surface of TiO₂ implants. Titanium dioxide is known to

activate upon exposure to ultraviolet light, where irradiation of the surface promotes electrons from the valence band to the conduction band leaving a positively charged hole. As electrons and holes migrate, the potent oxidative species, such as OH and O₂^{•-}, are produced (Ditta et al. 2008). In solution, these can further react to produce H₂O₂. These photocatalytic species are believed to attack the outer membrane of bacteria. It is generally believed that the surface bound hydroxyl radicals (•OH) play the main role in killing microorganisms, however its exact mode of action, whether they remains bound or diffuse into the bulk of the solution, the significance of other reactive oxygen species, such as H₂O₂ and O₂^{•-}, and the specific microorganism involved remains a subject of active debate (Cho et al. 2005). For instance, MS-2 phage were shown to be inactivated mainly by the free hydroxyl radical in the solution bulk, whereas *E. coli* cells were damaged by both the free and the surface-bound hydroxyl radicals, and other reactive species, namely O₂^{•-} and H₂O₂ (Ditta et al. 2008). As the bactericidal effect of illuminated TiO₂ has been positively correlated to the rate of cell adsorption onto the titanium surface, the properties of the liquid medium will also affect the killing efficacy of the surface (Gogniat et al. 2006). Addition of silver or copper can further enhance the antibacterial effectiveness of the photocatalytic coating, even under weak UV irradiation and lower exposure time. Compromised by the oxidative species, the cytoplasmic membrane is susceptible to the effectual permeation by the Ag or Cu ions (Hashimoto et al. 2005). Once within the bacterial cell, the Cu ions are thought to bind to specific sites in the DNA, particularly guanosine residues, causing single-strand breakage and base modification (Yates et al. 2008). The redox couples of Cu⁰/Cu²⁺ and Ag⁰/Ag⁺ can cause the transfer of electron leading to the O₂^{•-} generation even under dark conditions, enhancing the bactericidal activities via the synergy of the oxidation role of the O₂^{•-} and the bacteriostatic action of antibacterial ions (Hu et al. 2007).

Thin TiN/Ag composite films prepared using pulsed magnetron sputtering demonstrated an increase in *P. aeruginosa* and *S. aureus* inhibition, corresponding to an increase in silver content (Kelly et al. 2009). Heterogeneously distributed throughout the TiN matrix, the presence of the silver resulted in changes in the surface nanotopographies — surface features, grain sizes, and physicochemistry — with most profound changes observed at a silver concentration of 16.7 at.% (Whitehead et al. 2010). Recently, Ivanova et al. (2011) used magnetron sputtered nanoscopically thin silver films to show that while silver ion concentration may be the major determinant of bacterial viability, the extent of bacterial attachment and the patterns are largely affected by surface topography of the films (Ivanova et al. 2011). A strong correlation was also detected between total surface energy γ_{TOT} of the silver coating and *P. aeruginosa* adhesion, with the number of adhered

bacteria increasing linearly with γ_{TOT} but decreasing linearly with an increasing electron donor component γ^- (Shao and Zhao 2010a).

Plasma assisted micro- and nanofabrication methods are widely used to deposit a wide range of coatings, to pattern, and to selectively modify the properties of biomaterials (Hynek 2011). For deposition of nanocomposite coatings, plasma enhanced chemical vapour deposition is frequently employed to fabricate the organic matrix, in which particles are then introduced (Sardella et al. 2006). A combination of radiofrequency (RF) glow discharge and simultaneous sputtering from the silver RF electrode was used to deposit nanocomposite $Ag/C_xH_yO_z$ thin films characterized by sounds antibacterial properties (Favia et al. 2000). Similar plasma set up was used for the in situ formation of Ag nanoparticles within a functional hydrocarbon matrix, with the ability to control the concentrating of incorporated silver component (Körner et al. 2010, 2011). Silver nanoparticles bound to a plasma-deposited thin polymeric binding layer of allylamine were effective in preventing attachment of *S. epidermidis* and biofilm formation while supporting attachment and spreading of osteoblastic cells (Vasilev et al. 2009, 2010). Phosphine-stabilised silver maleimide complex deposited using plasma polymerization onto glass and non-woven polypropylene fabrics demonstrated cytocompatibility with Swiss mouse fibroblasts and human neonatal keratinocytes, while effectively limiting colonization by *P. aeruginosa* (Poulter et al. 2009).

Recently, RF plasma polymerization was used to fabricate thin film coatings from non-synthetic terpinen-4-ol, a major constituent of *Melaleuca alternifolia* essential oil attributed with the oil's broad spectrum antibacterial, antiviral, antifungal, and anti-inflammatory activity (Bazaka et al. 2010). The antifouling and bactericidal properties of the coatings were found to be strongly dependent on the fabrication conditions. Coatings deposited at lower input power that favoured condensation and only limited monomer fragmentation retained most of the activity of the monomer, effectively preventing adhesion and biofilm formation of *S. aureus*, *P. aeruginosa*, and *S. epidermidis*. On the other hand, films fabricated at conditions that resulted in high degree of monomer fragmentation, promoted adhesion of the aforementioned pathogens. In addition to differences in surface chemistry and hydrophobicity, surface nanoarchitecture was found to be a contributing factor to bacterial attachment and biofilm formation, with non-active films being significantly smoother compared to the unmodified substrate and bactericidal counterparts (Bazaka et al. 2011c).

Concluding remarks and future prospects

This review demonstrates that there are surface engineering methods that hold great potential in the fabrication of substrates

that can control microbial attachment and biofilm formation, particularly with regard to alleviating biomaterial associated infections. The presented physico-chemical modification methods have the advantages of high efficiency and minimal influence on the bulk properties of the biomaterials. Compared to the conventional antimicrobial-based methods for preventing and treating microbial colonisation, the approaches discussed in this review would be associated with low toxicity and minimal development of bacterial resistance. The latter would be afforded through modification of the bacterial cell–surface interactions rather than by simply killing the pathogen, hence lessening the selective pressure for antibiotic resistance. For the same reasons, such tailored surface modifications are an attractive means of mitigating the attachment and proliferation of bacterial strains that have already developed resistance to one or more currently available antibiotics. This area is of particular importance within the clinical setting, where nosocomial infections severely impede patient recovery and are recognised as the foremost cause of death in intensive care units (Livermore 2005). Lastly, these surfaces will provide scientists with a platform for in-depth investigation of the cell–surface interactions necessary for the effective management of microorganism colonisation in a variety of natural and engineered environments. With the ongoing advancement of manufacturing and characterisation technologies and significant research efforts dedicated to the area, nano-engineered surfaces with high degree of control at molecular and atomic length scales would evolve as a feasible alternative for ecological management of biofouling.

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