Review

Emerging rules for effective antimicrobial coatings

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In order to colonize abiotic surfaces, bacteria and fungi undergo a profound change in their biology to form biofilms: communities of microbes embedded into a matrix of secreted macromolecules. Despite strict hygiene standards, biofilm-related infections associated with implantable devices remain a common complication in the clinic. Here, the application of highly dosed antibiotics is problematic in that the biofilm (i) provides a protective environment for microbes to evade antibiotics and/or (ii) can provide selective pressure for the evolution of antibiotic-resistant microbes. However, recent research suggests that effective prevention of biofilm formation may be achieved by multifunctional surface coatings that provide both non-adhesive and antimicrobial properties imparted by antimicrobial peptides. Such coatings are the subject of this review.

Biofilms and implant infections

An aging population and advances in materials technology have brought about, over the past 50 years, an increase in the usage of biomaterials and medical devices such as catheters, cardiac pacemakers, hip implants, and contact lenses, which can restore function to diseased or damaged human tissue. However, the application of such devices involves some challenges – in particular, implant-associated infections resulting from the presence of biofilms. Biofilm formation typically results from peri-operative procedures (where organisms enter the wound or adhere to the implant during surgery) and post-operative procedures (where organisms infect the patient during hospitalization) [1,2]. Infectious diseases are responsible for tens of millions of deaths representing approximately 20% of all fatalities world-wide [3], and it is estimated that 80% of human infections are associated with biofilm formation. Furthermore, many of the causative organisms exhibit growing antimicrobial resistance [4].

A range of organisms has been specifically implicated in device/biomaterial-related infections, including many species of bacteria and major fungal pathogens affecting human health (Table 1) [1,2,5–18]. Device-related infection may result in substantial clinical complications, including death, as well as economic consequences such as increased healthcare costs generated by prolonged hospital stays or revision surgery. In the United States, recent estimates of direct costs for healthcare-associated infections were estimated to range from US$28 billion to $45 billion in 1 year with upward of 60% of these being related to medical devices [19].

Several medical interventions are currently used to treat device-related infections, including long-term antimicrobial strategies and combinations of antibiotics and surgical revision. Unfortunately, these interventions carry the risk of re-infection, often at a higher rate, and the development of antibiotic resistance. The application of non-adhesive and antimicrobial coatings has been researched and tested clinically as an alternative approach but has yet to find widespread application. Here, we review coating strategies combining low-fouling polymer coatings with antimicrobial peptides and the continued development required for the prevention of microbial biofilms on medical devices.

Low-fouling coatings

Effective control of biointerfacial interactions is the key to developing improved biomedical materials and devices, including infection-resistant medical implants. Many of these applications require surfaces that prevent non-specific interactions with the biological environment, in particular the adsorption of proteins and other biomolecules. Such 'low-fouling' coatings also reduce the ability of planktonic microbes to adhere, thereby interfering with the earliest stages of biofilm formation.

Various chemical approaches that are suitable for establishing such coatings have been described. These include self-assembled monolayers (SAMs), various polymer-based approaches [20–22], and, very recently, liquid-infused nanostructured surfaces that present a dynamic surface structure [23]. Although SAMs are easily applied, their

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Keywords: anti-infective coatings; antimicrobial peptides; biofilms; implant infections; low-fouling polymers.

0167-7799/$ – see front matter.
 http://dx.doi.org/10.1016/j.tibtech.2013.09.008

Box 1. Biofilm formation

The stages of biofilm formation (Figure I) by *Staphylococcus epidermidis*, a well-known biofilm-producer, are used here as a model.

Stage 1: attachment and monolayer formation. Free-floating cells attach within seconds after encountering an abiotic surface [69]. Non-specific interactions between bacteria and the surface are mediated by physiochemical forces, such as van der Waals forces, hydrophobic interactions, and polar and ionic interactions [70,71]. In addition, specific interactions can be mediated by a preformed 'conditioning film' of biomolecules derived from plasma components adsorbed to the device surface [72,73]. This conditioning film is thought to provide specific binding sites for bacterial surface proteins (adhesins), and the interactions often result in tight attachment of bacteria [74,75]. By the end of this stage, a confluent layer of *S. epidermidis* cells, referred to as an 'adherent monolayer', covers the device surface.

Stage 2: formation of microcolonies. Within the monolayer, bacteria multiply locally and then assemble to form a mound-shaped cellular aggregate — a 'microcolony'. In the case of *S. epidermidis*, microcolony formation depends on secreted and surface-adsorbed bio-macromolecules, including polysaccharide intercellular adhesin (PIA), surface proteins, teichoic acid, and extracellular DNA [76].

Stage 3: maturation and structuring. After the formation of microcolonies, *S. epidermidis* cells undergo further adaptation and development into a mature biofilm consisting of bacterial macrocolonies, which eventually converge, being encased by an extracellular polymeric substance (EPS) that is highly penetrated by channels [77]. PIA is still the key extracellular component of macrocolonies. Its expression during the maturation stage is regulated by quorum-sensing systems and other global gene regulators such as SarA, RsbU, and SigB [78–82]. Bacterial surfactant peptides and shear force (e.g., by flowing body fluids) also play a vital part in the biofilm shaping and maturation. All of these factors determine the density of the biofilm matrix, the overall cell density and the strength of surface attachment [83–85].

Stage 4: detachment and return to the planktonic growth model. During this last stage, bacteria return to the planktonic mode, causing a risk of spreading the infection. Low-level sloughing as well as active dispersion of bacteria generated from the biofilm occur synchronously [86]. Once a biofilm structure is formed, cells on the surface exit from the biofilm and re-enter the planktonic state in response to certain environmental cues and self-stress signals [77,87].

During attachment and the initial formation of colonies, microbes are relatively drug-sensitive and susceptible to immune cell response. Bacteria in biofilms are reportedly 100 to 1000 times more resistant than their planktonic counterparts. Drug resistance is provided through adaptive changes, genetic changes, production of a subpopulation of dormant cells, and physical protection from antibiotics by the matrix.

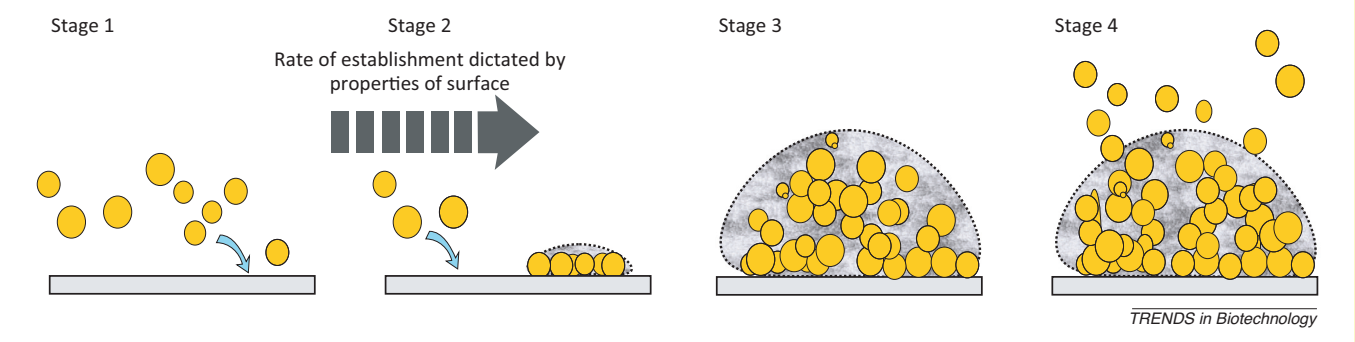

Figure I. The four stages of biofilm formation.

versatility with respect to substrates and stability is limited. By contrast, polymer coatings can be applied to almost any substrate material and provide a much broader range of architectures and properties. Among polymer based coating approaches, two techniques stand out in their ability to yield low-fouling surfaces: the 'grafting-to' technique (Figure 1A), in which polymers carrying suitable functional groups are synthesized in solution and then tethered to surfaces by reacting with complementary functional groups on the surface, and the 'grafting-from' technique (Figure 1B), in which polymer chains are grown from surface-immobilized initiators or chain transfer agents. Multiple factors, including the density and molecular weight of graft polymer chains, have been shown to determine the effectiveness of the coating [24]. An important feature of these graft polymers is that functional groups or biologically active signals can be introduced along the graft polymer chain or at its terminal end to further modulate the biological response [25].

Particularly effective low-fouling polymers include polyacrylamide (PAM) [26], polysaccharides such as dextrane [27], zwitterionic polymers such as poly(*N*-sulfobetaine methacrylamide) (PSBMA) [28,29], and poly(*N*-hydroxypropyl methacrylamide) (PHPMA) [30] (Figure 1C). However, polyethylene glycol (PEG)-based polymers and their low-fouling properties have received most of the attention to date. PEG polymers have been described with linear [31] and star-shaped architectures [32] as well as 'bottle brush'-type polymers with pendant PEG chains such as those based on poly(ethylene glycol) methacrylate (PEGMA) [33].

Impeding biomolecule adsorption disrupts a broad range of processes that require the interaction of proteins or other biomolecules with substrate materials, including cell attachment, platelet adhesion, and blood clot formation, as well as the foreign-body reaction and microbial colonization/biofilm formation [34,35]. However, even ultra-low fouling surfaces might eventually form substrates for the formation of biofilms – for example, due to degradation or inhomogeneity that might also be the result of damage during handling. Therefore, robust antimicrobial coatings would require more than one mechanism of defense.

A wide range of molecules that inhibit or disperse biofilms has been identified [36,37]. Among those that can be embedded or immobilized on surfaces, or combined with polymer coatings, silver [38], ammonium, and guanidinium salts, as well as peptides and proteins [39], have attracted much attention. In this context, cationic antimicrobial peptides (AMPs) have shown particularly promising results.