Progr Colloid Polym Sci (2006) 132: 138–144
DOI 10.1007/2882_026

Published online: 9 March 2006

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The Use of Positively Charged or Low Surface Free Energy Coatings versus Polymer Brushes in Controlling Biofilm Formation

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Abstract Biofilm formation on biomaterials implant surfaces and subsequent infectious complications are a frequent reason for failure of many biomedical devices, such as total hip arthroplasties, vascular catheters and urinary catheters. The development of a biofilm is initiated by the formation of a conditioning film of adsorbed macromolecules, such as proteins, followed by adhesion of microorganisms, where after they grow and anchor through secretion of extracellular polymeric substances. Adhesion of microorganisms is influenced by the physico-chemical properties of the biomaterial surface. Positively charged materials stimulate bacterial adhesion, but prevent growth of adhering bacteria. The use of low surface free energy materials did not always reduce in vitro adhesion of bacteria, but has been found beneficial in in vivo applications where fluctuating shear forces prevail, like on intra-oral devices and urine catheters. Polymer brushes have shown a very high reduction in in vitro adhesion of a great variety of microorganisms. However, for clinical application, the long term stability of polymer brushes is still a limiting factor. Further effort is therefore required to enhance the stability of polymer brushes on biomaterial implant surfaces to facilitate clinical use of these promising coatings.

Keywords Biofilm · Biomaterial centered infection · Microbial adhesion · Polymer brushes · Surface free energy

Introduction

Biomaterials are materials foreign to the human body that are used in medicine to replace, support or restore body function. Applications range from central venous and urinary catheters to more complex devices such as prosthetic joints and heart valves. The risk of biomaterial centered infection (BCI) is a key factor limiting their use [1]. The incidence of this type of infections varies for each application for instance 4% for hip prostheses [2] and 10–20% for urinary catheters (see Table 1). In BCI microorganisms are present in close association with the biomaterial surface forming a so-called biofilm. Different species of microorganisms are found in BCI that are often commensals of the skin or the intestines, for instance *Staphylococcus epidermidis*, often found in hip prosthesis infections and *Escherichia coli* in urinary catheter infections [2] (see Table 1). BCI can cause severe problems, from disfunctioning of the implanted device to lethal sepsis of the patient. Furthermore, treatment of BCI is complicated, as microorganisms in a biofilm are more resistant to antibiotics [3] than their planktonic counterparts [4]. As a consequence, the only remedy for a BCI is removal of the infected implant at the expense of considerable costs and patients suffering. A more convenient way to deal with this problem is to prevent the development of an infectious biofilm on the biomaterial surface. To achieve this, a thorough understanding of the development of biofilms is necessary.

Table 1 Incidences of infection of different biomedical implants and devices adapted from Dankert et al. [2]

Body site	Implant or device	Incidence (%)	Commonly causative bacterial species
Urinary tract	UT catheters	10–20	*Escherichia coli*
Percutaneous	CV catheters	4–12	*Staphylococcus epidermidis*
			Staphylococcus aureus
	Temporary pacemaker	4	*Staphylococcus epidermidis*
			Staphylococcus aureus
	Peritoneal dialysis catheters	3–5	*Staphylococcus epidermidis*
	Orthopedic pins*	50	*Staphylococcus aureus*
Subcutaneous	Cardiac pacemaker	1	*Staphylococcus epidermidis*
Soft tissue	Mammary prosthesis	1–7	*Staphylococcus aureus*
	Intraocular lenses	0.13	*Pseudomonas*
			Staphylococcus epidermidis
Circulatory system	Prosthetic heart valve	1.88	*Staphylococcus aureus*
			viridans streptococci
	Vascular graft	1.5	*Staphylococcus epidermidis*
			Staphylococcus aureus
			Gram negative bacteria
Bones	Prosthetic Hip	2.6–4.0	*Staphylococcus epidermidis*
			Staphylococcus aureus
	Total knee	3.5–4	*Staphylococcus epidermidis*
			Staphylococcus aureus

* Data obtained from [5] and [6]

Biofilm Formation

Although the function and appearance of biofilms in various environments may be different, all biofilms are formed according to the following basic sequence of events [7] (see Fig. 1).

1. Formation of a conditioning film of adsorbed macromolecular organic components (i.e. proteins and other organic molecules) on the substratum surface prior to microbial deposition.
2. Transport of microorganisms towards the substratum surface through diffusion, convection, sedimentation, or by intrinsic bacterial motility.
3. Initial microbial adhesion.
4. Strong attachment or anchoring of microorganisms to the substratum surface through the production of extracellular polymeric substances (EPS), mostly composed of polysaccarides [8] and proteins [9].
5. Surface growth of adhering microorganisms and continued secretion of EPS.
6. Localized detachment of isolated clumps of microorganisms caused by occasionally high fluid stress or other detachment forces operative in the environment of the biofilm.

Microbial adhesion is mediated by generic physico-chemical interactions forces as well as by specific interaction forces between cell surface structures and molecular groups on a substratum surface [10]. Generic, non-specific interaction forces include Lifshitz–Van der Waals forces and electrostatic forces, which both operate over a long range, and hydrophobic and acid-base interactions that act over a shorter range [11]. Specific interactions result in fact from non-specific forces acting on highly localized regions of the interacting surfaces over distances smaller than 1.5 nm [12].

Upon approach, organisms will be attracted or repelled by the biomaterial surface, depending on the resultant of the various interaction forces. Thus, the physico-chemical surface properties of the biomaterial, with or without conditioning film, and those of the microorganisms play a decisive role in this process. Because the size of microorganisms is in the μm range, adhesion can be described in terms of colloid science. Indeed, for several strains and species physico-chemical models like the Derjaguin-

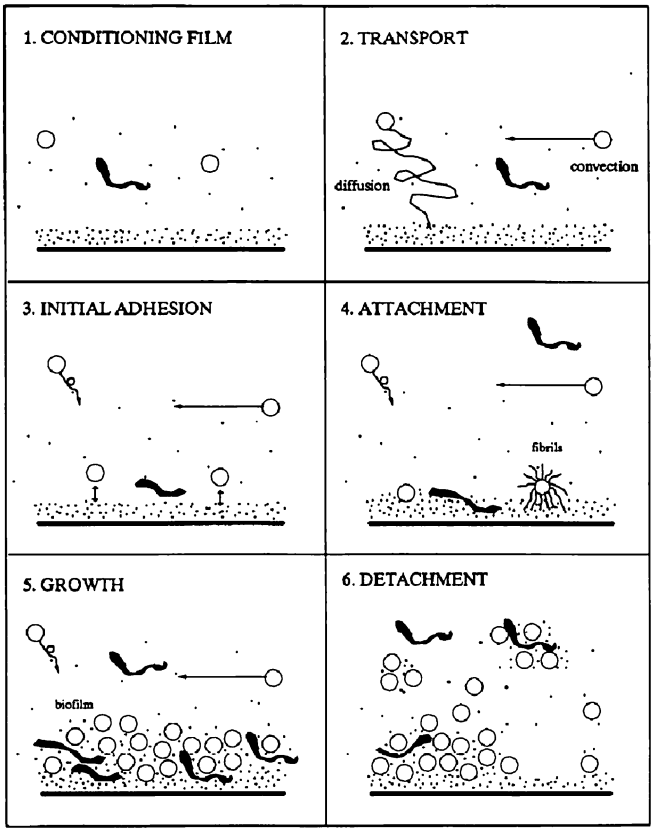

Fig. 1 Schematic, sequential presentation of the steps involved in biofilm formation. Reprinted from Meth. Enzymol. 310, Models for studying initial adhesion and surface growth in biofilm formation on surface, 523–533, Copyright 1999, with permission from Elsevier

Landau-Verwey-Overbeek (DLVO) theory of colloidal stability have been successful in qualitatively explaining microbial adhesion to solid substrata [8, 13].

Prevention of Biofilm Formation

Surgeons take considerable effort in preventing the contamination of implants with microorganisms during implantation. Although application of prophylactic antibiotics and better operation hygiene have reduced the incidence of BCI over the last four decades, still a significant number of patients suffer from such infections [2, 14].

Different strategies may prevent biofilm formation and thus BCI. In general, it is aimed to reduce the attractive force between microorganisms and a biomaterial surface by optimizing the physico-chemical surface properties of the biomaterial. For instance more negatively charged biomaterials [15], biomaterials coated with albumin [16], heparin [17, 18] or polysaccharide [19, 20] have shown to attract less bacteria. However, the most promising and most extensively studied methods to prevent biofilm formation are positively charged coatings, low surface free energy coatings and surfaces covered with a polymer brush. These three types of surfaces will be evaluated and compared in this review.

Positively Charged Coatings

Despite the fact that bacteria adhere more readily to positively charged surfaces than to negatively charged ones [21, 22], there are some aspects of biofilm formation on positively charged surfaces that deserve their further consideration. *E. coli* and *P. aeruginosa* hardly showed any growth after their adhesion to positively charged poly(methacrylate) surfaces [21], although *S. aureus* and *S. epidermidis* were able to grow on these surfaces. However, on negatively charged poly(methacrylate) surfaces, growth was found for all four species.

These observations may be explained by the strong binding of the negatively charged bacteria [23] to positively charged surfaces through attractive electrostatic interactions, impeding elongation and division needed for bacterial growth. It has indeed been demonstrated that as the binding strength of *P. aeruginosa* AK1 to substrata increases, their surface growth reduces [24].

Also in vivo, positively charged surfaces have appeared promising in certain applications. Differently charged poly(methacrylate) coated discs have been seeded with *E. coli* or *P. aeruginosa* and implanted in rats [25]. After 48 h, only 50% of the positively charged disks contained viable *E. coli*, while on all negatively charged disks viable bacteria were found. *P. aeruginosa*, however, was isolated from both positively and negatively charged surfaces, probably because this bacterium can circumvent the effect of the positive charge through production of extracellular polymeric substances.

Low Surface Free Energy Coatings

The surface free energy (s.f.e.) of a material is a measure for the work required to enlarge its surface (mJ m^{-2}). At constant pressure, constant temperature and if the surface composition remains constant, the s.f.e. equals the surface tension (γ_{SV}) of the material against its vapor [26]. To determine the value of γ_{SV} and hence of the s.f.e. of a solid, contact angles with several liquids with known s.f.e. (γ_{LV}) are required. Thomas Young's equation relates the surface free energies and the contact angle (θ) based on the force balance at the three-phase boundary [27]:

$$\gamma_{SV} = \gamma_{SL} + \gamma_{LV} \cos\theta \quad (1)$$

Equation 1 can only be solved using different methods for which additional assumptions are required. These methods are subject to serious controversy in surface science [26]

and lead to different γ_{SV} values for one and the same surface.

Therefore, literature values of γ_{SV} are mainly used as a relative measure for the s.f.e.

The type of forces determining the s.f.e. are Lifshitz–Van der Waals interactions and non-dispersive interactions like hydrogen bonding, stacking between π-electrons and ion pairing [26]. The value of γ_{SV} is determined mainly by the chemical nature and structure of the surface. Values for some constituent groups decrease in the order CH_2 ($36\,mJ\,m^{-2}$) > CH_3 ($30\,mJ\,m^{-2}$) > CF_2 ($23\,mJ\,m^{-2}$) > CF_3 ($15\,mJ\,m^{-2}$) [29]. The low s.f.e. of materials containing the CF_2 of CF_3 group may be explained by the highly electron-negative F atom that is able to withdraw electrons from the carbon backbone leading to a very inert, noble gas like configuration [30]. In water minimal interaction possibilities between this inert surface and a bacterium are expected, which should lead to minimal bacterial adhesion and easy removal of attached bacteria. From a thermodynamic viewpoint, it has also been predicted that bacteria with a s.f.e. higher than water, which constitute the majority of the bacterial population, in for instance dental plaque [31], have the lowest interaction energy with low s.f.e. surfaces [32].

Indeed fouling resistant gorgonian corals were found to have low s.f.e. ($23–27\,mJ\,m^{-2}$) [33]. Also, the first bacterial adhesion tests on materials with different s.f.e. showed lowest bacterial adhesion on materials with the lowest surface free energies [34]. In in vitro experiments, Everaert et al. [35] showed that fluoro-alkylsiloxane layers chemisorbed to silicone rubber surfaces reduced both yeast (*Candida albicans* and *Candida tropicalis*) and bacterial (*Streptococcus salivarius*, *S. epidermidis*) adhesion by 50 to 77% as compared to original silicone rubber. Furthermore, microorganisms were more easily detached when passing an air-liquid interface. Tsibouklis et al. [36] developed films of poly(perfluoroacrylate) and poly(methylpropenoxyfluoroalkylsiloxane) with surface free energies as low as 12.2 and $5.6\,mJ\,m^{-2}$ respectively. Retention of *Pseudomonas* and *S. aureus* after a washing step showed more than 95% reduction after 6 weeks of incubation as compared to glass controls. Adhesion in a stationary state of one of the initial colonizers of human teeth, *Streptococcus sanguis*, was determined on low s.f.e. fluoroethylenepropylene (FEP) and high s.f.e. glass [37]. On FEP adhesion was 0 to 94% lower than on glass depending on the buffer concentration and shear rate. Furthermore bacteria were more easily detached from the FEP surface through shear forces.

On the other hand, for low s.f.e. bacteria, a preference for low s.f.e. surfaces is predicted by the thermodynamic approach of Absolom et al. [32]. This effect may be explained mechanistically by hydrophobic interaction between the low s.f.e. surface and the low s.f.e. bacterium. Adhesion is driven by release of water from both the surface and the bacterium thus leading to an increase in entropy [38].

Indeed a bacterial preference for low s.f.e. surfaces has been shown by several authors [31, 32, 39]. However, in an in vitro study, low density poly(ethylene) films were treated with tetrafluoromethane plasma, which resulted in a dramatic reduction in s.f.e., but which did not show a significant reduction in bacterial adhesion [40].

In vivo studies mostly showed a beneficial effect of a low s.f.e. material. For instance in a study by Quirynen et al. [41], four surfaces with surface free energies ranging from 20 to $88\,mJ\,m^{-2}$ were attached to the teeth of healthy humans. The low s.f.e. surfaces attracted significantly less microorganisms after 9 days and those attached were less tightly associated with the surface as compared to bacteria adhering on high s.f.e. surfaces. Treatment of enamel surfaces with silicone oil, which lowered its s.f.e., resulted in a significant reduction in plaque formation [42]. Another study indicated, that after three months the exposed areas of low s.f.e. FEP coated abutments (transmucosal dental implants) displayed lower bacterial colonization and a lower plaque maturation as compared to bare titanium [43]. However, in the areas covered by the gingiva, where less mechanical shear forces are present, this effect was not apparent. Finally, modification of a silicone rubber voice prosthesis with perfluoroalkylsiloxane of 8 fluorocarbon units resulted in reduced biofilm formation in vivo [44].

In the in vivo examples mentioned above, low adhesion numbers were obtained when low s.f.e. surfaces were applied in combination with high shear forces, induced by high flow or passage of an air-liquid interface. Thus, in medical applications where high shear forces are operative, like oral devices, urine catheters and voice prostheses, low s.f.e. surfaces are promising. Fluoropolymers are especially suitable for use as coatings on biomaterials as they are known for their chemical and thermal stability [30]. However, applications that are completely internal like artificial hips, artificial veins and intraocular lenses are still prone to biofilm buildup and thus need a more general antiadhesive coating, like e.g. a polymer brush.

Polymer Brushes

Polymer brushes are polymer chains that are attached to a surface and stretch out into the surrounding medium. Brushes that are designed to prevent biofilm buildup are usually made from poly(ethylene oxide) (PEO), which are highly water soluble and non-toxic [45]. As the PEO chains are highly mobile [46] and attain extremely large exclusion volumes [47], they make the surface difficult to approach by incoming proteins or bacteria. Penetration or compression would lead to an increase in the local concentration of PEO, which, in turn, would lead to a repulsive osmotic interaction. Therewith, a PEO-brush forms a steric barrier preventing close approach, thus keeping the protein or bacterium at a distance where

the attractive Lifshitz–Van der Waals interaction is relatively low. The weak residual attraction generally leads to low adsorption and adhesion, if occurring at all, and to easy removal of biological matter from a brushed surface.

Protein adsorption on PEO brushes has been extensively studied and has recently been reviewed [48]. In general, PEO brushes greatly reduce and sometimes even completely prevent protein adsorption. Easy removal of proteins from PEO coatings has also been described [49, 50].

One of the first to study bacterial adhesion to PEO grafted surfaces were Bridgett et al. [51], who used copolymers of PEO and polypropylene oxide (PPO) of different lengths, where the PPO block anchored the PEO chains to the surface. All copolymers induced significant adhesion reductions (up to 97%) of three clinical isolates of *S. epidermidis*. Also another strain of *S. epidermidis* and the skin borne bacteria *Serratia marcescens* were reduced by about 90% applying a coating of this copolymer with 99 ethylene oxide (EO) units [52]. Coating with a copolymer of poly(*L*-lysine) and PEO with 47 EO units reduced adhesion of *Staphylococcus aureus* by 89 to 93% on titanium surfaces [53]. Self-assembled monolayers of only 6 EO units even showed more than 99.5% reduction of both a medical *S. epidermidis* strain and a marine *Delaya marina* strain [54]. Covalent attachment of PEO with 66 EO units to polyurethane resulted in 90 and 95% reduction of adhesion of *S. epidermidis* and *E. coli*, respectively [55]. Adhesion of *Pseudomonas* sp. was reduced by more than 99% by covalently attaching PEO chains with 110 PEO units to PET surfaces [56]. Reductions obtained in above mentioned studies are much higher than obtained with low surface energy coatings. In nearly all papers cited, the adhesion methodology employed some kind of washing step, which may cause detachment and contribute to the low adhesion numbers [57]. However, in a study by Razatos et al. [58], adhesion of *E. coli* was determined in situ without washing steps and still a reduction of more than 99% was found.

In our studies on well characterized covalently attached PEO brushes, adhesion of *S. epidermidis*, *S. aureus*, *S. salivaris* and *E. coli* [59] was reduced by more than 94%. Reductions of *Pseudomonas aeruginosa* and the yeast strains *Candida albicans* and *Candida tropicalis* amounted about 80%, thus indicating that different microbial species can adhere in different numbers to the same PEO brush. More hydrophobic microorganisms showed more adhesion and stronger adhesion of the yeast strains could be attributed to stronger Lifshitz–Van der Waals forces, due to their larger size. We have also shown that a PEO brush is effective at both 20 °C and 37 °C and that those microorganisms that do adhere can very easily be removed [60]. As for protein adsorption [61–64], the longest PEO chains were found to be most effective in preventing adhesion of bacteria and yeast [60]. This confirms the theory of attenuation of particle-substratum interaction forces by a brush [65–68].

Despite these excellent in vitro results, little progress has been made in vivo. For instance, under clinical conditions, blood proteins have been demonstrated to adsorb extensively to a PEO coated polymer, in contrast to in vitro results [69]. Also, in vivo research on PEO coatings in the oral cavity showed poor results [70], despite excellent in vitro reductions in salivary protein adsorption and oral bacterial adhesion to PEO coatings on glass and hydroxyapatite [70, 71]. Possibly, the durability of the thin layer of grafted PEO chains in the oral cavity was not sufficient over a clinically relevant time scale.

Recently we have developed a method to easily assess the stability of a PEO coating, by using a marker bacterium to directly determine the effectivity of a PEO coating [72]. We showed that our coating was only stable for at most 48 hours depending on the biological fluid it was exposed to. In another study, a PEO coating remained stable for less than a month [73]. In both cases, degradation most probably does not occur at the alkyl or ether bonds of the PEO polymer itself, as these can only be degraded by aggressive chemicals [74], high temperatures [75] or specific bacteria [76]. The grafting of the PEO chain to the surface is, in general, the weak point of a PEO coating. For instance, the often used organosilane linkage is susceptible to hydrolytic cleavage [77]. In our study, the PEO chains were coupled by a $Si-O-C$ linkage to the glass substratum, which may readily hydrolyze as well [78]. In case the polymer chains are grafted at the surface by physical adsorption, they can be expected to desorb over time, therewith losing their effectiveness.

Conclusions

This review has shown that positively charged materials, low s.f.e. materials and polymer brushes show potential in preventing BCI. Positively charged materials have shown to be effective in preventing growth of certain strains and species, while other stains readily adhere and grow on them. Possibly, positively charged surfaces can only be applied in situations where there is little supply of new organisms, such as for totally implanted surfaces, because a large supply of new organisms will cause adhesion of viable organisms on the organisms killed or hampered in their growth by the positive charge. Also low s.f.e. materials are not generally applicable as some bacterial strains are able to adhere to them or even show preference for these materials. However, for applications where fluctuating shear forces are operative, excellent in vivo results have been obtained. Theoretically, polymer brushes should be able to reduce adhesion of any bacterium and, indeed, have shown a very high effectiveness in preventing bacterial adhesion in vitro. Whether or not those bacteria that do successfully adhere to a polymer brush will

also grow and form a biofilm, has not yet been established. Problems concerning durable attachment of the polymer chains to the surface preventing successful in vivo applications with these coatings could be overcome by developing a stable coupling between a surface and the brush.

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