Chapter 22
Inhibition of Bacterial Adhesion on Medical Devices

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Abstract Microbial infections resulting from bacterial adhesion to biomaterial surfaces have been observed on almost all medical devices. Biofilm infections pose a number of clinical challenges due to their resistance to immune defence mechanisms and antimicrobials, and, regardless of the sophistication of the implant, all medical devices are susceptible to microbial colonisation and infection. Research efforts are currently directed towards eliminating or reducing infection of medical devices. Strategies to prevent biofilm formation include physiochemical modification of the biomaterial surface to create anti-adhesive surfaces, incorporation of antimicrobial agents into medical device polymers, mechanical design alternatives, and release of antibiotics. Nevertheless, the success of these alternatives has been modest, mainly due to the various environments into which devices are placed and the diversity of ways in which organisms can colonise surfaces. Biosurfactants have been reported as a promising strategy as they effectively inhibit bacterial adhesion and retard biofilm formation, and are thus potentially useful as a new generation of anti-adhesive and antimicrobial coatings for medical devices.

22.1 Introduction

Microbial adhesion and biofilm formation on medical devices is a common event that can have important medical and economic consequences. The use of temporary or permanent implants or prosthetic devices fabricated from polymeric biomaterials has increased dramatically in recent years. It is estimated that over 5 million medical devices or implants are used per year in the United States alone (Bryers, 2008). Medical devices are responsible for about 60–70% of hospital-acquired infections, particularly in critically ill patients (Bryers, 2008; Darouiche, 2001).

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Bacterial colonisation of an indwelling device, followed by biofilm formation, can be a prelude to infection and consequently to tissue destruction, systemic dissemination of the pathogen and dysfunction of the device, resulting in serious illness and death (Hall-Stoodley et al., 2004). The process by which microorganisms colonise open and closed implants or prosthetic devices is fairly complicated and involves a series of steps starting with deposition of host substances (macromolecules such as collagen and fibronectin) onto the material (Costerton et al., 1999). Device-associated infections are resistant to immune defence mechanisms and are difficult to treat with antimicrobial agents because the organisms are encased within a protected microenvironment hampering the prevention and treatment of established biofilms (Habash and Reid, 1999). Bacteria that grow in association with medical devices always form slime-enclosed biofilms within which they are protected, to a large extent, from the bactericidal activity of chemical biocides and antibiotics. Removal of the device may be necessary, resulting in both attendant distress to the patient and cost. Considerable research endeavour is currently directed towards reducing, if not eliminating, infection of medical devices. Strategies under investigation include physiochemical modification of the biomaterial surface to create anti-adhesive surfaces, incorporation of antimicrobial agents into medical device polymers and the use of electric fields to improve antibiotic therapy (Darouiche, 2001; Hall-Stoodley et al., 2004; Hetrick and Schoenfisch, 2006). Nevertheless, the efforts to reduce adhesion using specially developed materials, with hydrophilic or heparin coated surfaces, have had only modest clinical success (Habash and Reid, 1999). The reason, at least for the most part, is the various environments into which devices are placed and the diversity of ways in which organisms colonise surfaces. A better understanding of the process is required, as well as the development of new alternatives to the traditional surface-modifying preventive approaches, which have largely focused on antimicrobial coating of devices and on employment of antibiotics (Darouiche, 2001; Habash and Reid, 1999).

This chapter focuses on adhesion and biofilm formation by bacteria on medical devices, strategies to inhibit bacterial adhesion and the potential use of biosurfactants as anti-adhesive and antimicrobial surface coatings.

22.2 Medical Biofilms

More than half of the infectious diseases that affect mildly immune-compromised individuals involve bacterial species that are commensal in humans or are common in our environment (Costerton et al., 1999). For example, the skin bacterium *Staphylococcus epidermidis* and the soil bacterium *Pseudomonas aeruginosa* can cause devastating chronic infections in immune-compromised hosts. Microbial infections have been observed on most, if not all, medical devices or implants including: prosthetic heart valves, orthopaedic implants, intravascular catheters, artificial hearts, left ventricular assist devices, cardiac pacemakers, vascular prostheses, cerebrospinal fluid shunts, urinary catheters, voice prostheses, ocular prostheses and

contact lenses, and intrauterine contraceptive devices (Bryers, 2008; Darouiche, 2001; Rodrigues et al., 2007). In non-surgical indwelling medical devices, such as central venous and urinary catheters, biofilm colonisation may originate either from the skin at the point of insertion, or by migration of the organism(s) through or around the catheter once implanted. As for surgical devices, tissue damage and clot formation associated with surgical implantation are correlated with enhanced rates of microbial biofilm colonisation (Donlan and Costerton, 2002; Hetrick and Schoenfisch, 2006; Lynch and Robertson, 2008; Zilberman and Elsner, 2008). At the cellular level, implant-associated infections are the result of bacterial adhesion to a biomaterial surface. Upon implantation, there is competition between integration of the material into the surrounding tissue and adhesion of bacteria to the implant surface (Gristina, 1987). For a successful implant, tissue integration occurs prior to appreciable bacterial adhesion, thereby preventing colonisation at the implant. However, host defences often can not prevent further colonisation if bacterial adhesion occurs before tissue integration (Gristina, 1987). A 6 h post-implantation "decisive period" has been identified during which prevention of bacterial adhesion is critical to the long-term success of an implant (Poelstra et al., 2002). Over this period, an implant is particularly susceptible to surface colonisation. Virtually all medical devices or tissue engineering constructs are susceptible to microbial colonisation and infection (Castelli et al., 2006).

Upon adhesion to a surface, replicating adherent bacteria secrete mostly insoluble gelatinous exopolymers, forming a biofilm (Bryers, 2008; Darouiche, 2001). From a medical perspective, both commensal and pathogenic microorganisms form biofilms that are associated with the epithelial or endothelial lining: embedded in the lung, intestinal or vaginal mucus layer; attached to the teeth or medical implant surfaces; or formed intracellularly (Costerton et al., 1999; Hall-Stoodley et al., 2004; Pizarro-Cerdá and Cossart, 2006; Reid 1999). Microorganisms involved in biofilms related to human infections are compiled in Lynch and Robertson (2008). Biofilm formation and persistence has profound implications for the patient, because microorganisms growing as biofilms are significantly less susceptible to antibiotics and host defenses than the planktonic forms of the same microorganisms (Bryers, 2008; Costerton et al., 1999). Sessile bacterial cells release antigens and stimulate the production of antibodies, but the antibodies are not effective in killing bacteria within biofilms and may cause damage to surrounding tissues (Costerton et al., 1999). Even in individuals with excellent cellular and humoral immune reactions, biofilm infections are rarely resolved by host defence mechanisms and commonly manifest themselves as chronic or recurrent infections (Bryers, 2008; Costerton et al., 1999; Gristina, 1987; Gottenbos et al., 2004). Biofilm infections constitute a number of clinical challenges, including disease, chronic inflammation, impaired wound healing, rapidly acquired antibiotic resistance, and the spread of infectious emboli (Bryers, 2008; Hall-Stoodley et al., 2004; Pizarro-Cerdá and Cossart, 2006). A number of physical, biological and chemical processes are involved in biofilm formation, with the relative contribution of each changing throughout biofilm development and depending on environmental and hydrodynamic conditions (Habash and Reid, 1999; Hall-Stoodley et al., 2004). Therefore, non-fouling biomaterials

ought to be developed; otherwise protein deposition onto the surfaces will occur with subsequent microbial adhesion and biofilm formation.

22.3 Prevention of Medical Device-Associated Infections

Prophylactic use of antibiotics and biocides can reduce the incidence of biofilm-associated infections with indwelling medical devices (Lynch and Robertson, 2008). Strategies to prevent biofilm formation range from systemic approaches controlling any bacterial invasion of sterile sites to local biofilm inhibition on medical devices (Fux et al., 2003). The latter focuses on the elimination of planktonic cells before they adhere to the surface and initiate biofilm formation. Both material properties and host factors determine bacterial adhesion to medical devices (Rodrigues et al., 2004a, 2006b, c, 2007). Bacterial adherence to silicone, for example, has been found to be significantly higher than to polyurethane or Teflon® (Lopez-Lopez et al., 1991). Host factors, such as fibronectin, fibrinogen or platelets may be deposited on the foreign body material and provide specific ligands for bacterial adhesins (Shenkman et al., 2002). A variety of approaches have proven to be effective in reducing biofilm-related infections by preventing bacterial adhesion, at least in high-risk populations (Fux et al., 2003). They include device coatings, device immersion, anti-septic irrigation of the surgical site, antibiotic loaded cements in orthopedic surgery (Zilberman and Elsner, 2008), and antibiotic catheter lock therapy containing vancomycin and heparin (Carratalà et al., 1999) or minocycline and EDTA (Raad et al., 2002). In antibiotic catheter lock therapy, a concentrated antibiotic solution is placed in a catheter in a volume adequate to fill the lumen. The catheter is then "locked" into place for an extended period while the catheter is not in use, with the goal of preventing it from becoming colonised and thereby reducing the risk of infection (Lynch and Robertson, 2008). Impregnation of catheter surfaces with antiseptics (Veenstra et al., 1999) or antibiotics (Zilberman and Elsner, 2008) has been shown to delay bacterial colonisation. Although the use of antibiotic prophylaxis is controversial because of its potential to increase antimicrobial resistance, it is increasingly common in high-risk patient groups (Lynch and Robertson, 2008). With regard to device coatings, Falagas and co-workers (2007) conducted a recent and comprehensive meta-analysis of randomised controlled trials of rifampin-impregnated central venous catheters and found that they are both safe and effective in reducing the rate of catheter colonisation and catheter-related bloodstream infections. Similarly, Manierski and Besarab (2006) performed six independent studies on the efficacy of antibiotic lock therapy in the prevention of catheter-related bloodstream infections in haemodialysis patients, and found an overall reduction of 64–100% in catheter-related bloodstream infections.

Furthermore, Rodrigues and collaborators (2004, 2006b, c, 2007) have shown that impregnation of silicone rubber surfaces with biosurfactants produced by several lactobacilli inhibits the adhesion of several microorganisms. In the specific case of voice prostheses, it is well-known that biofilms are resistant to a range of

antifungal agents currently in clinical use, including amphotericin B and fluconazole, thus new prophylactic treatments are being explored to prolong their lifetime. As antimicrobial resistance is becoming a source of concern in modern medicine and health-improving functional foods are gaining in popularity, the development of alternative prophylactic and therapeutic agents, including probiotics, has been investigated (Free et al., 2001). Lactobacilli are one of the most well-known probiotic bacterial genera and play an important role in the maintenance of a healthy intestinal and urogenital tract (Velraeds et al., 1998; Reid, 1999, 2000). Other bacterial genera known to have probiotic effects are *Lactococci*, *Enterococci* and *Streptococci*. The mechanisms by which probiotic bacteria exert their beneficial effects are not yet entirely understood. Possible mechanisms are competitive adhesion (Busscher et al., 1997), activation of the immune system (Perdigon et al., 1986), or nutrient competition (Free et al., 2001). Some strains are able to release biosurfactants, while others are known to have antimycotic effects by producing lactic acid or hydrogen peroxide.

22.4 Treatment of Medical Device-Associated Infections

Traditional treatment of microbial infections is based on compounds that inhibit growth of the microbe or kill it (Bryers, 2008). The major concern with this approach is the frequent development of resistance to antibiotics. For details on agents for treatment or prophylaxis of biofilm-associated infections, the reader is directed to the review by Lynch and Robertson (2008). Bacterial biofilms are inherently resistant to antimicrobial agents and the host immune system, and tend to be significantly less responsive to antibiotics and antimicrobial stressors than planktonic organisms of the same species (Bryers, 2008; Costerton et al., 1999). Prolonged and high-dose antibiotic therapy and the elimination of infected foreign-body material are the basis of successful therapy. Antibiotic treatment of bacterial endocarditis was shown to be more successful when serum antibiotic levels were held at least tenfold above the minimal bactericidal concentration (Joly et al., 1987); but even with 8 weeks of treatment, few patients have been cured by antimicrobial therapy alone (Hancock, 1994). The combination of rifampicin and a fluoroquinone has proven especially successful in the treatment of various *S. aureus* biofilm infections, ranging from infections of orthopaedic prostheses (Habash and Reid, 1999) to right-heart endocarditis (Heldman et al., 1996). On the other hand, the study reported by Bagge and co-workers (2004) showed that sub-lethal doses of β-lactam antibiotic can actually enhance biofilm formation by *P. aeruginosa*, increasing its volume and polymer matrix, which can lead to adverse consequences when treating cystic fibrosis patients. Also, Hoffman et al. (2005) reported that sub-inhibitory concentrations of aminoglycoside antibiotics induced biofilm formation by *P. aeruginosa* and *E. coli*.

As a consequence of this increase in resistance, researchers have turned to a number of alternatives to synthetic antibiotics including disinfectants (Hetrick and

Schoenfisch, 2006; Zilberman and Elsner, 2008), bacteriophage (Sulakvelidze et al., 2001) and bacteriophage lytic enzymes (Fischetti, 2005), probiotics (Hong et al., 2005), and human antimicrobial peptides (defensins, cathelicidins, and histatins) (De Smet and Contreras, 2005). Nevertheless, these are mostly synthetic compounds with the ability to inactivate or kill suspended bacteria but with poor efficacy when applied to biofilm infections (Stewart, 2002). It has been recently proposed that substances that specifically inhibit bacterial virulence should be developed. Such "antipathogenic" drugs, in contrast to antibacterial drugs, do not kill bacteria or stop their growth and are assumed not to lead to the development of resistant strains (Bryers, 2008). A very elegant approach comprises the inhibition of regulatory systems that govern the expression of a series of bacterial virulence factors: for example, anti-adhesion therapy [passive antibody therapy (Casadevall et al., 2004), and synthetic peptide vaccine and antibody therapy (Cachia and Hodges, 2003)], inhibition or negation of cell–cell signalling (Otto, 2004), negation of biofilm formation by disrupting iron metabolism (Kaneko et al., 2007), and up-regulation of biofilm detachment promoters (rhamnolipids) (Boles et al., 2005).

Replacement or removal of an infected indwelling medical device, combined with systemic antibiotic and/or antifungal therapy, is the most effective treatment in most settings (Trampuz and Zimmerli, 2006). For managing indwelling medical device infections in non-surgery patients, long-term antimicrobial suppressive therapy remains the only option (Lynch and Robertson, 2008). Recent reviews summarise current recommended practices for the treatment of infections of prosthetic joints (Trampuz and Zimmerli, 2006), arterial prostheses (Goeau-Brissonnière and Coggia, 2000), vascular catheters (Castelli et al., 2006), prosthetic heart valves (Karchmer, 2000), central nervous system shunts (Yogev and Bisno, 2000), pacemakers and defibrillators (Eggimann and Waldvogel, 2000), endotracheal and tracheotomy tubes (Dever and Johanson, 2000), and hemodialysis and peritoneal hardware (Oliver and Schwab, 2000), as well as treatment of foreign body infections of the urinary tract (Hessen et al., 2000).

22.5 Development of Anti-adhesive Biomaterials

The remarkable resistance of biofilms to conventional antibiotic therapy has prompted a great deal of research to produce anti-infective and anti-adhesive devices or implants by either (a) mechanical design alternatives, (b) modification of material surface features (biosurfactants, plasma, brushes), (c) anti-infective agents bound to the surface of the material (silver, quaternary ammonium, synthetic antibiotics, biosurfactants), or (d) release of soluble toxic agents (chlorhexidine, antibiotics) into the adjacent surroundings (Bryers, 2008; Hetrick and Schoenfisch, 2006). Mechanical design alternatives have had only marginal success and are only applicable for short-term indwelling catheters (Bryers, 2008). Coatings have been developed that reduce bacterial adhesion by altering the physicochemical properties of the substrate so that conditioning films do not form and/or bacteria-substrate

interactions are not favoured. These "passive" coatings include surfaces modified with poly(ethylene glycol) (Kingshott et al., 2003), poly(ethylene oxide) brushes (Kaper et al., 2003), and hydrophilic polyurethanes (Nagel et al., 1996), among many others. Unfortunately, the effectiveness of passive coatings for reducing bacterial adhesion is limited and varies greatly depending on the bacterial species (Hetrick and Schoenfisch, 2006). The physicochemical properties of the coating can be masked by an adsorbed conditioning film, thereby diminishing their effectiveness. Additionally, surface-bounded anti-infective agents are only toxic to the initial wave of incoming bacteria and provide little residual effects once layers of dead cells accumulate, which are also inflammatory (Bryers, 2008). Nevertheless, there are studies pointing to some success in retarding bacterial adhesion, which in turn inhibits or delays biofilm formation. One specific example is the development of anti-adhesive silicone rubber surfaces for voice prostheses. Voice prostheses are continuously exposed to saliva, food, and drinks that, together with the oropharyngeal microflora, contribute to valve failure and the need to replace the implant frequently (Mahieu et al., 1986). Therefore, improvement of the antifouling properties of the silicone rubber material is desirable. Rodrigues and collaborators (2007) reviewed the different approaches that have been undertaken to modify the silicone rubber surface as an obvious strategy to inhibit biofilm formation and consequently to prolong the lifetime of voice prostheses.

A recent alternative approach to reducing bacterial adhesion is based on coatings that actively release antibacterial agents. Such "active" coatings have been designed to temporarily release high initial fluxes of antibacterial agents during the critical short term post-implementation period (several hours) to inhibit adhesion of bacteria. Continued release beyond this short term period is desirable because protective fibrous capsule formation and tissue integration occur over a longer time period (weeks to months) (Anderson, 2001). Zilberman and Elsner (2008) reviewed the latest developments on antibiotic-eluting medical devices for various applications.

For example, non-degradable polymethylmethacrylate (PMMA) bone cements and spacer beads loaded with antibiotics have been employed clinically in various forms in joint replacements and in prevention or treatment of deep bone infections (osteomyelitis) (Webb and Spencer, 2007). Such systems slowly release the soluble drug from the solidified PMMA bone cement surrounding the implant over time. Moreover, the use of a bioactive ceramic coating containing hydroxyapatite (HA), calcium phosphate and other osteo-conductive materials as antibiotic carriers offers the added value of providing the physiochemical environment and structural scaffold required for bone-implant integration. In vitro release of antibiotics from HA-coated implants has been reported for several antibiotics (Teller et al., 2007). The conventional plasma spraying technique for HA-coating is associated with high processing temperatures and therefore does not enable the incorporation of antibiotics in the process. Most reported work therefore focuses on soaking antibiotics onto plasma-sprayed HA. Gollwitzer and collaborators (2003) studied biodegradable polymeric coatings made from polylactic acid and its copolymers with glycolic acid. An additional advantage of such coatings is the relative ease with which the polymer can be

applied to both alloys and plastics with polished, irregular or porous surfaces using a simple dip-coating technique (Schmidmaier et al., 2006).

Other examples of antibiotic-eluting medical devices include intravascular devices and vascular grafts. Infection of intravascular devices for vascular access and vascular prostheses for the replacement or bypass of damaged arteries is a rare but serious event (Zilberman and Elsner, 2008). The infection of a vascular graft is a rare complication, with an estimated incidence of 0.5–2.5% of bypass procedures. However, the mortality and morbidity rates due to this complication are high (Bryers, 2008). Once a prosthetic graft is infected, it must almost always be excised and replaced with a new prosthetic bypass. The development of infection-resistant vascular prostheses may therefore contribute to the prevention and treatment of this complication. PET (polyethyleneterephtalate, Dacron^{TM}) and ePTFE (expanded polytetrafluorethylene) vascular prostheses soaked in an antibiotic solution produce a wash-out release of antibiotics within minutes after placement (Blanchemain et al., 2005). Several approaches have been proposed for extending release over days and weeks. Antibiotics have been "bonded" by soaking collagen, albumin, and gelatin sealed grafts to produce extended antibacterial activity (Zilberman and Elsner, 2008).

Finally, regardless of the type of "drug release" method used (passive versus active), release of a toxic agent from a biomaterial of a soluble anti-infective agent will inevitably stop once the entrapped agent is depleted. Further, as discussed above, delivery of sub-lethal dosages of antibiotics can lead to accelerated biofilm formation and induced virulence factor expression (Bryers, 2008).

22.6 Biosurfactants: A Powerful Tool to Inhibit Bacterial Adhesion

Biosurfactants are microbial amphiphilic compounds with both hydrophilic and hydrophobic moieties and with a distinct tendency to accumulate at interfaces. For all interfacial systems, it is known that organic molecules from the aqueous phase tend to immobilise at the solid interface. There they eventually form a conditioning film, which will change the properties (wettability and surface energy) of the original surface (Neu, 1996). In an analogy to organic conditioning films, biosurfactants may interact with the interfaces and affect the adhesion and/or detachment of bacteria. In addition, the substratum surface properties determine the composition and orientation of the molecules conditioning the surface during the first hour of exposure. After about 4 h, a certain degree of uniformity is reached and the composition of the adsorbed material becomes substratum-independent (Neu, 1996). Adsorption of charged biosurfactants to interfaces is governed by a range of interactions that are not only hydrophobic. Most interfaces have an overall negative or, rarely, positive charge. Gottenbos et al. (2001) showed that positively charged biomaterial surfaces exert an antimicrobial effect on adhering Gram-negative, but not on Gram-positive bacteria.

Microbial surfactants constitute a diverse group of surface-active molecules and are known to occur in a variety of chemical structures, such as glycolipids, lipopeptides and lipoproteins, fatty acids, neutral lipids, phospholipids, and polymeric and particulate structures (Rodrigues et al., 2006a). Their use and potential commercial application in the medical field has increased during the past decade (Muthusamy et al., 2008; Rivardo et al., 2009; Rodrigues et al., 2006a), due to their antibacterial, antifungal and antiviral activities, which make them useful for combating many diseases and as therapeutic agents. In addition, their role as anti-adhesive agents against several pathogens indicates their utility as suitable anti-adhesive coating agents for medical insertional materials, leading to new and effective means of combating colonisation by pathogenic microorganisms without the use of synthetic drugs and chemicals (Rodrigues et al., 2006a). Mireles and collaborators (2001) pre-coated vinyl urethral catheters by running the surfactin solution through them before inoculation with media and found a decrease in the amount of biofilm formed by *Salmonella* Typhimurium, *Salmonella enterica*, *E. coli* and *Proteus mirabilis*. Moreover, the use of lactobacilli as a probiotic for the prevention of urogenital infections has been widely studied (Reid, 2000; Boris and Barbés, 2000). Velraeds et al. (1998) reported the inhibition of biofilm formation by uropathogens and yeast on silicone rubber with biosurfactants produced by *Lactobacillus acidophilus*. Also, Heinemann et al. (2000) showed that *Lactobacillus fermentum* RC-14 releases surface-active components that can inhibit adhesion of uropathogenic bacteria. Efforts to develop strategies to prevent the microbial colonisation of silicone rubber voice prostheses have been reported by Rodrigues and co-workers (2004a, 2006c). Biosurfactants obtained from *Lactococcus lactis* 53 and *Streptococcus thermophilus* A have been evaluated as anti-adhesive agents against several microorganisms isolated from explanted voice prostheses. Over 90% reductions in the initial deposition rates were achieved for most of the bacterial strains tested. The biosurfactant obtained from *S. thermophilus* A was more effective against *Rothia dentocariosa* GBJ 52/2B, which is one of the strains responsible for valve prosthesis failure. Nevertheless, the effect of the adsorbed biosurfactant was less pronounced for the initial deposition rates of the yeast strains. The authors also demonstrated that, when rinsing flow chambers designed to monitor microbial adhesion with a rhamnolipid solution, the rate of deposition and adhesion was significantly reduced for a variety of microorganisms (Rodrigues et al., 2006a, b). Thus, this rhamnolipid may be useful as a biodetergent solution for cleaning prostheses, prolonging their lifetime and directly benefiting laryngectomised patients.

Furthermore, the biosurfactants produced by the same strains (Rodrigues et al., 2004b) and by *Lactobacillus paracasei* ssp. *paracasei* A20 (Gudiña et al., 2010a, b) were found to possess antimicrobial and anti-adhesive activity against several microorganisms. For contact lenses (CL), maintaining the optical properties might limit the use of biosurfactants as coating agents. Consequently, Rodrigues and collaborators evaluated the influence of biosurfactants on refractive index (RI) and transmittance (T) (*unpublished data*). One conventional hydrogel (Etafilcon A) and two silicone-hydrogel (Galyfilcon and Lotrafilcon B) contact lenses were tested (Table 22.1). Prior adsorption of biosurfactants to silicone-hydrogel lenses had no

Table 22.1 Refractive index and transmittance in the visible spectrum of contact lenses with and without an absorbed biosurfactant layer. Biosurfactants from *Lactococcus lactis* (BS1), *Lactobacillus paracasei* ssp. *paracasei* A20 (BS2) and *Streptococcus thermophilus* A (BS3) were tested at 2 different concentrations (10 and 50 g/L). One conventional hydrogel CL (Etafilcon A) and two silicone-hydrogel (Galyfilcon and Lotrafilcon B) lenses were used. Experiments were done in triplicate. Refractive index values correspond within 1–2% and transmittance values correspond within 2–5%

		Treatment with biosurfactant						
		BS1 (g/L)		BS2 (g/L)		BS3 (g/L)		Untreated contact lenses
	Contact lenses	10	50	10	50	10	50	
Refractive index	Galyfilcon	1.408	1.411	1.408	1.409	1.410	1.411	1.408
	Lotrafilcon B	1.422	1.424	1.423	1.423	1.422	1.424	1.422
	Etafilcon A	1.414	1.436	1.406	1.418	1.408	1.418	1.398
Transmittance in the visible spectra (%)	Galyfilcon	86.2	82.8	89.7	88.5	83.0	82.2	91.0
	Lotrafilcon B	82.4	80.1	85.8	81.5	82.5	81.6	83.9
	Etafilcon A	90.3	86.5	89.1	88.9	90.2	81.6	88.7

effect on the RI. However, for the biosurfactant-conditioned hydrogel CL, a higher RI was obtained compared to the untreated lenses. This increase in RI is a consequence of the dehydration observed with the adsorption of the biosurfactants, which is not desirable. All treated contact lens types showed a decrease in transmittance levels in the visible spectra, the effect being more pronounced for higher biosurfactant concentrations as a result of their colour. Although the results obtained for the transmittance experiments were promising, further characterisation and purification of the biosurfactants is required to enable the use of lower concentrations, more active and colourless fractions.

In another study, the same authors explored the possibility of using the biosurfactant produced by *S. thermophilus* A to pre-condition silicone rubber surfaces to inhibit the adhesion of the two most frequent fungi isolated from maxillofacial prostheses, *Candida albicans* MFP 22-1 and *Candida parapsilosis* MFP 16-2 (*unpublished data*). Adhesion assays showed a reduction of 60–80% in the initial deposition rates (Fig. 22.1). These results represent progress towards designing new strategies for preventing microbial adhesion to silicone rubber maxillofacial prostheses.

Besides the screening of lactobacilli as biosurfactant producers, Rodrigues and collaborators (2006d) also characterised the anti-adhesive activity of these biosurfactants against several microorganisms including Gram-positive and Gram-negative bacteria and filamentous fungi (Gudiña et al., 2010a, b). For example, the biosurfactant produced by *L. paracasei* A20 showed anti-adhesive activity against *Streptococcus sanguis* (72.9%), *S. aureus* (76.8%), *S. epidermidis* (72.9%) and *Streptococcus agalactie* (66.6%) (Gudiña et al., 2010a). Additionally, the anti-adhesive activity of two biosurfactants produced by *Candida sphaerica* UCP 0995

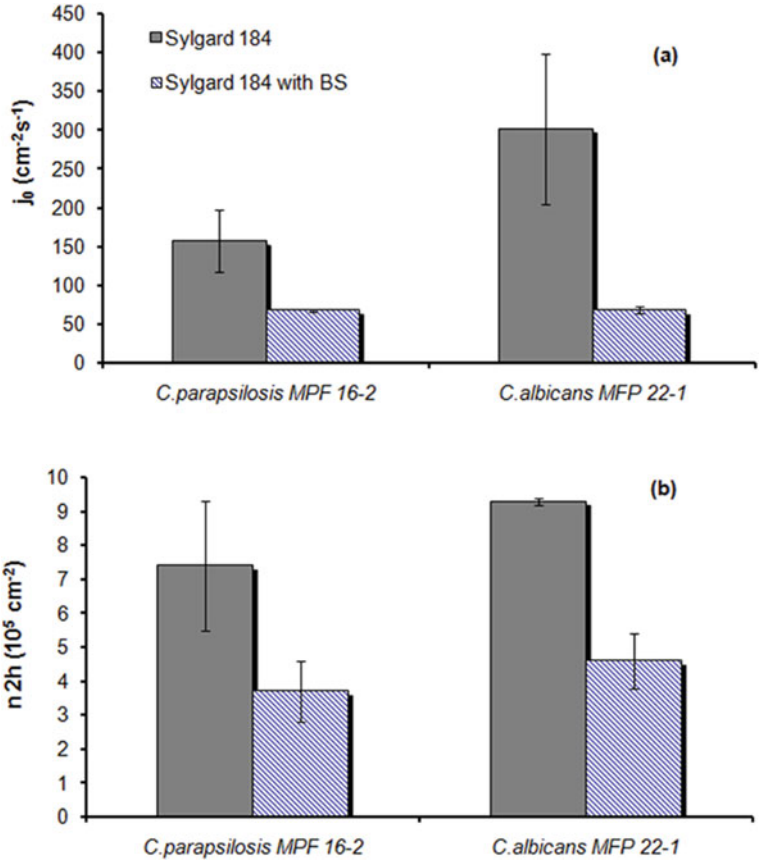

Fig. 22.1 (**a**) Initial deposition rates (j_0, cm^{-2} s^{-1}) of *Candida parapsilosis* MFP 16-2 and *Candida albicans* MFP 22-1 isolated from maxillofacial prostheses on Sylgard® 184 silicone rubber with and without an adsorbed biosurfactant (BS) layer; (**b**) Number of microorganisms adhering after 2 h ($n_{2\,h}$) on Sylgard® 184 with and without an adsorbed biosurfactant (BS) layer. Biosurfactant was produced by *Streptococus thermophilus* A, (see Rodrigues et al., 2006c). Results are averages of triplicate experiments and the standard deviation represented by *error bars*

and *Candida lipolytica* UCP 0988 was studied (*unpublished data*). The biosurfactant from *C. sphaerica* UCP 0995 was found to inhibit the adhesion of *P. aeruginosa*, *S. agalactiae*, *S. sanguis*, *C. tropicallis*, *E. coli*, and *S. salivarius* by between 80 and 92%. Inhibition of adhesion with percentages near 100% occurred for the higher concentrations of biosurfactant used (Table 22.2). Although less pronounced, similar results were obtained with the biosurfactant produced by *C. lipolytica* UCP 0988 for some of the microbial strains studied (Fig. 22.2). All these results open prospects for the use of biosurfactants against the adhesion of microorganisms responsible for diseases and infections in the urinary, vaginal and gastrointestinal tracts, as well as in the skin.

Table 22.2 Anti-adhesive properties of crude biosurfactant produced and extracted from *Candida sphaerica* UCP 0095. Negative controls were set at 0% to indicate the absence of biosurfactant. Positive percentages indicate the reductions in microbial adhesion when compared to the control, and negative percentages indicate increased microbial adhesion. Results are expressed as percentage means from triplicate experiments and correspond within 1–3%

	[Biosurfactant] (mg/L)				
Microorganism	0.3	0.6	2.5	5	10
Candida tropicalis	80	85	87	98	100
Escherichia coli	89	93	96	97	99
Pseudomonas aeruginosa	80	82	83	89	92
Streptococcus agalactiae	80	86	88	92	100
Streptococcus sanguis	80	83	87	98	100
Streptococcus salivarius	92	93	95	97	100

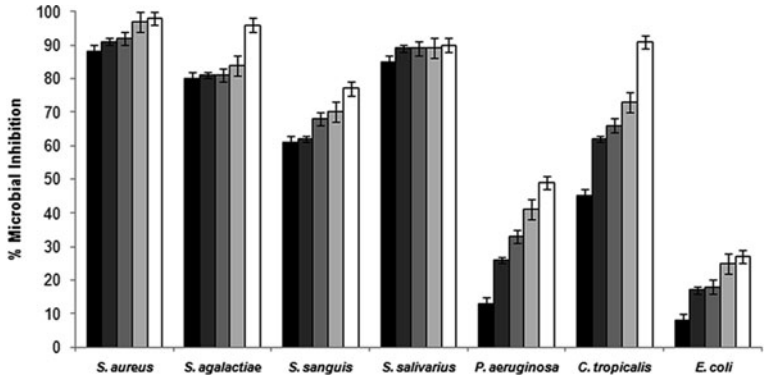

Fig. 22.2 Microbial inhibition percentages obtained from the anti-adhesion assays with the crude biosurfactant produced by *Candida lipolytica* UCP 0988 at different concentrations (0.75 mg/L [■], 1.5 mg/L [■], 3 mg/L [■], 6 mg/L [■] and 12 mg/L [■]). Results are averages of triplicate assays and *error bars* represent standard deviations

Based on the above, biosurfactants can play an important role in the development of anti-adhesive coatings for silicone rubber as they effectively inhibit bacterial adhesion and retard biofilm formation. Therefore, surface and bulk modification techniques, laser-induced surface grafting and the sequential method for interpenetrating polymer networks should be explored as ways to link the biosurfactants more strongly with the silicone rubber surfaces, thus avoiding their washout from the surfaces and prolonging their effect. Furthermore, biosurfactants are a suitable alternative to antimicrobial agents, and could be used as safe and effective therapeutic agents or probiotics. The use of biosurfactants as antimicrobial agents is currently of particular interest, since an increasing number of drug-resistant microorganisms are being encountered and there is a need for alternative lines of therapy. Some biosurfactant activities could be exploited by developing an alternative therapy for treating patients (Rodrigues et al., 2006a). Nevertheless, although the replacement

of synthetic surfactants by biosurfactants would provide advantages such as biodegradability and low toxicity, their use has been limited by their relatively high production cost, as well as scarce information on their toxicity in humans. The main limiting factor, however, for commercialisation of biosurfactants is the high cost of large-scale production. Several strategies have been adopted to reduce costs (Rodrigues et al., 2006e). The use of agro-industrial wastes as substrates, optimisation of medium and culture conditions, and efficient recovery processes all help. However, to compete with synthetic surfactants, effective microorganisms must be developed for biosurfactant production. The use of biosurfactant hyper-producer strains allows increasing biosurfactant production and reduces production costs. Strains producing higher amounts of biosurfactants can be obtained by screening high biosurfactant-producing microorganisms from the natural environment, or by engineering strains for biosurfactant production. Therefore, knowledge of the genes required for production of biosurfactants is critical for their application in industry. Once the genes have been indentified and isolated, they can be expressed in other microorganisms (e.g. to prevent pathogenicity), or they can be modified or placed under regulation of strong promoters to increase their expression and so enhance production. This knowledge will also allow the production of novel biosurfactants with specific new properties (designed by metabolic engineering) for different industrial applications. Genetic engineering of the known biosurfactant molecules could produce potent biosurfactants with altered antimicrobial profiles and decreased toxicity against mammalian cells.

22.7 Concluding Remarks

The processes governing biofilm formation are rather complex, involving several steps and almost all surfaces are susceptible of being colonised. Bacterial colonisation and subsequent biofilm formation on an indwelling device can lead to infection with severe economic and medical consequences. Device-associated infections are resistant to immune defense mechanisms and are difficult to treat with antimicrobial agents because the organisms are encased within a protected microenvironment. Therefore, non-fouling biomaterials ought to be developed. Several strategies based on the modification of the physicochemical properties of the substrate have been pursued. Nevertheless, the effectiveness of these coatings has been found to be limited and varies greatly depending on bacterial species, mainly due to the diverse environments into which the devices are placed and the multiplicity of ways in which organisms can colonise surfaces. Development of alternatives to the traditional surface-modifying preventive approaches, which have largely focused on antimicrobial coating of devices and employment of antibiotics, is required. Biosurfactants represent an interesting approach because it may be possible to modify the surface properties to make it simultaneously anti-adhesive and give it antimicrobial activity. However, although some studies have demonstrated the potential of biosurfactants in biomedical applications, the genetics and structure-function relationships of biosurfactants, and methods of binding them to surfaces, require further exploration.

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