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MINIREVIEW

Prevention and control of biofilm-based medical-device-related infections

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Abstract

Biofilms play a pivotal role in healthcare-associated infections, especially those related to the implant of medical devices, such as intravascular catheters, urinary catheters and orthopaedic implants. This paper reviews the most successful approaches for the control and prevention of these infections as well as promising perspectives for the development of novel devices refractory to microbial adhesion, colonization and biofilm formation.

Introduction

Biofilms play a pivotal role in healthcare-associated infections (HAIs), especially those related to the implant of medical devices, such as intravascular catheters, urinary catheters and orthopaedic implants.

As reported in 2008 by the European Center for Disease Prevention and Control [http://ecdc.europa.eu/en/healthtopics/pages/healthcare-associated_infections_(hcai).aspx], annually, approximately 4 100 000 patients are estimated to acquire an HAI in European hospitals. The number of deaths occurring as a direct consequence of these infections is estimated to be at least 37 000.

In the United States, 1 737 125 HAIs have been estimated for the year 2002, including 417 946 in intensive care units (ICUs) and 1 266 851 outside ICUs. The number of associated deaths was 98 987; of these, 30 665 were from bloodstream infections (BSIs), 13 088 from urinary tract infections (UTIs) and 8205 from surgical site infections including those associated with orthopaedic implants (Klevens *et al.*, 2007). Of particular interest is the recent analysis by Scott (2009), an economist at the US Centers for Disease Control and Prevention, about the costs of HAIs in US hospitals and the benefits of prevention.

Central venous catheters (CVCs) are responsible for the highest proportion of BSIs, their wide use being associated with a substantial risk of infectious complications that prolong hospital stay and increase healthcare costs. More than five million CVCs are implanted each year in the United States, and there are approximately 200 000 cases of BSIs related to their use (Mermel *et al.*, 2001). According to data provided by the European Sepsis Group, 28% of CVC infections in ICU patients are associated with sepsis, 24% with severe sepsis and 30% with septic shock, the remaining 18% being unclassified (Alberti *et al.*, 2002; Donelli, 2006). Not surprisingly, catheter-related infections are the most common cause of nosocomial endocarditis (Corey & Lalani, 2008; Falcone *et al.*, 2009), the duration of catheterization and the anatomical location of CVC being among the major risk factors.

The urinary tract is the most common site of nosocomial infections, catheter-associated urinary tract infections (CAUTIs) affecting an estimated 449 334 patients year^{-1} in US hospitals (Klevens *et al.*, 2007).

Among surgical site infections, those related to the implant of orthopaedic devices are of great relevance for public health due to the increasing number of aged and disabled patients requiring this type of surgical intervention. In the United States, primary total hip arthroplasty is associated with a 90-day deep-infection rate of 0.24% (Mahomed *et al.*, 2003), while primary total knee arthroplasty has a 90-day deep-infection rate of 0.4% (Mahomed *et al.*, 2005). The 1–3% incidence of hip implant failures due to infections is associated with significant morbidity and mortality indices of 2.7–18% (Berbari *et al.*, 1998).

Because of the large number of patients suffering from biofilm-based device-related infections, a number of strategies for their prevention have been developed in the last two decades.

Here, we review the most successful approaches for the control of these infections as well as some promising perspectives for the development of novel devices fully refractory to microbial adhesion, colonization and biofilm formation.

Microbial biofilms: resistant enemies

A worrying feature of biofilm-based infections (Bryers, 2008; Hall-Stoodley & Stoodley, 2009) is represented by the higher resistance of bacterial and fungal cells growing as biofilms as compared with planktonic cells (Aslam, 2008; Ramage *et al.*, 2009). Biofilms represent an ideal niche for plasmid exchange among bacteria. In fact, the conjugation frequency appears to be higher in bacteria growing in the sessile mode than in the planktonic mode. Thus, because some plasmids contain genes coding for multidrug resistance, microbial biofilms provide a suitable environment to amplify both naturally occurring and induced antibiotic-resistance phenomena.

The mechanisms involved in the increased drug resistance of biofilms (Lewis, 2008) presumably include the following: **(1)** Slow or incomplete penetration of antimicrobial agents through the biofilm matrix, even though it has been demonstrated that daptomycin is able to penetrate *Pseudomonas aeruginosa* biofilm rapidly (Stewart *et al.*, 2009). Thus, although the presence of the matrix undoubtedly retards the diffusion of antimicrobial agents, the poor penetration does not fully account for the observed drug resistance.
(2) Physiological response of microorganisms to the heterogeneous chemical environment existing in biofilms (Stewart & Franklin, 2008). In fact, the growth conditions in biofilms are quite different at lower layers, where nutrients and oxygen are limited, and microbial waste products can be toxic; for example, oxygen can be completely exhausted in the biofilm surface layers, while in the deep layers, anaerobic niches can be present (Borriello *et al.*, 2004). Also, nutrient depletion by slowing the growth rate of microorganisms can significantly reduce the number of targets for antimicrobial molecules.
(3) Onset of subpopulations of persister or dormant bacterial cells in a spore-like, nondividing state (Lewis, 2007; Stewart & Franklin, 2008). The susceptibility of active and dormant cell populations from *P. aeruginosa* biofilms to nonantibiotic antimicrobial agents such as chlorine, hydrogen peroxide and silver ions in comparison with antibiotics has been determined recently. Results indicated that dormant cells were more tolerant to tobramycin and silver ions, whereas active cells were significantly more tolerant to chlorine (Kim *et al.*, 2009). By contrast, recent studies of *Candida* biofilms suggested that persister cells alone cannot account for antimicrobial resistance (Al-Dhaheri & Douglas, 2008).

Another critical issue of biofilm-based infections is that biofilms are polymicrobial communities in which both bacteria and fungi often occur. In fact, biofilms were also estimated to be responsible in humans for a large proportion of fungal infections, *Candida* spp. being the fourth most common cause of nosocomial BSIs in North America (Klotz *et al.*, 2007). *Candida* spp. have also been demonstrated to be able to form *in vivo* polymicrobial biofilms with *Staphylococcus aureus* (Harriott & Noverr, 2009; Shirtliff *et al.*, 2009). Microorganisms have been shown to gain a fitness advantage when growing in a mixed-species biofilm. In fact, *in vitro* studies on polymicrobial biofilms comprising *Candida albicans* and *Staphylococcus epidermidis* demonstrated an altered sensitivity of each species to antimicrobial agents as a result of their mutual interaction: the *S. epidermidis* extracellular polymer inhibited fluconazole penetration, while *C. albicans* appeared to protect *S. epidermidis* against vancomycin (Adam *et al.*, 2002).

The occurrence of polymicrobial infections has significant implications for patient management owing to the related difficulties in selecting the most appropriate antimicrobial therapy, especially when multidrug-resistant pathogens are involved.

Do we need novel antibiofilm biomaterials, new synergistically acting antimicrobial compounds or both?

Knowledge of the mechanisms supporting biofilm development is of pivotal importance to address any preventive or controlling strategy.

According to the three-stage process of biofilm formation (Fig. 1), possible antibiofilm strategies should be based on: (1) inhibition of microbial adhesion to the surface and of colonization; (2) interference with the signal molecules modulating biofilm development; and (3) disaggregation of the biofilm matrix.

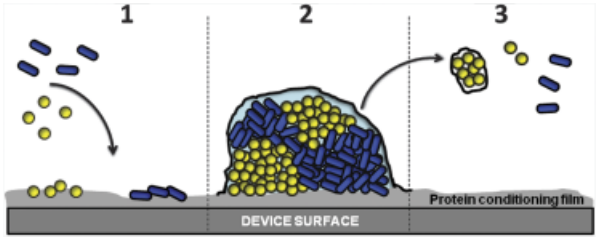

Fig. 1. Schematic representation of biofilm formation. Stage 1: microbial adhesion to the surface. Stage 2: exopolysaccharide production and three-dimensional biofilm development. Stage 3: detachment from biofilm of single and clustered cells.

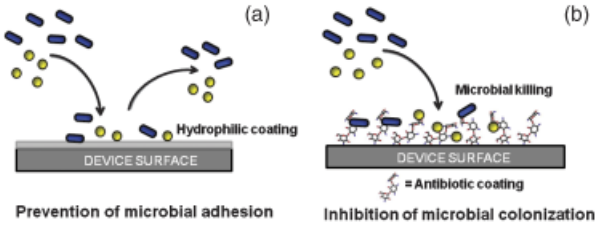

Fig. 2. Prevention of microbial adhesion by hydrophilic coating (a) and of microbial colonization of medical device surfaces by antibiotic coating (b).

Inhibition of microbial adhesion to device surfaces

Microbial adhesion depends strongly on the physicochemical properties of the materials constituting medical devices, their hydrophilicity and surface charge being the most important.

Microbial adhesion is a dynamic and biphasic process involving an early stage in which microorganisms reversibly interact with the device surface by van der Waals forces and H-bonds, and a second step with irreversible microbial adhesion mediated by specific adhesins able to recognize the host proteins (fibronectin, fibrin, etc.) layered as a conditioning film on the device surface. Once adhered, microorganisms duplicate and develop as microcolonies over the entire surface, these representing the 'building blocks' of the biofilm.

Given the hydrophobic nature of microbial surfaces (van der Mei *et al.*, 1988), prevention of bacterial adhesion can be achieved by coating the device surface with hydrophilic polymers (Fig. 2a). To avoid the growth of already adhered and/or colonizing microorganisms, research efforts have focused in the last two decades on the surface adsorption of, or material bulk impregnation with, one or two antimicrobial agents (Fig. 2b).

Hydrophilic coatings

Hydrophilic polymers such as hyaluronic acid (Cassinelli *et al.*, 2000) and poly-*N*-vinylpyrrolidone (Boelens *et al.*, 2000), originally used to coat polyurethane catheters and silicon shunts, respectively, have been demonstrated to successfully reduce the adhesion of *S. epidermidis*.

Various hydrogel coatings, especially of ureteral stents, have also been developed for their ability not only to reduce bacterial adhesion, due to their hydrophilic properties, but also for their propensity to uptake and release antibiotics, as a consequence of their high water potential (John *et al.*, 2007).

Heparin coatings or bindings have also been shown to prevent microbial adhesion and colonization *in vitro* and *in vivo* (Appelgren *et al.*, 1996). Heparin binding reduces fibronectin deposition on vascular catheter surfaces and makes the catheter negatively charged, thus preventing thrombosis and reducing microbial colonization (Russell *et al.*, 1987). This antiadhesive activity of heparin results in a significant reduction of catheter-related infections, as recently confirmed by a randomized-controlled clinical trial of heparin-coated and uncoated non-tunnelled CVCs inserted in 246 patients (Abdelkefi *et al.*, 2007) and a retrospective comparative analysis of 89 coated and 86 uncoated tunnelled dialysis catheters (Jain *et al.*, 2009).

Antimicrobial agents

Research in this field is very active. A PubMed survey on papers published since the year 1990 yields > 750 results just with 'antibiotic-coated devices' as keywords and approximately 1150 papers with 'medicated devices'.

The coating of device surfaces with one or two antimicrobial substances (Fig. 2b) or entrapping of these agents within the device material are the approaches most often used to obtain devices with different antimicrobial spectra and durations of the antimicrobial effect (Donelli & Francolini, 2001; Darouiche, 2008; Zilberman & Elsner, 2008).

Antimicrobial-coated urinary catheters

Recent studies have shown that the underlying cause of CAUTIs is the colonization of the catheter surfaces by microorganisms able to develop as a biofilm community (Stickler, 2008), the most common species present in these often mixed-population biofilms being *Enterococcus faecalis*, *P. aeruginosa*, *Escherichia coli* and *Proteus mirabilis* (Macleod & Stickler, 2007).

The Cochrane Database of Systemic Reviews reported, in 2004, a comprehensive evaluation of eight differently designed trials comparing silver alloy with standard urinary catheters in hospitalized adults. According to the pooled results, the risk of asymptomatic bacteriuria as well as of symptomatic UTIs was significantly reduced at < 1 week of catheterization in the silver alloy group, but at a lesser degree for > 1 week of catheterization (Schumm & Lam, 2008).

Further research has suggested a role in preventing UTIs for catheters impregnated with different antibiotics, including nitrofurazone, gentamicin, norfloxacin and minocycline–rifampicin (MR).

In fact, in addition to silver alloy-coated latex catheters, currently marketed antimicrobial urinary catheters include nitrofurazone-coated silicon catheters; the efficacy of both in preventing CAUTIs has been evaluated in clinical trials vs. latex and silicon standard catheters, respectively. Thus far, no trials have directly compared nitrofurazone and silver alloy-coated latex catheters. A recently published systematic review (Johnson *et al.*, 2006) considered 12 clinical trials performed in the period 1986–2004, three of which investigated nitrofurazone-coated silicon latex catheters and the remaining investigated nine silver alloy-coated latex catheters. In comparison with silicon or latex control catheters, these two types of coated catheters appear to reduce the development of asymptomatic bacteriuria during short-term use (< 30 days), while the effect of both types on morbidity, including BSIs, has not yet been defined.

A study performed in a rabbit model on the efficacy of gentamicin-releasing uretheral catheters showed inhibition of CAUTIs for 5 days, suggesting a role for these devices in short-term catheterization (Cho *et al.*, 2003). The fluoroquinolone hydrophobic antibiotic norfloxacin was evaluated for long-term catheterization using blends of copolymer of ethylene and vinyl acetate (EVA) and polyethylene oxide (PEO) as catheter coatings. A continuous delivery of norfloxacin was obtained up to 30 days with growth inhibition of *E. coli*, *Klebsiella pneumoniae* and *Proteus vulgaris* for 10 days (Park *et al.*, 2003). Bladder catheters impregnated with minocycline and rifampicin were shown to significantly reduce the rate of gram-positive-associated bacteriuria, while similar rates of gram-negative bacteriuria and candiduria were found in the two groups of patients receiving control or MR-coated catheters (Darouiche *et al.*, 1999).

Antimicrobial-coated orthopaedic implants

The most frequent infections affecting orthopaedic implants are those affecting knee and hip replacement despite strict hygienic protocols and intraoperative antibiotic prophylactics. Thus, attempts have been made in the last few decades to prevent and treat orthopaedic implant infections using antibiotic-releasing polymethylmethacrylate bone cements and spacers. To prevent the formation of both gram-positive and gram-negative microbial communities on prosthesis surfaces, antibiotics absorbed on bone cements or spacers should have a broad antibacterial spectrum and, in particular, good activity against the most commonly involved bacterial species, as well as an optimal water solubility to facilitate their release from the polymethylmethacrylate matrix. From the early 1970s, a number of antibiotics, including gentamicin, rifampicin, vancomycin and tobramycin, have been investigated either alone or in combination for their physicochemical features and antimicrobial activity, the most suitable being gentamicin (van de Belt *et al.*, 2001). Neut *et al.* (2009) compared the widely used gentamicin-loaded polymethylmethacrylate beads with a novel biodegradable gentamicin-releasing poly-(trimethylene carbonate), and demonstrated an 80% inhibition of *S. aureus* biofilm formation over at least 14 days (Neut *et al.*, 2009). This is a promising antibiotic delivery system for the local treatment of osteomyelitis.

Antimicrobial-coated CVCs

With regard to CVCs, systematic reviews and meta-analyses of statistically significant clinical trials since 2000 have shown that either antibiotic or chlorhexidine/silver sulphadiazine (CH/SSD)-coated CVCs were anti-infective only for short-term insertion (approximately 5–8 days), with a lack of relevant clinical data for long-term catheterization (Donelli & Francolini, 2001; Walder *et al.*, 2002). The most efficacious antimicrobial-coated CVCs were MR coated, as they exhibited significant activity against numerous gram-positive and gram-negative bacteria, but not against *P. aeruginosa* and *Candida* spp. either *in vitro* or *in vivo* (Sampath *et al.*, 2001).

Since 2003, clinical trials have been performed on the new generation of medicated CVCs in which the antimicrobial agents (MR or CH/SSD) were present on both the external and the luminal surfaces of catheters.

A low rate of colonization by coagulase-negative staphylococci, but a significant increase in *Candida* spp. colonization has been demonstrated in a prospective randomized double-blind controlled multicentre trial in which catheters coated with MR on both sides were implanted for > 3 days in ICU patients (Leon *et al.*, 2004).

Two other large randomized clinical trials on either long-term non-tunnelled (Hanna *et al.*, 2004) or tunnelled (Darouiche *et al.*, 2005) CVCs, both MR impregnated, have shown their efficacy in reducing catheter-related bloodstream infections (CRBSIs) up to a catheterization time of approximately 60 days. A systematic review and meta-analysis by Casey *et al.* (2008) summarized data on the efficacy of currently marketed, medicated catheters in reducing microbial colonization and preventing BSIs. According to this meta-analysis, silver-alloy-coated, silver-impregnated and silver-iontophoretic CVCs were not able to reduce CRBSIs. This lack of effect is presumably due to the poor silver antimicrobial activity against gram-positive bacteria, the most frequently involved species in CVC-related infections.

Rifampicin-miconazole, benzalkonium chloride and chlorhexidine/silver sulphadiazine-treated CVCs were also

taken into consideration by the Casey review. A first clinical trial performed to assess the efficacy of rifampicin-miconazole-impregnated CVCs showed a significantly reduced colonization for a catheterization period of 7.5 days: from 36% of patients with standard CVCs to 5% of patients with rifampicin-miconazole CVCs (Yucel *et al.*, 2004). A more recent cohort study, performed on 184 femoral (73 RM-CVCs vs. 111 standard) and 241 jugular central venous catheters (114 RM-CVCs vs. 127 standard), showed a statistically significant reduction in the incidence of catheter-related bacteremia in patients with short-term catheterization (Lorente *et al.*, 2008).

A small clinical trial (Jaeger *et al.*, 2001), including only 25 patients in each group, failed to show a decrease in the occurrence of colonization or CRBSIs with benzalkonium chloride-coated CVCs.

With regard to CH/SSD CVCs, considering 662 second-generation catheters (having a threefold higher concentration of chlorhexidine, with silver sulphadiazine on the external and chlorhexidine on the intraluminal catheter surfaces) from the three most recent clinical trials (Brun-Buisson *et al.*, 2004; Ostendorf *et al.*, 2005; Rupp *et al.*, 2005), a marked decrease in the colonization of these catheters was evidenced even if no significant reduction of CRBSIs was detected.

According to the systematic review by Gilbert & Harden (2008), MR-coated CVCs were confirmed to be able to significantly reduce BSIs. They also underlined the antibiofilm effect of heparin-coated catheters as well as the lack of efficacy in preventing CRBSIs of both silver-impregnated and CH/SSD CVCs, even if the latter appeared to be able to reduce the risk of colonization.

On the basis of the above data, it is possible to make some remarks and to propose possible strategies to face the most critical issues. One of the main drawbacks of most available antimicrobial-coated devices is the burst release of the adsorbed antibiotics in the first few hours, followed by a long-lasting phase of slow release at very low concentrations. This behaviour can be associated with the development of antimicrobial resistance even if *in vitro* and *in vivo* studies, focused on minocycline and rifampicin, have seemingly ruled out the risk possibly associated with the prolonged use of MR-coated catheters (Munson *et al.*, 2004).

The development of an innovative catheter with long-lasting antibiofilm activity depends on the ability of the catheter constitutive polymer to adsorb large amounts of antibiotic molecules and on their long-term release at relatively constant concentrations. In this regard, our group has developed properly functionalized polymers that are able to adsorb large amounts of antibiotic by introducing into the polymer side chains acidic or basic groups able to interact with different classes of drugs (Donelli *et al.*, 2002; Piozzi *et al.*, 2004).

Looking for alternative antimicrobial agents for device coating/impregnation, and preferably not used in clinics for therapy and poorly soluble in biological fluids, we loaded polyurethanes with usnic acid, demonstrating that this dibenzofurandione adsorbed on polymer surfaces is able to inhibit *S. aureus* biofilm formation (Francolini *et al.*, 2004).

The antimicrobial combination for an ideal antibiofilm catheter should also contain an antifungal drug as it is well known that yeasts, particularly *Candida* spp., account for about 15% of CRBSIs. We therefore performed a combined entrapment in functionalized polyurethanes of fluconazole and albumin, as a pore-forming agent, in order to obtain good and controlled release over time of the antifungal drug, thus inhibiting *C. albicans* growth and biofilm formation on polymeric surfaces for up to 8 days (Donelli *et al.*, 2006).

In fact, to increase and control drug release from the polymer matrices, we experimented with two pore-forming agents at different molecular weights, polyethylene glycol (PEG; molecular weight = 2000, 10 000 and 35 000) and bovine albumin (molecular weight = 69 000), which were incorporated into the polymer bulk together with antibiotic or antifungal molecules, thus obtaining different degrees of impregnation and release. We demonstrated that polyurethanes containing PEG 10 000 + cefamandole + rifampicin were active against a rifampicin-resistant *S. aureus* strain for up to 23 days (Ruggeri *et al.*, 2007).

Given the well-known decreased antibiotic susceptibility of bacteria growing in the sessile mode, we carried out experiments with *S. epidermidis* and *S. aureus* grown as biofilms on untreated or Dispersin B-treated polyurethanes. As Dispersin B is a β-*N*-acetylglucosaminidase able to dissolve the staphylococcal exopolysaccharide matrix (Kaplan *et al.*, 2003), it has been demonstrated that this enzyme is able to promote the antimicrobial and antibiofilm activity of cefamandole nafate (Donelli *et al.*, 2007), sodium dodecyl sulphate (Izano *et al.*, 2007) and triclosan (Darouiche *et al.*, 2009).

Prevention of biofilm formation

Gram-positive and gram-negative bacteria communicate with each other using small diffusible signal molecules called autoinducers. The most common classes of signal molecules are oligopeptides in gram-positive bacteria, *N*-acyl homoserine lactones in gram-negative bacteria and a family of autoinducers known as AI-2 in both gram-negative and gram-positive bacteria.

This communication process among cells, known as quorum sensing (QS), plays a significant role in modulating not only the expression of genes associated with the production of specific enzymes, virulence factors and metabolites but also the development of microbial communities as biofilms. In fact, QS is a regulatory mechanism allowing

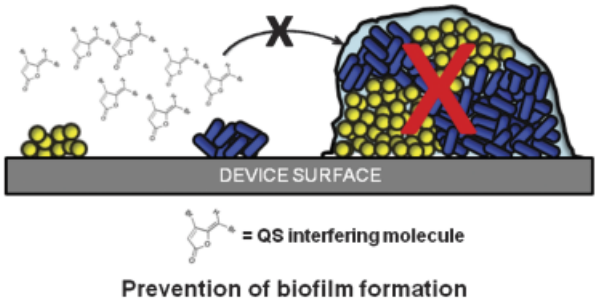

Fig. 3. Prevention of biofilm formation using agents that interfere with QS.

sessile microorganisms to respond to needs that are related to the increasing population density through the expression of specific sets of genes. For recent reviews on QS, see Costerton *et al*. (2007) and Williams (2007).

In many bacterial species, QS has been shown to play a significant role in biofilm survival (Donlan & Costerton, 2002; Parsek & Greenberg, 2005; Kiran *et al*., 2008).

Furthermore, it has been demonstrated recently in *P. aeruginosa* that the Las QS system is involved in the development of antibiotic tolerance, this Las-system-induced tolerance being regulated by the *rpoS* gene (Kayama *et al*., 2009).

According to Rasmussen & Givskov (2006), there are basically three different targets in gram-negative bacteria: (1) the signal generator, (2) the signal molecule and (3) the signal receptor, the latter being the most investigated for application purposes. In fact, QS can be prevented by inhibiting the signal molecule from binding to the receptor via analogues of the signal molecules (Zhang & Dong, 2004; Gonzalez & Keshavan, 2006).

Thus, the use of molecules interfering with QS is a promising strategy to counteract microbial adaptation to the host environment (Fig. 3) and the establishment of infectious processes (Bjarnsholt & Givskov, 2008; Kaufmann *et al*., 2008; Martin *et al*., 2008). In fact, QS inhibitors and antagonists represent the most promising therapeutic tools for the treatment of biofilm-based infections.

Potent inhibitors of gram-negative QS are the halogenated furanone purified from *Delisea pulchra* (Givskov *et al*., 1996) and a series of related synthetic derivatives (Hentzer *et al*., 2003), reported to be efficacious as anti-infective drugs in animal models (Wu *et al*., 2004).

Usnic acid, a naturally occurring dibenzofuran derivative, was demonstrated by our group to be able to affect the morphology (thickness and roughness) of *P. aeruginosa* biofilm without inhibiting bacterial growth, this phenomenon presumably indicating its interference with bacterial signalling pathways (Francolini *et al*., 2004).

In gram-positive bacteria, the QS inhibitor RNAIII-inhibiting peptide (RIP) has been demonstrated to be very efficacious in preventing and treating staphylococcal infections associated with CVCs, orthopaedic implants and ureteral stents.

Using a rat model, Cirioni *et al*. (2006) reported that RIP-coated CVCs significantly reduced bacterial load and enhanced the effect of tested antibiotics in the treatment of CVC-associated *S. aureus* infections. When exposed to RIP, biofilm *S. aureus* cells become as susceptible to antibiotics as planktonic cells (Cirioni *et al*., 2006).

Regarding orthopaedic implants, RIP-loaded polymethylmethacrylate beads were implanted in rats and were demonstrated to be able to prevent *in vivo* methicillin-resistant *S. aureus* (MRSA) biofilm formation either alone or combined with vancomycin, highlighting this QS inhibitor as an alternative or an additional agent to be used for the prevention of orthopaedic infections (Anguita-Alonso *et al*., 2007).

Ureteral stents coated with the QS inhibitor RIP were implanted in rat bladders and shown to inhibit *S. aureus* biofilm formation on the stent surfaces. In addition, stent coating with RIP and teicoplanin increases the antibiotic efficacy in preventing ureteral stent-associated staphylococcal infections (Cirioni *et al*., 2007).

Promoting microbial killing within an established biofilm

Considering the occurrence of multispecies biofilms in device-related infections and the increasing antimicrobial resistance of the microorganisms involved, there is a need for continuous updates in the strategies of microbial killing. In fact, we need to be able to counteract microbial communities inhabited by either gram-positive or gram-negative bacteria as well as fungal species. Thus, the treatment of biofilm-based infections must rely on the combined use of drugs with different antimicrobial spectra and modes of action.

In this regard, Raad *et al*. (2007) demonstrated that linezolid and vancomycin, administered alone, were less effective in decreasing the viability of biofilm-embedded *S. aureus* than daptomycin, minocycline and tigecycline. However, when rifampicin was added to linezolid or vancomycin, an enhancement of their activity in biofilm killing was observed (Raad *et al*., 2007).

Kim *et al*. (2008) showed that combined and sequential treatments with tobramycin and silver enhanced antimicrobial efficacy by $> 200\%$ in *P. aeruginosa*. The authors concluded that the use of combinations of agents that have similar antimicrobial behaviours, but that are not too oxidative, i.e. silver and tobramycin, might be an effective strategy for preventing microbial adaptation and facilitating the antimicrobial action of agents.

Similarly, the combination of silver/ciprofloxacin was demonstrated very recently by our group to have a syner-

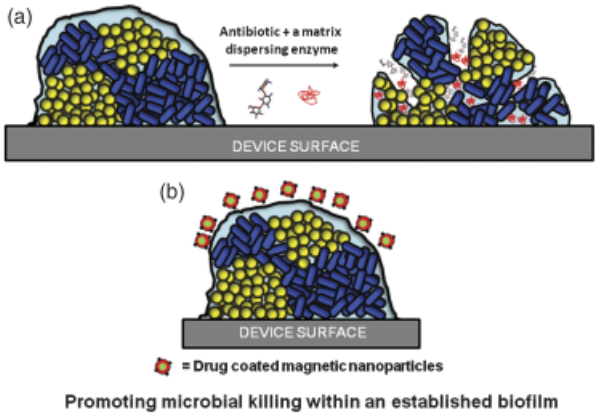

Fig. 4. Promoting microbial killing within an established biofilm using a combination of an antibiotic and a matrix-dispersing enzyme (a) or drug-coated magnetic nanoparticles properly targeted to a localized area in which the drug release is planned to occur (b).

gistic effect in killing *S. epidermidis* growing as biofilms (Donelli *et al.*, 2009; Francolini *et al.*, 2010).

Other combinations of antibiotics and antifungal drugs exhibiting synergistic activity include: (1) aminoglycosides and fosfomycin against *P. aeruginosa* biofilm in a rat model (Cai *et al.*, 2009); (2) anprocide and bacitracin or oxacillin against *S. aureus* and *S. epidermidis* biofilms *in vitro* (Pettit *et al.*, 2009); and (3) amphotericin B, caspofungin or fluconazole in combination with a high-dose doxycycline against *C. albicans* biofilms *in vitro* (Miceli *et al.*, 2009).

Another way to enhance the activity of antibiotics is their use in combination with QS-interfering molecules or biofilm matrix-degrading substances (Fig. 4a). In particular, the combination of furanones, as *P. aeruginosa* QS-inhibiting agents, and tobramycin has been demonstrated to enhance biofilm susceptibility to this antibiotic both *in vitro* and *in vivo* (Hentzer *et al.*, 2003).

The efficacy of *N*-acetyl-cysteine in combination with thiamphenicol in sequential therapy of upper respiratory tract infections sustained by bacterial biofilms has also been demonstrated (Macchi *et al.*, 2006; Serra *et al.*, 2007). More recently, *N*-acetylcysteine, EDTA, ethanol and recombinant human talactoferrin, in combination with fluconazole, amphotericin B, vancomycin and nafcillin, were used successfully as catheter lock solutions to salvage colonized catheters. In fact, these combinations were able to inhibit monomicrobial and polymicrobial biofilms of *S. epidermidis* and *C. albicans* (Venkatesh *et al.*, 2009).

Presumably because of its activity in rapidly dissolving *S. epidermidis* biofilm matrix, Dispersin B was shown to be able to promote the antimicrobial activity of cefamandole nafate against *S. epidermidis* (Donelli *et al.*, 2007) and of sodium dodecyl sulphate against *Aggregatibacter actinomycetemcomitans* (Izano *et al.*, 2007).

In vitro experiments have shown that the application of an appropriate electric current can enhance the activity of some antimicrobial agents against some bacterial species growing as biofilm (Ehrlich *et al.*, 2005). This so-called 'bioelectric effect' on microbial biofilms was confirmed recently by del Pozo *et al.* (2009), who reported a statistically significant effect against MRSA biofilms either when vancomycin plus 2000 μA electric current or daptomycin and erythromycin in combination with 200 or 2000 μA were used.

Also, ultrasound waves combined with gentamycin entrapped in bone cements were able to reduce up to 70% biofilm formation in a rabbit model (Ensing *et al.*, 2005).

Very recently, Di Poto *et al.* (2009) demonstrated a significant inactivation of *S. aureus* cells in biofilm when simultaneously exposed to a photosensitizer drug, the cationic tetra-*N*-methyl-pyridyl-porphine, and to visible light. This strategy, known as photodynamic treatment (PDT), was based on the combined action of visible light and a photosensitizer drug that generates cytotoxic reactive oxygen species and free radicals that are bactericidal. Furthermore, PDT of *S. aureus* biofilms, followed by incubation with 1 × minimum inhibitory concentration of vancomycin resulted in a fivefold decrease in viable bacteria compared with samples only exposed to PDT.

A further novel strategy that represents a promising, but still poorly investigated tool for biofilm eradication from device surfaces and surrounding tissues is represented by the use of nanoparticles able to target antimicrobial agents, alone or possibly in combination with QS-interfering agents or enzymes.

In fact, nanoparticles, either polymeric or inorganic, can be properly targeted to a localized area in which the drug release is planned to occur (Fig. 4b). In this regard, magnetically driven nanoparticles can be easily guided to a specific body area by the application of an appropriate magnetic field (Corchero & Villaverde, 2009; Xie *et al.*, 2009).

Thus far, magnetic nanoparticles have been applied for imaging, delivery and targeting in cancer therapy (Gindy & Prud'homme, 2009) and, very recently, were also demonstrated to be able to lower *in vitro* the OD of *S. epidermidis* broth cultures as well as to promote bacterial death for up to 48 h (Taylor & Webster, 2009).

Our group is now experimenting with the use of magnetic nanoparticles to concentrate antimicrobial agents exclusively in the infected area surrounding the implanted medical device, thus potentiating the activity of the antimicrobial agents against the biofilm. We have developed iron oxide-based magnetic nanoparticles exhibiting antimicrobial activity against both gram-positive and gram-negative bacteria after their coating with a silver-containing polymer and ciprofloxacin as a model of an antimicrobial agent (Francolini & Donelli, 2009).

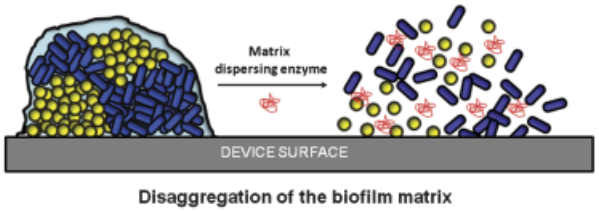

Fig. 5. Disaggregation of the biofilm matrix using an enzyme.

Disaggregation of the biofilm matrix

The use of substances able to destroy the physical integrity of the biofilm matrix is an attractive antibiofilm approach (Fig. 5) as the consequent loss of the highly protective barrier, represented by the exopolysaccharide matrix, exposes sessile microbial cells to antibiotics as well as to the innate host immune defence (Kaplan, 2009).

For staphylococcal species, numerous studies have shown that biofilm-forming strains produce a linear poly-*N*-acetyl-1,6-β-glucosamine that plays a key role in biofilm formation and accumulation. Our group showed that Dispersin B, a soluble β-*N*-acetylglucosaminidase purified from *A. actinomycetemcomitans* by Kaplan *et al.* (2003), was able to prevent staphylococcal biofilm formation (Donelli *et al.*, 2007).

Chaignon *et al.* (2007) tested the susceptibility of five clinical staphylococcal strains associated with orthopaedic infections to different enzymatic treatments, including proteinase K, trypsin, pancreatin and Dispersin B, finding that enzymatic detachment depended on the nature of the biofilm constituents. In fact, the heterogeneity of the biofilm matrix suggests that at least two successive treatments, for example Dispersin B, followed by a protease (proteinase K or trypsin), may be necessary for the entire degradation of staphylococcal biofilms (Chaignon *et al.*, 2007).

Engineered bacteriophages able to express Dispersin B were also successfully tested against *E. coli* biofilms (Lu & Collins, 2007), demonstrating that the use of bacteriophages as antibiofilm weapons can both dissolve the biofilm matrix and kill microbial cells within the biofilm.

Finally, the use of bacteriophages has been reported recently (Fu *et al.*, 2009) as a promising approach in the control of *S. epidermidis* and *P. aeruginosa* biofilm formation when catheters are pretreated with a cocktail of bacteriophages, thus reducing the 48-h mean biofilm cell density by 99.9%, even if few biofilm isolates were reported to be resistant to these phages.

Concluding remarks

In a historical perspective, we should seriously consider the final sentence of the chapter 'Infections caused by intravascular devices used for infusion therapy: pathogenesis, prevention and management' in Maki (1994): 'Future research must strive to better understand the biologic forces governing cutaneous colonization in order to develop more effective strategies to suppress it with new antiseptics that exhibit much higher and more prolonged levels of surface activity; to delineate fully the molecular mechanism of microbial adherence to prosthetic surfaces in order to develop new materials intrinsically resistant to colonization; to design devices that intrinsically deny microbial assess, to identify new technology to allow rapid detection of contamination of infusate, device colonization, and BSI; and to devise more cost-effective programmes for care of intravascular devices'.

In fact, even just 15 years later, although great advances have been made from both the scientific and the technological points of view, most of the targets listed by Maki remain unreached, even though an enormous number of papers have been published in the last decade on these critical issues. Producing medical devices that are refractory to microbial colonization and biofilm formation remains an uphill task and it is necessary to establish closer collaborations between scientists working in universities or research institutes and industrial investigators to hasten achievement of the above objectives and find more advanced solutions to prevent medical device-related infections.

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