Nanotechnology applied to medical biofilms control

C. Sousa, C. Botelho and R. Oliveira*

IBB-Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal
*To whom correspondence should be addressed: roliveira@deb.uminho.pt

Nanoscience and Nanotechnology (N&N) are new approaches to research phenomena at atomic, molecular and macromolecular scales, where properties differ significantly from those at a larger scale. This new approach can be applied to microbial biofilms, which are formed when bacterial and/or yeast cells adhere to abiotic and biotic surfaces. It is well known that microorganisms in biofilms have a different behaviour from their planktonic counterparts, demonstrating a general recalcitrance to medical therapy. Therefore, biofilm-associated infections on indwelling medical devices, such as catheters and prostheses, may persist even after suspension of antibiotic therapy and hence may require the removal of the device. In order to reduce patient`s morbidity and mortality, as well as high economical costs associated to medical biofilms, several attempts have been made to develop novel mechanisms of biofilm prevention and/or elimination. In this mini-review, the current knowledge on the features of biofilm formation and their relevance to medical device-associated infections are enclosed, as well as the new anti-biofilm approaches based on nanotechnology.

Keywords nanotechnology; biofilm control; biomedical device infection

Introduction

A microbial infection either from bacteria and/or yeast can lead to high morbitidy and even mortality of patients worldwide. Microbial adhesion to medical devices surfaces is considered the base of the pathogenic mechanism, so a continuous concern to the medical community is the high rate of infection of biomaterials which are inserted into or in contact with the human body. As an example on the orthopaedic field, the percentage of implant failure due to infection is approximately 1.5–2.5% of all implants (3000–6000 incidents per year) (Teterycz *et al*., 2010). The failure of such devices relies on the ability of microorganisms to adhere to submerged surfaces and produce extracellular substances that facilitate adhesion and provide a structural matrix, forming multicellular communities - the biofilms (Cotter *et al*., 2011). The main problem is that biofilms are extremely resistant to host defence mechanisms and antibiotic treatment, comparing to their planktonic analogues, making biofilm-related infections a major cause of morbidity and mortality. Frequently, the only solution to solve this problem is to surgically remove the infected implant which bears additional morbidity to patient as well high economic costs. In order to control biofilm formation on medical devices and all costs associated, a large number of new strategies and approaches have been developed in the last few years, including: antimicrobial locks (in the case of catheters) (Bookstaver *et al*. 2009); surface modification of biomaterials with antimicrobial coatings (Knetsch and Koole, 2011); the use of quorum sensing (QS) inhibitors (Lönn-Stensrud *et al*., 2009); antimicrobial peptides as a new class of antibiotics (Batoni *et al*., 2011); enzymes that dissolve biofilms (Donelli *et al*., 2007), nitric oxide (Regev-Shoshani *et al*., 2010), electrical (Del Pozo *et al*., 2009) or ultrasound (Hazan *et al*., 2006) enhancement of antimicrobial activity, or even the application of light activated antimicrobial agents (Perni *et al*., 2009) Nevertheless, nanoscale materials have recently appeared as one of the most promising strategies to control biofilm infections related to indwelling medical devices, especially due to their high surface area to volume ratio and unique chemical and physical properties (Rai *et al*., 2009). A nanomaterial has a diameter ranging from1 and 100 nm, and they can be made from different materials, like copper, zinc, titanium, magnesium, gold, alginate and silver. The use of silver nanoparticles (NPs) is now considered as one of the most promising strategies to combat biofilm infections related to indwelling medical devices (Gong *et al*., 2007). Drug delivery nanocarriers systems, such as liposomes (Tamilvanan *et al*., 2008) and polymer-based (Martinelli *et al*., 2011) carriers have also arisen as appealing methods with a great potential in the treatment of biofilm infections, due to several factors especially good biocompatibility and ample range and extent of drugs that they can carry. Another important factor is the protection provided by the encapsulation of the drug in the biological milieu, decreasing toxicity and allowing the drug to reach the specific site. On the case of bacterial biofims, the use of bacteriophages is another nanostrategy studied for the prevention and control of clinical biofilms, especially because phages have a very strong bactericidal activity and specificity; they can also replicate at the site of infection, being available in abundance where they are most required (Azeredo and Sutherland, 2008). These characteristics put forward the potential of applying phages, especially phage cocktails, to the surfaces of indwelling medical devices, in order to reduce biofilm formation by clinically relevant organisms.
Therefore, this review highlights the current knowledge about biofilms as well as the nanotechnologies in use or in development to prevent or decrease the risk of biomedical devices infections due to colonization and/or biofilm formation on their surfaces.

1. Biofilms

Microorganisms have primarily been characterized as unicellular life forms, living as planktonic, freely-suspended cells and it is undeniable the importance of the use of microbial pure cultures growing in liquid medium in the understanding of microbial pathogenesis and physiology (Davey and O'Toole, 2000). However, recent advances in microscopy and molecular technologies have made possible the direct observation of a wide variety of natural habitats, establishing that the majority of microorganisms persist attached to surfaces within a structured biofilm ecosystem and not as free-floating organisms (Costerton *et al.*, 1995).

From an historical point of view, the discovery of microbial biofilms can be attributed to Antonie van Leeuwenhoek, who first observed microorganisms in the plaque on his own teeth, using his simple microscope, in the 17th century. Later, in the 20th century, Heukelekian and Heller (1940) and Zobell (1943) showed that bacterial growth and activity were considerably enhanced by the presence of a surface to which bacteria could attach and that the number of microorganisms on surfaces was significantly higher than in the surrounding medium. The study of Characklis (1973), about microbial slimes in industrial water systems, revealed their high cohesiveness as well as their strong resistance to disinfectants, but it was Costerton *et al.*, in 1978, which postulated the general theory of biofilm predominance.

In natural world, more than 99% of all microorganisms exist as biofilms (Costerton *et al.*, 1987) and therefore this ubiquity among diverse ecosystems suggests a strong survival and/or selective advantage for sessile cells over their planktonic counterparts. This advantage arises from the fact that microorganisms attached to a surface are in a most favourable environment in terms of nutrients availability, metabolic cooperativity and protection against external factors (Davey and O'Toole, 2000). It is therefore well established that sessile cells are physiologically distinct from microorganisms growing in planktonic state (Hall-Stoodley *et al.*, 2004; Dufrêne, 2008) and that biofilms exhibited a distinct phenotype from their free-floating counterparts. The main phenotypic alterations are related to gene transcription, growth rate, respiration rate, rate of oxygen uptake, electron transport activity, synthesis of extracellular polymers, substrate uptake rates, rate of substrate breakdown, heat production and ability to resist to antimicrobial treatments (Donlan, 2002; Wilson, 2001; Růzicka *et al*., 2007). In fact, it is estimated that sessile bacteria within biofilms are up to 1,000-fold more resistant to antibiotics and to the host immune defence system than their planktonic counterparts (Ceri *et al.*, 1999). However, cells growing in biofilms are not only physiologically distinct from planktonic cells, but also differ from each other, both spatially and temporally, as biofilm development proceeds. The metabolic activities of the cells within a biofilm, together with diffusion processes, have as outcome gradients of nutrients concentration, signalling molecules and microbial waste. Thus, microorganisms respond to these gradients, adapting to the local chemical conditions, which can modify over time as biofilms develop and, as a result, biofilms exhibit considerable heterogeneity (Stewart and Franklin, 2008). These factors need to be taken in consideration on applying antimicrobial therapy. Therefore, a biofilm can be defined as a community of microorganisms that is irreversibly attached to a biotic or abiotic surface and that is enclosed in a matrix of exopolymeric products (Costerton *et al.*, 1999; Prakash *et al.*, 2003; Blankenship and Mitchell, 2006).

1.1 Biofilm formation

Biofilm formation is a step-wise process involving two main distinct phases: primary attachment of the microorganisms to the surface, and the formation of multi-layered cell clusters with cell-to-cell adhesion depending on the production of an extracellular "slimy" matrix (O'Toole *et al.*, 2000; Blankenship and Mitchell, 2006; Finkel and Mitchell, 2011). In the case of bacteria, on the first stage of colonization, the cell approaches the surface so closely that its motility is slowed forming a transient association with the surface. The solid-liquid interface between a surface and an aqueous medium provides an ideal environment for the attachment and growth of microorganisms (Costerton *et al.*, 1999). This initial, reversible microbial adherence is mostly dependent on bacterial cell surface characteristics and on the nature of the material surface (von Eiff *et al.*, 2002). However it is mainly due to the physicochemical interactions that bacteria firmly adhere to the biomaterial surface during the adhesion process (Oliveira *et al.*, 2003). These comprise Lifshitz-van der Waals forces, Lewis acid-base, and electrostatic forces. The presence of bacterial surface-associated proteins it is also associated with cell surface hydrophobicity and initial adhesion (von Eiff *et al.*, 2002). However, in natural environments, bacteria mostly adhere to the layer of adsorbed molecules that coats the surface, the so called "conditioning film", and not directly to the substratum. In this case, attachment is mainly dependent of specific interactions between bacterial adhesins called MSCRAMMs (*i.e.,* microbial surface components recognizing adhesive matrix molecules) and their complementary receptors present on molecules on the substratum surface (Whittaker *et al.*, 1996). After the initial adhesion to the foreign body surface, the bacteria multiply forming microcolonies and accumulate as multilayered cell clusters, a step that involves intercellular adhesion and the synthesis of extracellular matrix molecules, such as proteins and polysaccharides. Chemical signals that communicate via QS mechanisms start to being released, among the bacterial cells, activating genetic mechanisms responsible for exopolysaccharide production (Costerton *et al.*, 1999). Therefore, further growth of the attached microorganisms occurs, leading to the formation of dense bacterial aggregates embedded in the exopolymeric matrix, typical of mature biofilms. Mature biofilms can then undergo a detachment process, due to the exposure to strong mechanical and hydrodynamic forces and to QS regulation,

releasing planktonic bacteria that can then colonize another region of the substratum to form new microcolonies (Karatan and Watnick, 2009). Bacterial biofilms and their role in disease have been studied extensively, while only recently there has been a particularly focus on the study of the yeast biofilm formation and its importance. It is known that the adhesion is the first step for the yeast biofilm formation, being clear that cell–substrate, cell–cell interactions, extracellular matrix production and hyphal differentiation are key steps in biofilm development (Blankenship and Mitchell, 2006). In fact, the ability of yeasts to form hyphae or pseudohyphae is key factor, which does not occur on bacterial biofilms, allowing them to form biofilms with different structures, from very loose to very dense biofilms. It is also known that the adhesion and biofilm formation is also depend on the material to which the yeasts are adhering (Hawser and Douglas, 1994) as well as the medium where they are grown; *e.g.*, a yeast biofilm formed on a high glucose solution is different from a biofilm formed in saliva (Nikawa, *et al*., 1996; Chandra, *et al*., 2001).

1.2 Biofilm structure

The application of advanced microscopy, such as confocal laser scanning microscopy, molecular and electrochemical high-resolution methods has provided insights into the structural organization and function of biofilm communities. Therefore, a mature biofilm is seen as a very heterogeneous arrangement, with a basic community structure consisting of microcolonies of microorganisms encased in an extracellular polymeric substance (EPS) matrix separated by water channels. Nevertheless, although some structural attributes can generally be considered universal, every microbial biofilm community is unique (Flemming and Wingender, 2010). This is due to the fact that a biofilm structure can be influenced by several conditions, such as surface and interface properties, nutrient availability, the composition of the microbial community, the three-dimensional architecture of the matrix (the dense areas, pores and channels), and hydrodynamics, making the exact structure of any biofilm probably a sole feature of the environment in which it develops. A porous architecture, *e.g*., allows a convectional flow through the depth of the biofilm while within the EPS matrix, only diffusional transport is possible. Therefore, organisms at the bottom of the biofilm can access nutrients without competing with those at the interface to the bulk water phase. Strong gradients can occur in biofilms, caused, for instance, by actively respiring aerobic heterotrophic organisms, which consume oxygen faster than it can diffuse through the matrix. This generates anaerobic habitats just below highly active aerobic colonies in distances of less than 50 μm. Other gradients, such as pH-value, redox potential and ionic strength are known within biofilms (Stewart and Franklin, 2008)

Hence, the structure of a biofilm can range from a smooth and dense biofilm model (Wimpenny and Colasanti, 1997), to a heterogeneous mosaic model (Keevil and Walker, 1992) or to one consisting of a more complex organization involving mushroom-like aggregates separated by water channels, normally considered the most typical biofilm architecture (Costerton *et al*., 1994). This structure is characteristic of biofilms formed under low nutrient concentration, high hydrodynamic shear stress and the absence of mechanical, abrasive and compressive forces.

Concerning the biofilm composition itself, water is considered to be the major component of the biofilm matrix - up to 90% of total volume, being essential as medium for efficient transport of nutrients to microorganisms and also for cell membrane integrity of microbial cells. Microorganisms occupy only between 10% (in most biofilms) and 50% of the total volume of the biofilm, whereas EPS can account for over 90% of the total organic carbon of biofilms (Flemming and Wingender, 2010). Besides polysaccharides, proteins, or phospholipids, non cellular materials such as mineral crystals, corrosion particles or blood components, depending on the environment in which the biofilm has developed, may also be found in the biofilm matrix (Donlan, 2002). The matrix is also reservoir of genetic material, namely extracellular DNA, which is now acknowledged as a considerable proportion of the EPS components (Decho *et al*., 2005). The water channels that separate the matrix-enclosed microcolonies are vital to biofilm maintenance, providing a nutrient flow system within it that delivers nutrients deep within the complex community and allows the exchange of metabolic products with the bulk fluid layer (Donlan and Costerton, 2002). The hydrodynamic flow of liquid over and through the biofilm can also promote the separation of some fragments with viable organisms away from the surface, which can be carried with the flow and deposited elsewhere for further colonization (Flemming, 2011). Besides protection, EPS matrix immobilizes biofilm cells and keeps them in close proximity, thus allowing cells to exchange information and the formation of synergistic microconsortia by QS molecules - chemical signals, used to regulate cell density-dependent gene expression (Flemming, 2011). Therefore, this complex level of structural organization helps to explain the remarkable metabolic efficiency of microbial biofilms.

1.3 Biofilms and medical device associated infections

As already mentioned, microbial adhesion and biofilm formation onto biomedical devices surfaces are considered the essential pathogenic mechanisms of implant infections. During the past 20 years it has been reported that between 6 and 14% of patients that enter general hospitals develop a nosocomial infection (Vazquez-Aragon *et al.*, 2003), *i.e.*, an infection that was not present or incubating at the moment of patient admission at a hospital. Overall, a large percentage of biofilm-related infections are associated with indwelling medical devices: about 1 million cases - an estimated 60% of nosocomial infections are due to biofilms that have formed on indwelling devices (Darouiche, 2004). Taking into account the aging population and the increasing number of implantable medical devices available, it is expected that the

number of infections associated with biofilms will increase. Medical implants that are more prone to biofilm formation include: artificial voice prostheses, replacement joints, prosthetic heart valves, cardiac pacemakers, cerebrospinal fluid shunts, endotracheal tubes, urinary catheters, peritoneal dialysis catheters, central venous catheters (CVCs), contact lenses, dental implants and implanted prosthetic devices for erectile dysfunction (Hall-Stoodley *et al*., 2004; von Eiff *et al.*, 2005). For instance, infection associated with CVCs is a major cause of bacteraemia in hospitalized patients and its main source of microbial contamination is the skin insertion site, from where bacteria can migrate down the intracutaneous tract on the external surface of the catheter leading to subsequent colonization of the catheter or sepsis (Hanna *et al*., 2006). The failure of such devices relies on the ability of microorganisms to form biofilms on their surfaces, which are extremely resistant to host defence mechanisms and antibiotic treatment (Donlan and Costerton, 2002). When a biofilm infection occurs, the host establishes an immune response to antigens released from the biofilm. However, not only does the host immune response fails to eradicate the biofilm, but it may also result in damage to surrounding tissues (O'Gara and Humphreys, 2001). The high resistance of biofilm cells can arise from factors such as phenotypic changes that result in resistance within the biofilm environment, inactivation of the antibiotics by extracellular polymers or modifying enzymes and nutrient limitation resulting in slowed growth rate (Fernández *et al*., 2011).

The main microorganisms responsible for biofilm formation on indwelling medical devices are: Gram-positive (*Enterococcus faecalis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus viridans*) and Gram-negative (*Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*) bacteria, as well as yeasts (Davey and O'Toole, 2000). *Candida* species are in fact emerging as important nosocomial pathogens, and approximately 80% of patients with candidemia possess a CVC, a fact which highlights the importance of yeasts from *Candida* spp., as common causes of CVC related infections (Ben-Ami *et al*., 2008). It is also important to emphasize that the colonizing biofilms can be mixed biofilms containing yeasts and bacterial species and, therefore, extensive interactions between the prokaryotic and eukaryotic cells can occur.

The sources of contamination of the medical devices by these organisms can be the skin of patients or healthcare workers, tap water to which entry ports are exposed or other sources in the environment (Donlan, 2001) All these facts make biofilm-related infections a major cause of morbidity and mortality and frequently the only solution to an infected implanted device is its surgical removal which bears additional economic and health costs (Cotter *et al*., 2011).

1.4 Control of biofilm biomaterial infections

The treatment of medical devices associated infections is usually performed using conventional antimicrobial agents which are based on standardized antimicrobial susceptibility test results, and are often revealed to be unsuccessful, requiring the removal of the implant. The problem related to these standardized antimicrobial susceptibility tests is that they are usually performed with planktonic cells, while biofilm cells are much less susceptible to be killed by antimicrobial agents (Nadell *et al*., 2009). In order to try to control microbial adhesion, biofilm formation and the consequent medical device infection, several attempts, including strict hygienic rules during implantation of medical devices as well as antibiotic prophylaxis in the course of introduction of implants have been applied. Nevertheless, the prophylactic use of antibiotics should be under strict control and even discouraged due to the possibility of antibiotic-resistant microorganisms to emerge. In order to control medical biofilm infections, new strategies and approaches have been developed in the last years. New materials which could be resistant to microbial adherence and colonization have been tested (von Eiff *et al.*, 2005; Wilson, 2001) and modifications of biomaterial surfaces have also been studied (Poortinga *et al.*, 2002; Price *et al.*, 2005; Camargo *et al*., 2009). Surface modification of biomedical devices generally requires a complete modification of the surface, mostly with hydrophilic polymeric surface coatings, in order to achieve a non-biofilm forming surface (Knetsch and Koole, 2011). Despite the high number of research related to biomedical devices surfaces modification, not many of these studies pass to the next step, the clinical tests. Chemical modifications including the incorporation of antimicrobial agents into polymers (antibiotics, antiseptics, metals), physical modifications or production of very smooth surfaces, have been performed, although none has been totally successful (Fux *et al.*, 2005; Kumon *et al.*, 2001; von Eiff *et al.*, 2005), especially concerning long-term activity results (Bayston *et al*., 2009). It must be noted that all steps in the pathogenesis of biofilm formation may act as possible targets for the development of strategies to eliminate or prevent its formation. Besides surface modification, other approaches have been investigated, namely, the use of antimicrobial peptides with therapeutic potential (Piper *et al*., 2009), QS interfering molecules (Lönn-Stensrud *et al*., 2009), enzymes targeting the EPS (Donelli *et al*., 2007), nitric oxide (Regev-Shoshani *et al*., 2010), electrical (Del Pozo *et al*., 2009) or ultrasound (Hazan *et al*., 2006) enhancement of antimicrobial activity, and the use of light-activated antimicrobial agents (Perni *et al*., 2009), among others. Nevertheless, it has been extremely difficult to achieve a completely anti-adhesive material mainly due to thermodynamical aspects and to the fact that, *in vivo*, any material surface is rapidly covered by plasma and matrix proteins toward which bacteria display specific adhesins (Arciola *et al.*, 2005). Therefore, despite the great efforts in implantation techniques, material improvements and antimicrobial therapies, biofilm associated infections are still difficult to eradicate. It is in this context that nanotechnologies have arisen has one of the most promising strategies to control medical biofilms.

2. Nanotechnology

I want to build a billion tiny factories, models of each other, which are manufacturing simultaneously. . . The principles of physics, as far as I can see, do not speak against the possibility of maneuvering things atom by atom. It is not an attempt to violate any laws; it is something, in principle, that can be done; but in practice, it has not been done because we are too big. — Richard Feynman, Nobel Prize winner in physics. Therefore, taking advantage of this new field, nanotechnology can be applied in several areas from medicine to security.

The first description of nanotechnology is from the scientist who envisioned this area, Richard Feynman, in 1959, and it can be defined as the engineering of functional systems at the molecular scale. In 2005, the European Commission Communication defined an action plan for Europe, where it was clarified that *Nanosciences and Nanotechnologies (N&N) are new approaches to research and development that concern the study of phenomena and manipulation of materials at atomic, molecular and macromolecular scales, where properties differ significantly from those at a larger scale.* Furthermore, nanoscience and nanotechnology can be applied into several fields including medicine, materials science and security. On the case of medical applications, it can go from the development of biosensors to the improvement of biomaterials with increased biocompability or even with antimicrobial properties. Although, of all advantages of nanotechnology, several concerns have been raised, namely the high surface reactivity and the ability of nanoparticles to cross cell membranes that in some cases can be beneficial, like in cancer treatment, but it may also have negative health and environmental impacts. This type of technology can also be employed to study microbial cells at the nanoscale level, using *e.g.* atomic force microscopy (AFM), which allows the study *in situ* of membrane proteins and live cells at high resolution. It is also possible to apply this technique to determine, for instance, adhesion forces, or to map surface properties and receptor sites on cells, as well to measure cellular interactions at the single-cell and single-molecule levels (Dufrêne, 2008). As mentioned previously, the single cell interaction is the first step for the adhesion and biofilm formation and, therefore, using this approach, it is possible to analyse step by step the formation of a biofilm and understand the initial phenomenon.

Concerning biomedical biofilms, nanotechnology is emerging as one of the most auspicious methodologies for its prevention and control. The main nano approaches that have demonstrated the most promising results include: silver nanoparticles, drug delivery nanocarriers or phage therapy.

2.1 Silver nanoparticles

Antimicrobial activity of silver, copper and other metal ions is well known and, of all the elements, silver has been described as the one with the highest levels of toxicity for microorganisms and the lowest toxicity for animal cells (Guggenbichler *et al*., 1999). In fact, this metal has a broad antimicrobial activity spectrum against both Gram-positive, and Gram-negative bacteria (Elliott, 2007), as well as yeasts (Saulou *et al*., 2010; Despax *et al*., 2011). On the case of bacteria it is known that inhibits replication by binding to the microbial DNA and it also switches off important enzymes, leading to microbial death (Chaiyakunapruk *et al*., 2002).

The nanoscale materials have recently appeared as new antimicrobial agents due to their high surface area to volume ratio and unique chemical and physical properties (Morones *et al*., 2005; Kim *et al*., 2007; Rai *et al*., 2009). Silver nanoparticles (NPs), which are clusters of silver atoms (Chaloupka *et al*., 2010), exhibit very strong bactericidal activity against both Gram-positive and Gram-negative bacteria, including multiresistant strains (Morones *et al*., 2005; Panacek *et al*., 2006). There are few studies regarding the mechanism behind the ability of silver NPs or nanocoatings to control yeast biofilm. According to the literature, the silver NPs have affinity for proteinaceous compounds, where they combine with SH groups inducing protein denaturation and corresponding enzyme inactivation (Gordon *et al*., 2010; Liau *et al*., 1997; Feng *et al*., 2000). On a model for *Saccharomyces cerevisiae* it was demonstrated that a nanosilver based treatment induces the formation of clusters at the cell wall periphery composed by silver and sulphur, with significant levels of phosphorus, showing a specific reactivity of silver species to phosphorus-containing compounds (Despax *et al*., 2011). Naik *et al*. (2002) suggested that the carbonyl groups from amino acid residues of proteins are able to bind to metal and, additionally, Ag^{+} ions interact with amide groups with a preferential binding to amide carbonyl oxygen (Ng *et al*., 2004). This may cause disarrangement on the secondary structures of proteins, indicating the presence of inactive conformations (Saulou *et al*., 2010).

Given the increasing microbial resistance and consequent development of resistant strains to traditional antimicrobial agents, silver NPs or nanocoatings constitute nowadays an important antimicrobial agent with numerous applications in medicine (Gong *et al*., 2007; Chaloupka *et al*., 2010). Indeed, silver NPs have not been shown to cause bacterial resistance, which is presumably due to the fact that, unlike antibiotics, silver NPs do not exert their antibacterial effects only in a particular site but at several degrees such as bacterial wall, proteosynthesis and DNA (Morones *et al*. 2005; Gogoi *et al*. 2006; Shrivastava *et al*. 2007). These nanosilver coatings exert their antimicrobial properties *in vivo* by slowly releasing Ag^{+} ions (Furno *et al*. 2004; Roe *et al*. 2008). The considerable surface-to-volume ratio of the NPs enables a constant local supply of Ag^{+} ions at the coating-tissue interface and also allows an improved contact with the

microorganisms (Chen and Schluesener, 2008; Rai *et al*., 2009). As a result, prevention of microorganisms adhesion and biofilm formation is more prolonged than in other antimicrobial approaches involving silver ions or metallic silver impregnation. Thus, the advantage of impregnation of medical devices with silver NPs is that it protects both outer and inner surfaces of devices and there is continuous release of silver ions providing antimicrobial activity (Wilcox *et al*., 1998; Darouiche *et al*., 1999).

Nanosilver, as particles, coating, or even impregnated on the medical device are thus emerging as a next-generation of antimicrobial agents (Chen and Schluesener, 2008). Although, some studies have raised some concerns regarding silver NPs biosafety (Johnston *et al*., 2010), there are studies demonstrating the efficacy of silver NPs in reducing or preventing biofilm formation on catheter-materials both *in vitro* (Samuel and Guggenbichler, 2004) and in animal models (Roe *et al*., 2008, Hsu *et al*., 2010). Studies with patients are still scarce.

2.2 Novel drug delivery carrier systems

Even though the efficacy of nanocoatings and silver NPs to prevent biofilm formation on catheters, there is an important limitation: the ability of the material to adsorb always the same concentration of the drug and also the ability to control their release, which in most cases results in a non-controlled elution of the drug in the first hours subsequent to the insertion (Crisante *et al*., 2009). Drug delivery has been a subject of intense studies over the recent years. The objective is to accomplish sustained (or slow) and /or controlled drug release and therefore improve efficacy, safety, and/or patient comfort (Varshosaz, 2007).

These new drug delivery carriers can be considered as a way to prevent the colonization and biofilm formation, and the most exploited ones for elimination of microbial biofilms on biomedical devices are lipid- and polymer-based carrier systems (Tamilvanan *et al*., 2008).

2.2.1 Liposomes as drug carriers

In 1961, Alec D Bangham firstly described the liposomes at the Babraham Institute, in Cambridge. Liposomes are artificially prepared vesicles made of lipid bilayer that can be used as drug carriers. They are appealing drug carrier systems, especially against colonizing microorganisms, due to several factors such as: good biocompatibility; they are able to carry drugs with very different characteristics (from hydrophobic to hydrophilic drugs, *e.g*); the encapsulation of the drug protects it from the biological milieu; and also allows the drug to be transported to a specific target site (Vyas *et al*., 2007; Tamilvanan *et al*., 2008). Several studies have been performed concerning the interaction between liposomes and bacterial biofilms (Jones, 2005; Kim and Jones, 2004). Halwani *et al*., (2008) showed that this new strategy can be used to deliver two agents at the same time in order to prevent *P. aeruginosa* biofilm formation and resistance *in vitro*. DiTizio et al. (1998) developed a strategy of ciprofloxacin delivery consisting of a liposomal hydrogel that reduced bacterial adhesion to silicone catheter in a rat model of persistent *P. aeruginosa* peritonitis. This technique opened new perspectives for the development of new antimicrobial peritoneal dialysis catheters or as well other types of catheters (Finelli *et al*., 2002). Buckler *et al*. (2008) reported that liposomal antifungal lock therapy can be considered as a possible alternative to catheter removal. In fact, this therapy was previously tested with success in an animal model of *C. albicans* biofilm-associated CVC infection (Schinabeck *et al*., 2004). A different study using liposomal amphotericin B (LAMB) at the minimal inhibitory concentration in a catheter continuous flow model for *Candida* showed that after 24-h treatment with LAMB, the growth of hyphae diminished in 20% in comparison with the traditional antifungal therapy; additionally, the thickness of extracellular matrix was imperceptible (Seidler *et al*., 2010). In 2008, eleven liposomal delivery systems were approved for clinical used, being two of them against fungi (Abelcet and Ambisone). So, the use of liposomes as drug carriers seems to be advantageous over other therapies used to prevent biofilm formation on biomedical surfaces. Liposomes can target matrix or biofilm by specific attachment, allowing the drug to be released in the vicinity of the microorganisms, although in the case of yeast cells adhesion to human cells there is the need to deepen the knowledge regarding the ability of this systems to prevent the adhesion but not to affect adhered cells. So, this nanotechnology is indeed a promising research area, but it requires more studies to fully understand the mechanism behind the antimicrobial activity.

2.2.2 Polymer carriers

For the past years, the use of biocompatible or biodegradable polymers has gained importance on the medical field. Polymer carriers from both natural and synthetic origins, have several advantages over other types of drug nanocarriers, such as the liposomes which can be taken up by macrophages, and accumulate in the liver and spleen, even when the liposomes are coated with polyethyleneglycol (PEG) (Henry, 2002). Therefore, the use of polymers as drug carriers for antimicrobial agents, associated to implants infections, can be an extremely valuable alternative. Among these products, polymeric microspheres, polymer micelles, and hydrogel-type materials have been shown to be effective nanocarriers in enhancing drug targeting specificity, lowering systemic drug toxicity, improving treatment absorption rates, and providing protection for the pharmaceuticals against biochemical degradation (Şanlı *et al*., 2008). These polymer drug delivery systems are based on "nanocarriers" which are formed by mixing polymeric chemical compounds with drugs,

forming complex and large molecules, which "carry" the drug across physiological barriers. Poly(rhylene-glycol)-poly(alpha, beta-asparic acid), carboxylates, and heterobifunctional polyethylene glycol, constitute examples of these polymeric compounds (Varshosaz, 2007). Another advantage of this system is the possibility to add a pore forming polymer, which will increase the amount of drug loaded on the carrier, as described by Ruggeri *et al*. (2007), which developed an antimicrobial polyurethane system containing two antibiotics, cefamandole nafate and rifampin and PEG as a pore forming polymer. Recently, Crisante *et al*. (2009) developed a nanostructured polymer system for antibiotic delivery using bovine serum albumin or polyallylamine as pore forming substances. Their results were corroborated by the work of Martinelli *et al*. (2011), who hypothesize that this system possesses the suitable features for the manufacturing of different types of antimicrobial medical devices, including intravascular catheters. However, studies *in vivo* are still required to test the efficacy of this antibiotic delivery carrier system. De Prijck *et al*. (2010) showed that polydimethyl siloxane (PDMS) can be mixed with different antifungal agents to induce reduction on *Candida* biofilm formation. Using a similar strategy described for bacterial biofilms, Donelli *et al*. (2006) used polyurethane loaded with fluconazole and pore-former agents in order to promote the release of the antifungal drug. The entrapment of a antifungal drug in the polymer of PEG as a pore former significantly improved fluconazole release, while the use of polymer with a higher molecular weight, as porogen albumin resulted in a tighter controlled drug release with an improved antifungal activity over time. In order to diminish *Candida*-associated denture stomatitis, which is a serious clinical problem, a similar nanotechnological approach was tested: antifungal drugs, such as miconazole, were incorporated in polymethacrylic acid (PMAA) which can be covalently bound onto diurethane dimethacrylate, a denture resin. This system was very effective, as the PMAA-resin discs drug-containing were sustained for a prolonged period of time (weeks and months) (Cao *et al*., 2010).The combination of antimicrobial drugs in polymers with pore formers leads to a controlled release over time, which is a promising approach in the development of medical devices.

2.3 Phage therapy

The use of bacteriophages (phages) has been another strategy studied for the prevention and control of biomedical biofilms. Since they were first recognized, early in the 20th century (Twort, 1915; d`Herelle, 1917), phages have been the focus of significant attention. Considering the increasing apprehension with antimicrobial resistance in hospitals worldwide nowadays (Kaplan *et al*. 1988; Tenover, 2001; Sieradzki *et al*. 2003), there is a renewed interest in phage therapy. Phage therapy has been successfully used to treat experimental infections, including bloodstream infections and meningitis, in poultry and animals (Smith *et al*., 1987; Soothill, 1992; Barrow *et al*., 1998), and antimicrobial-resistant infectious diseases in humans such as dysentery, skin infections, lung infections, meningitis, wound infections and osteomyelitis, caused by a range of organisms including *Staphylococcus*, *Streptococcus*, *Escherichia coli*, *P. aeruginosa*, *Shigella* and *Salmonella* (Cislo *et al*. 1987; Weber-Dabrowska *et al*. 2000; Sulakvelidze *et al*. 2001; Weber-Dabrowska *et al*. 2001; Wood *et al*. 2001). The use of phages to control infections caused by biofilms has advantages over treatment with other conventional antimicrobial agents, namely by the fact that phages have a very strong bactericidal activity and can replicate at the site of infection (Smith and Huggins, 1982). Doolittle and colleagues described, in 1996, a progeny phage which propagates radially throughout a biofilm, suggesting that a single dose of phage could treat a biofilm infection. The progeny phage infects adjacent cells and degrades the biofilm matrix (Doolittle et al., 1996). Besides, it was demonstrated that some phages are able to produce enzymes (depolymerises) that hydrolyse and degrade EPS matrix of a biofilm (Sutherland, 1967; Hughes *et al*., 1998; Hanlon *et al*., 2001; Deveau *et al*., 2002; Kimura and Itoh, 2003). Curtin and Donlan (2006) demonstrated, using an *in vitro* model system, that a phage active against *S. epidermidis* could be incorporated into a hydrogel coating on a catheter and significantly reduce biofilm formation on its surfaces. Recently, Fu *et al*. (2010) studied, *in vitro*, the effect of pre-treating hydrogel-coated catheters with *P. aeruginosa* phages on biofilm formation, and observed a significant reduction in the number of biofilm cells. This shows that the combination of two nanotechnological approaches can improve the final outcome: the control of biofilm formation on indwelling devices. However, despite these results, some aspects still must be considered prior to the use of phage therapy in humans, such as, bacterial resistance to phage, inactivation by the patient's immune system, impure phage preparations that could contain endotoxins or phage-encoded virulence genes that can incorporate into the host bacterial genome (Donlan, 2009). The use of phage mixtures or engineered phages can provide successful strategies to overcome these problems.

Conclusions

Medical biofilms still pose as a critical issue for the clinical community, as most of the traditional therapies are not effective, due to the recalcitrant cells within these communities and the emergence of new highly resistant strains. New nanotechnological strategies are being developed in order to overcome the problems associated with bacterial or /and fungi biofilm formation. Although, at this point few of this therapies are being applied sistematically by the medical community. Even so, the nanotechnology approaches seem to be at the moment the most promissing field of research to control/erradicate biomedical biofilms, most especially for the multiresitant microorganisms strains.

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