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Review

Silver nanoparticles as an alternative strategy against bacterial biofilms

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Biofilms are complex bacterial communities that resist the action of antibiotics and the human immune system. Bacteria within biofilms are the cause of numerous, almost impossible to eradicate, persistent infections. Biofilms can form on many medical devices and implants, and so have an enormous impact on medicine. Due to the lack of effective anti-biofilm antibiotics, novel alternative compounds or strategies are urgently required. This review describes some of the latest approaches in the field of biofilm treatment. New anti-biofilm technologies target different stages in the biofilm formation process. Some act to modify the colonized biomaterials to make them resistant to biofilm formation. One potentially important candidate treatment uses silver nanoparticles that show anti-bacterial and anti-biofilm activity. The biological action of nano-silver is complex and seems to involve a number of pathways. However, there have been few reports on the anti-biofilm activity of silver nanoparticles and the precise mechanism underlying their action remains unresolved. Here, we describe some anti-biofilm approaches employing AgNPs and consider the challenges and problems that need to be addressed in order to make silver nanoparticles a part of an effective anti-biofilm strategy.

Key words: biofilm, antibacterial, silver nanoparticles

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INTRODUCTION

In recent years, the number of infections associated with antibiotic-resistant bacteria has increased. Many of these infections are caused by microorganisms growing in biofilms. Both Gram-negative and Gram-positive bacteria can form biofilms on indwelling medical devices such as catheters, mechanical heart valves and prosthetic joints. The most common biofilm-forming bacteria associated with human disease are *Enterococcus faecalis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus viridans*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis* and *Pseudomonas aeruginosa* (Donlan, 2001). Biofilm-related diseases are typically persistent infections characterized by slow development, an ability to resist host immune defenses and a transient response to antimicrobial therapy (Parsek & Singh, 2003). Chronic infections are believed to be caused by a sub-population of cells within biofilms known as "persistent" that can survive prolonged antibiotic treatment and then detach from mature biofilms and spread to other organ systems (Lewis, 2010). The well known antibiotic resistance of biofilms may be caused by poor antibiotic penetration within the biofilm matrix, an altered microenvironment or an adaptive bacterial response. Such mechanisms acting together can raise the antibiotic resistance of biofilms by up to 1000 times in comparison with free living bacterial cells (for a review see Mah & O'Toole, 2001). The aforementioned attributes of biofilms place them amongst the most serious problems currently facing medicine and considerable effort is being made to identify novel technologies that could form the basis of anti-biofilm therapies that are superior to current antibiotic treatment strategies. This review describes new approaches developed to prevent or treat biofilms, particularly focusing on the anti-biofilm activity of silver nanoparticles (AgNPs).

BACTERIAL BIOFILMS

Biofilms were first described by Antonie van Leeuwenhoek and they remain a subject of great interest to many researchers. Ongoing studies have greatly increased our knowledge of the genetic and physiological bases of biofilm formation and their structure for a wide range of bacteria. Various bacterial activities can influence the structure and attributes of biofilms, including cell growth or death, nutrient acquisition, waste product accumulation, motility mechanisms and exopolysaccharide synthesis (Karatan & Watnick, 2009; Haussler & Fuqua, 2013).

Biofilms represent bacterial communities embedded in self-produced extracellular polymeric matrix that is attached to a surface. The microbial population comprising a biofilm can be made up of single or multiple bacterial species. Bacteria develop biofilms on various surfaces such as natural aquatic systems, water pipes, living tissues, tooth surfaces, indwelling medical devices and implants (Donlan, 2002). The extracellular matrix is an intermediate environment for biofilm bacteria that stabilizes the three-dimensional biofilm structure and mediates bacterial adhesion (Flemming & Wingender, 2010). The composition of the matrix, which directly affects the biofilm architecture, is controlled by enzymes secreted in response to nutrient availability (Gjermundsen *et al.*, 2005).

Biofilm formation is initiated when bacterial cells attach and adhere to a surface. The switch between a planktonic and sessile lifestyle is associated with the recognition and transmission of particular signals from the environment. Signals favoring the early settlement of bacteria may include (i) the presence of an appropriate surface, (ii) increased levels of extracellular iron and fer-

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Abbreviations: AgNPs, silver nanoparticles; c-di-GMP, cyclic di-guanosine monophosphate; ROS, reactive oxygen species

ritin that induce the biofilm phenotype in *P. aeruginosa* in the sputum of cystic fibrosis patients (Reid *et al*., 2002); (iii) the presence of compounds such as indole that stimulate biofilm formation of many Gram-negative bacteria (including *E. coli*, *Klebsiella oxytoca*, *Citrobacter koseri*), or other chemicals including polyamines, calcium or bile salts that modulate biofilm formation by *Vibrio cholerae*, *Yersinia pestis*, *Pseudomonas putida* and *S. aureus* (Di Martino *et al*., 2003; Kataran *et al*., 2005; Patel *et al*., 2006; Karatan & Watnick, 2009). Bacteria recognize environmental signals by specific sensory systems: primarily two-component systems and the c-di-GMP-mediated signal transduction network. Two-component systems are not typically implicated in controlling the switch between planktonic and sessile lifestyles, but such systems are involved in the production of specific compounds that comprise the extracellular biofilm matrix. For a diverse group of bacterial species, the intracellular secondary messenger cyclic diguanosine monophosphate (c-di-GMP) has been implicated in the transition to the sessile form (Simm *et al*., 2004). It was suggested that the c-di-GMP signaling network is responsible for inhibiting cell motility and promoting biofilm development (Valle *et al.,* 2013). However, some biofilm-forming bacteria lack the enzymes required for c-di-GMP synthesis and some species such as *S. aureus* and *Listeria monocytogenes* use c-di-AMP as an alternative secondary messenger (Woodward *et al*., 2010; Corrigan *et al*., 2011).

Adhesion of bacterial cells to surfaces may be facilitated by the production of multiple adhesive factors. Some species, e.g. *S. aureus*, produce adhesins that bind to host factors or plasma to mediate bacterial attachment to host tissues and implant surfaces (Menzies, 2003; Ní Eidhin *et al*., 1998). Bacterial attachment onto a surface becomes irreversible and this is accompanied by changes in the physiology, gene expression and protein profile of the cells. In the early stage of biofilm formation, the attached bacteria proliferate, aggregate and form characteristic microcolonies. Cells from the surrounding area are recruited, bind to existing structures and become embedded in extracellular matrix. The steps leading to biofilm development have been defined in detail (for a review see Kostakioti *et al*., 2013). The mature biofilm structure has a complex architecture and is permeated by channels. However, complicated biofilm structures, such as three-dimensional mushroom-shaped structures formed by *P. aeruginosa*, have so far been observed only *in vitro* (Deligianni *et al*., 2010). Within mature biofilm, the microbial community actively exchanges and shares products required to provide a favorable living environment for the bacteria in order to maintain the biofilm architecture. However, these structures are not static and cells may detach, leading to dispersion of the biofilm. Besides passive dispersal occurring as a result of shear stresses, bacteria have mechanisms that sense environmental changes and may be impelled to resume a planktonic lifestyle (Kostakioti *et al*., 2013). Subsequent stages of biofilm development are presented in Fig. 1.

Although a great deal is known about biofilm development, there are still unresolved questions concerning the initiation of biofilm formation on the surface of host tissues and the factors that determine the immune response towards the biofilm. Some recent studies have revealed that cyclic dinucleotides such as c-di-GMP, are recognized by the pattern-recognition receptors of the innate immune system, which activates type I interferon production by the host (McWhirter *et al*., 2009; Woodward *et al*., 2010; Blander & Sander, 2012). The innate immunity is the first line of host defense against infection that recognizes and provides a rapid response to pathogens. It has been suggested that bacterial cyclic dinucleotides act as a signal of the presence of an incipient biofilm that triggers the immune response (Valle *et al*., 2013).

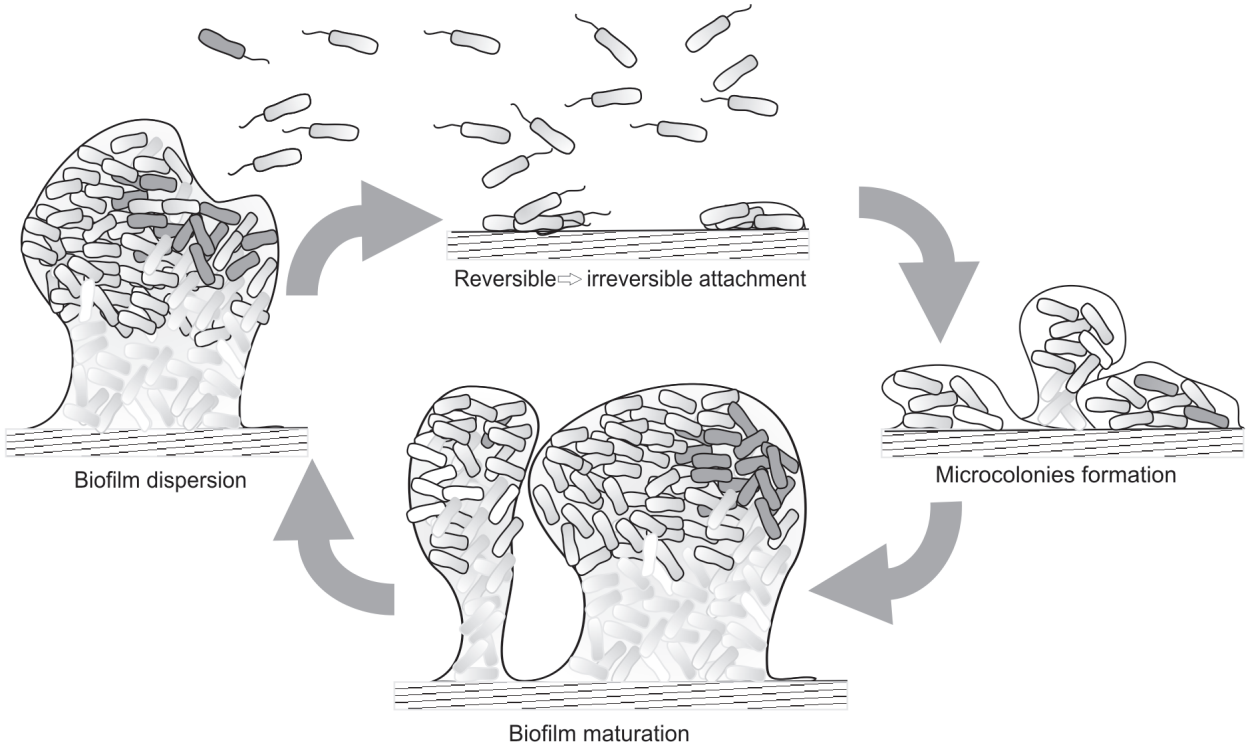

Figure 1. Subsequent stages of biofilm development.

NEW ANTI-BIOFILM STRATEGIES

Due to the limited efficacy of antibiotics in preventing or treating biofilms, a number of alternative strategies has been devised. Chen and coworkers proposed the division of the new anti-biofilm technologies into two groups: (i) treatments that specifically inhibit the biofilm formation process and (ii) modified biomaterials for use in medical devices to make them resistant to biofilm formation (Chen *et al*., 2013). Examples of strategies designed to target different stages in biofilm formation are shown in Table 1. There are also numerous reports concerning new approaches to the design of devices with surfaces that are able to limit microbial adhesion and/or growth (for a review see Desrousseaux *et al*., 2013).

Large-scale screens that permit examination of a huge number of agents have been conducted to expedite the identification of compounds with anti-biofilm properties. High-throughput screening of small molecule libraries for novel bacterial biofilm formation inhibitors has identified a chemical series of molecules that inhibit the formation of or kill *S. epidermidis* or *V. cholerae* biofilms (Peach *et al*., 2011; Panmanee *et al*., 2013). New candidate compounds arising from the application of these large-scale screening techniques are likely to provide the basis for therapeutic agents that may be considered as alternatives to antibiotics after further examination.

One strategy for biofilm control that is currently receiving serious consideration is based on interference with bacterial cell-to-cell communication (quorum sensing). Because quorum sensing plays a vital role in infections caused by human, animal and plant pathogens, the identification of mechanisms that disrupt this system is a hot topic in microbiology. Quorum sensing inhibitors are likely to be effective in controlling bacterial infections, while having no effect on human cells (Defoirdt *et al*., 2013). A few studies have successfully applied high-throughput assays to identify inhibitors of quorum sensing (Christensen *et al*., 2013; Tan *et al*., 2013).

One class of compounds that show great promise as inhibitors of biofilm formation are anti-biofilm polysaccharides synthesized by bacteria. The first such polysaccharide was discovered in 2006 while studying interactions between uropathogenic and commensal strains of *E. coli* in mixed *in vitro* biofilms. It was found that the biomass of biofilms produced by the commensal strain was reduced in the presence of the uropathogenic strain (Valle *et al*., 2006). Recent studies have revealed the existence of polysaccharides that inhibit biofilm formation in a wide spectrum of bacteria and fungi grown both *in vitro* and *in vivo* (Rendueles *et al*., 2013).

Another approach to the control of biofilm formation is a bacteriophage therapy. Lytic phages may penetrate biofilm to directly infect and kill specific host bacteria or produce enzymes to degrade the exopolysaccharide matrix causing disruption of the biofilm structure (Donlan, 2009; Hanlon *et al*., 2001). A combination of phages and the chlorine disinfectant was successfully used in the control and removal of a *P. aeruginosa* biofilm (Zhang & Hu 2013). Plant-derived compounds have been proved to be the other very promising anti-biofilm agents (for a review see Kurek *et al*., 2011).

Nanotechnological approaches to combat biofilm formation are based on the use of nanoparticles to functionalize the surface of biomaterials by coating (Roe *et al*., 2008; Applerot *et al*., 2012; Lellouche *et al.,* 2012), impregnation (Flemming *et al*., 2000; Shi *et al*., 2006) or by embedding nanomaterials (Beyth *et al*., 2008). One such technology with great potential is generation by the use of sonochemistry of nano-antibiotics that are more active and more effective than classical antibiotics against drug-resistant pathogens. The reason for the increased efficacy of these nano-compounds is likely to be due to improved permeability through the cell envelope (Haussler & Fuqua, 2013).

ANTI-BIOFILM ACTIVITY OF SILVER NANOPARTICLES

Among metal nanoparticles with proven antimicrobial activity, those made of silver are particularly effective bactericidal agents (Seil & Webster, 2012). The antibacterial properties of silver have long been known and nanoparticles of this metal (AgNPs) are believed to be less toxic than silver ions. In recent years, the application of AgNPs in various fields has expanded considerably. AgNPs have been successfully used in medical and pharmaceutical nano-engineering for the delivery of therapeutic agents, in chronic disease diagnostics, and as part of sensors (Wong & Liu, 2010; Thiwawong *et al*., 2013). The comparison of the various nano-silver activities that have been studied is difficult because of differences in the chemistry and physical properties of the particles employed. Furthermore the bactericidal effect of AgNPs is dependent on the size and shape of the particles (Panáček *et al*., 2006; Pal *et al*., 2007). The specific surface area of a dose of AgNPs increases as the particle size decreases, allowing greater material interaction with the surrounding environment. In addition, triangular-shaped particles of silver display more bacterial killing activity than rods or spherical particles (Pal *et al*., 2007). Other characteristics affecting the biological activity of nanoparticles are zeta potential and particle chemistry, with the former likely to play a significant role in the ability of particles to penetrate into the cell (Seil & Webster, 2012).

Silver nanoparticles probably have multiple mechanisms of antibacterial action, but due to the current dearth of knowledge on this subject, the exact basis for the activity of AgNPs is still uncharacterized. Some studies have shown that AgNPs release Ag^+ ions in the presence of water (Santoro *et al*., 2007; Asharani *et al*., 2008; Damm & Münstedt, 2008). Lok and coworkers calculated that approximately 12% of the silver is present in the ionic form, tightly associated with the oxidation layer (Lok *et al*., 2007). However, their experimental design makes it difficult to distinguish between the mechanisms of action of AgNPs and dissolved Ag^+ ions. Hence, it was suggested that nano-silver affects bacterial membrane permeability by attaching to the cell membrane surface and modifying the cell potential. Observation of large numbers of nanoparticles inside bacteria suggests that this is important to the antibacterial mechanism (Morones *et al*., 2005). Proteomic analysis (2-DE and MS identification) of *E. coli* cells revealed that short-exposure to AgNPs resulted in the accumulation of envelope precursors, which is indicative of the dissipation of the proton motive force. Proteins whose expression was found to be stimulated by AgNPs over 1.8-fold were the inclusion body binding proteins A and B (IbpA and IbpB), which serve as molecular chaperones, and 30S ribosomal subunit protein S6 (Lok *et al*., 2006). Furthermore, AgNPs have been shown to interact with bacterial membrane proteins, intracellular proteins, phosphate residues in DNA, and to interfere with cell division, leading to bacterial cell death (Sondi & Salopek-Sondi, 2004; Xu *et al*., 2004). Presence

of biocidal Ag^+ ions released from the nanoparticle surfaces evokes bacterial DNA conglomeration defense mechanisms, which protect the cell from toxic effects, but simultaneously compromises its replication ability. Thus microbial responses to ionic silver and nanoparticles are different, and knowledge of both is required for a complete understanding of the antibacterial activity of AgNPs. Some studies have reported that nano-silver causes oxidative damage, leading to the production of reactive oxygen species (ROS), *i.e.* free-radicals (Kim *et al.*, 2007; Hwang *et al.*, 2008), and it has been suggested that the production of ROS is one of the primary mechanisms of nanoparticle toxicity (Khan, 2012). Schematic representation of the effect of silver nanoparticles on microbial cell targets and biofilm formation is presented in the Fig. 2.

The anti-biofilm activity of silver nanoparticles has been demonstrated in a number of studies. Small but significant decreases in the biomass of 24-hour *P. putida* biofilms were observed by Fabrega and coworkers in the first report that discussed interactions between well quantified and characterized bacterial biofilms and silver nanoparticles (Fabrega *et al.*, 2009). The average diameter of the AgNPs employed in this study (65±30 nm) was quite high considering that nanoparticles are within the range 1–100 nm. Another study found that AgNPs (mean diameter 50 nm) at a concentration of 100 nM almost completely prevented biofilm formation by *P. aeruginosa* and *S. epidermidis* by impeding the initial step: bacterial adhesion to the surface (Kalishwaralal *et al.*, 2010). More recent studies have tended to employ smaller AgNPs with greater biological activity. Nano-silver (average particle diameter 25.2 ± 4 nm) was found to effectively prevent the formation of *P. aeruginosa* biofilms and kill bacteria in established biofilm structures (4-log reduction in the number of colony-forming units), suggesting that it could be used for the prevention and treatment of biofilm-related infections (Martines-Gutierrez *et al.*, 2013). Another recent study showed that AgNPs (average diameter 12.6±5.7 nm) are also effective against *Mycobacterium* spp. biofilms. The colonization and growth of *M. smegmatis* biofilms on membranes coated with nano-silver at a concentration of 100 μM were decreased by over 98.7%. In addition, the presence of silver nanoparticles reduced survival of this bacterium to only 0.03% (Islam *et al.*, 2013). It is important to note that differences in the chemical and physical properties of nano-silver used in the aforementioned studies may have caused some of the observed variation in its antimicrobial and anti-biofilm efficacy.

Because of the relatively low stability of colloidal solutions, some researchers propose the usage of stabilized AgNPs. This impedes the aggregation of particles into larger forms that can significantly decrease their activity. Radzig and colleagues found that AgNPs (about 8 nm in diameter), stabilized by hydrolyzed casein peptides, strongly inhibited biofilm formation by some Gram-negative bacteria. A decrease in the bacterial mass in biofilms of *E. coli*, *P. aeruginosa* and *Serratia proteamaculans* was observed when the AgNP concentration was between 5 and 10 micrograms per milliliter (Radzig *et al.*, 2013). Mohanty *et al.* showed that starch-stabilized nanoparticles (about 20 nm in diameter), at very low concentrations of 1–2 mM, decreased *P. aeruginosa* and *S. aureus* biofilm formation by 65% and 88%, respectively (Mohanty *et al.*, 2012). Park and coworkers demonstrated the antimicrobial activity of citrate-capped silver nanoparticles (47 nm in diameter) against *P. aeruginosa* biofilms. Interestingly, they found that the inactivation of biofilms was greatly influenced by stirring. Therefore, these authors suggested that AgNPs inactivate biofilms in a biosorption-dependent manner (Park *et al.*, 2013). The recent report

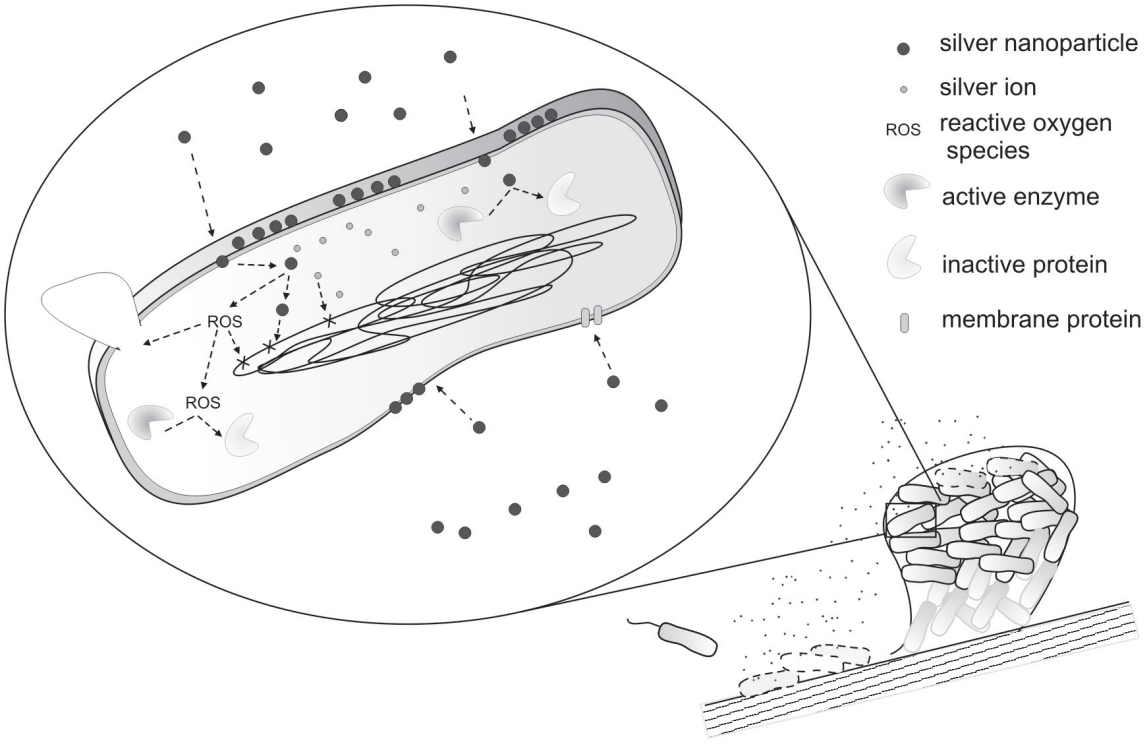

Figure 2. Schematic representation of the silver nanoparticle mechanism of action on the biofilm forming microbial cell. Description in the text.

of Hartmann *et al.* introduced a new chip-calorimetric approach to detect bacterial metabolic changes with high resolution and reasonable throughput. With this system, they revealed a complete inhibition of the cell metabolic activity in a bead-grown biofilm of *P. putida* treated with commercially available silver nanoparticles (exact diameter not given) at a concentration of 0.5 micrograms per milliliter (Hartmann *et al.*, 2013).

Silver nanoparticles, as it was previously shown, can also enhance the anti-bacterial and anti-biofilm activity of conventional antibiotics. There are reports describing synergistic activity between AgNPs and *e.g.* ampicillin, kanamycin, streptomycin or vancomycin against *E. coli* and *P. aeruginosa* (for review see Wolska *et al.*, 2012). Additionally it was already demonstrated by our research group that nano-silver acts synergistically with streptomycin against *P. aeruginosa* (data not shown). Other studies have revealed synergy of AgNPs with compounds other than antibiotics. For example Ammons *et al.* (2011) showed that a silver wound dressing combined with the immune molecule lactoferrin and the rare sugar-alcohol xylitol, reduced biofilm viability more effectively than standard silver hydrogel.

As mentioned above, some new anti-biofilm approaches are based on the coating of medical devices or improvement of the properties of biomaterials. Silver has been proposed as a component of coatings that may have potential in combating biofilm formation. Roe and coworkers examined the efficacy of nano-silver (mean average diameter of 10 nm) as an anti-biofilm agent used to coat the surface of catheters (Roe *et al.*, 2008). Notable anti-biofilm effects against Gram-positive and Gram-negative bacteria, and also *Candida albicans*, were found when catheters coated with AgNPs were tested *in vitro*. After 72 hours of incubation, these authors observed almost complete prevention of biofilm formation by *E. coli, S. aureus* and *C. albicans*, and more than 50% inhibition in the case of *Enterococcus* sp., coagulase-negative staphylococci and *P. aeruginosa*. In addition, no significant accumulation of silver was detected in the major organs of mice fitted with the treated catheters, suggesting that these are non-toxic devices that permit the targeted and sustained release of bactericidal silver at the implantation site (Roe *et al.*, 2008). Subsequently Cheng and coworkers created dental composites containing silver nanoparticles that can kill oral bacteria such as *S. mutans*. These new nanocomposites significantly reduced the metabolic activity and lactic acid production of *S. mutans* biofilms, when compared with two commercial composites (Cheng *et al.*, 2012).

Besides the aforementioned primary studies of the anti-biofilm effects of AgNPs, some nano-silver-coated medical devices are already at the stage of clinical trials. The most prominent examples are catheters, drains and wound dressings containing silver nanoparticles. Some studies have confirmed the beneficial effect of nano-silver as a component of coatings (Knetsch & Koole, 2011). Two reports evaluating large prospective randomized studies emphasize the promising outcome of trials with biomaterials containing nano-silver (Lackner *et al.*, 2008; Gravante & Montone, 2010). In some cases the successful use of silver nanoparticles has already been confirmed, e.g. for coating surgical masks (Li *et al.*, 2006). The impregnation of such medical devices with silver nanoparticles has the advantage that it protects both the outer and inner surfaces, providing continuous antimicrobial activity.

CHALLENGES FOR THE ANTI-BIOFILM APPLICATION OF SILVER NANOPARTICLES

The application of silver nanoparticles as an effective antimicrobial agent should not cause microbial resist-

Table 1. Examples of new strategies to inhibit or disrupt biofilms at different stages of their development.

Stage of biofilm development	Strategy to inhibit or disrupt biofilm formation	Reference
Reversible/irreversible attachment	Anti-adhesion agents, *e.g.* mannoside, pilicides, curlicides	Han *et al.*, 2010 Cegelski *et al.*, 2009
	Anti-biofilm polysaccharides	Rendueles *et al.*, 2013
	Signal transduction interference, *e.g.* quorum sensing and two-component signaling	Roy *et al.*, 2013 Gotoh *et al.*, 2010
Microcolony formation and biofilm maturation	Silver nanoparticles	Kalishwaralal *et al.*, 2010
	Lytic phages	Carson *et al.*, 2010
	Enzymes degrading extracellular matrix, *e.g.* Dispersin B	Lu & Collins, 2007
	Anti-biofilm polysaccharides	Rendueles *et al.*, 2013
	Antimicrobial peptides	Pompillo *et al.*, 2011; Kharidia & Liang, 2011
	Signal transduction interference, *e.g.* quorum sensing and two-component signaling	Roy *et al.*, 2013 Gotoh *et al.*, 2010
	Chelating agents	Percival *et al.*, 2005b; Shanks *et al.*, 2006; Abraham *et al.*, 2012
Dispersion	c-di-GMP engineering to promote motility vs. sessility	Ma *et al.*, 2011
	Introduction of dispersal signals, *e.g.* D-amino acids, norspermidine	Kolodkin-Gal *et al.*, 2010; Kolodkin-Gal *et al.*, 2012

ance even after long-term usage. However, there have been reports concerning bacterial resistance to silver compounds determined by genes carried on plasmids. The resistance mechanism involves a periplasmic multimetal-binding protein, a chemiosmotic efflux pump and an ATPase efflux pump encoded by a single toxic metal cation resistance gene cluster (Silver, 2003). Resistance is conferred by the action of plasmid-encoded pumps that promote the active efflux of Ag^+ ions out of the cell. The widespread usage of silver nanoparticles (e.g. as a component of disinfectants) might promote the spread of silver-resistant bacterial strains. However, prolonged exposure of bacteria to silver nanoparticles has not resulted in the development of resistant mutant cells. Silver nanoparticles as biocides tend to target multiple sites on or within bacterial cells and hence have broad-spectrum activity. Furthermore, this property of AgNPs means that they can overcome existing microbial drug resistance mechanisms, including decreased uptake and increased efflux from the microbial cell, and biofilm formation (Pelgrift & Friedman, 2013). The evolution of silver resistance seems to be slow and this problem is less of a concern than resistance to other antibacterial agents (Percival *et al.*, 2005a).

It should be noted that there also have been some disappointing clinical trials of medical devices coated with nano-silver (Knetsch & Koole, 2011). One shortcoming of bactericidal surfaces is that their antimicrobial function may be negated if they become covered by macromolecules or dead microorganisms. Another problem is the reduced efficacy of metallic silver on medical devices contacting the blood, as the coating wears off (Rai *et al.*, 2009). Therefore improved methods of coating or incorporating AgNPs are required to prolong the efficacy of such devices. On the other hand, there are reports confirming that biofilm formation is almost completely prevented on catheters coated with nano-silver (Knetsch & Koole, 2011; Chen *et al.*, 2013).

In depth knowledge concerning the biocompatibility of silver nanoparticles is essential before they become widely used as coating for medical devices or incorporated into biomaterials. The interaction between nano-silver and the human body will determine the clinical success of medical devices containing AgNPs. The biosafety of silver nanoparticles is currently uncertain. Some of the mechanisms mediating the biological activity of AgNPs are not specific for the cells of bacteria or fungi, but are conserved in many organisms, potentially also in humans. So far, there have been very few reports on the effects of AgNPs on human health, although some studies have revealed *in vivo* bio-distribution and toxicity in rats and mice. AgNPs administered by inhalation, ingestion or injection were subsequently detected in the blood and caused toxicity in several organs including the brain (for a review see Ahamed *et al.*, 2010). However, other reports suggested that the toxicity of nano-silver for mammalian cells is low. In experiments using therapeutic doses of silver nanoparticles, only very low levels (below 0.5 micrograms per gram of an organ) could be detected in the organs of mice, suggesting that nano-silver is safe at these low concentrations (Wong & Liu, 2010). Other studies have confirmed the dose-dependent toxicity of nanoparticles. Lee *et al.* recently demonstrated unambiguous stage- and dose-dependent toxic effects of AgNPs on embryonic development in zebrafish (Lee *et al.*, 2013). Another report implied that AgNPs at high doses could have genotoxic and cytotoxic effects on human cells (Jena *et al.*, 2012). In *in vitro* experiments, Park and coworkers showed that the potency of silver nanoparticles to induce cell damage, compared to silver ions, is cell type and size-dependent (Park *et al.*, 2011). Another recent study revealed the dose-dependent influence of AgNPs on the bioactivity of bone-forming cells and the possibility of nanoparticle uptake by human mesenchymal stem cells and human osteoblasts (Pauksch *et al.*, 2014). In clinical practice, features such as wound exudation might increase the applicability of silver nanoparticles, because the high protein content of such exudates is likely to neutralize nano-silver tissue toxicity (Wong & Liu, 2010). Undoubtedly the possible side effects of nanoparticles have not received sufficient attention and detailed studies are urgently required before the clinical use of AgNPs is approved.

CONCLUSIONS

Bacterial biofilms are a serious medical problem. Due to the increasing ineffectiveness of conventional antibiotics, numerous alternative methods to combat bacterial biofilms are being considered. Silver nanoparticles have recently received an increased attention for their antimicrobial effects and possible clinical applications. Despite numerous studies conducted over the last decade there are still considerable gaps in our knowledge about the antimicrobial properties of AgNPs. Furthermore, the precise basis of their antibacterial activity has yet to be defined. This is mainly due to the pleiotropic effects of nano-silver on bacterial cells, which suggests multiple mechanisms of action on several cellular targets. Nonetheless, the strong anti-biofilm effect of AgNPs is indisputable. Several studies have demonstrated the inhibition of *in vitro* biofilm formation by a variety of bacterial species at specific nanoparticle concentrations. This raises the intriguing possibility of treating infections caused by biofilm-forming bacteria with AgNPs. However, the toxicity of nanoparticles to eukaryotic cells is a legitimate concern and still remains uncharacterized. One way of avoiding this potential drawback might be to target AgNPs to the specific site of an infection so that toxic silver concentrations are localized. In addition, improvements in the way that AgNPs are incorporated into medical devices could increase their efficacy and diminish any side-effects, but considerable research effort is still required to perfect this technology.

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