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Review

The growing importance of materials that prevent microbial adhesion: antimicrobial effect of medical devices containing silver

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ABSTRACT

Research has clarified the properties required for polymers that resist bacterial colonisation for use in medical devices. The increase in antibiotic-resistant microorganisms has prompted interest in the use of silver as an antimicrobial agent. Silver-based polymers can protect the inner and outer surfaces of devices against the attachment of microorganisms. Thus, this review focuses on the mechanisms of various silver forms as antimicrobial agents against different microorganisms and biofilms as well as the dissociation of silver ions and the resulting reduction in antimicrobial efficacy for medical devices. This work suggests that the characteristics of released silver ions depend on the nature of the silver antimicrobial used and the polymer matrix. In addition, the elementary silver, silver zeolite and silver nanoparticles, used in polymers or as coatings could be used as antimicrobial biomaterials for a variety of promising applications.

1. Introduction

Antimicrobial polymers that contain silver represent a great challenge for academics and industry [1]. These materials capture attention because of their novelty in being a long-lasting biocidal material with high temperature stability and low volatility [1]. The large increase in the number and occurrence of antibiotic-resistant bacterial strains has prompted a renewed interest in the use of silver as an antibacterial agent [2].

Silver is a metal known for its broad-spectrum antimicrobial activity against Gram-positive and Gram-negative bacteria, fungi, protozoa and certain viruses [3], including antibiotic-resistant strains [2,4]. It can be used to reduce infections in the treatment of burned areas [5–7], to prevent bacterial colonisation on medical devices [7–12] as well as in textile fabrics [7,13] and for water treatment [14]. Silver, as an antiseptic agent, has been effective in a variety of materials, including glass, titanium and polymers [15].

The antimicrobial activities of commercially available silver-impregnated dressings and catheters have been reported [8,16,17]. It has been suggested that impregnation of silver into a coating can be more effective than direct surface coating alone, since surface silver can be readily deactivated by protein anions [2,18]. This impregnation of silver ions (SI) would also be beneficial in protecting the inner and outer catheter surfaces against bacterial attachment [2,19].

However, the use of medical devices containing silver must be undertaken with caution, since a concentration-dependent toxicity has been demonstrated. Braydich-Stolle et al. [20] assessed the suitability of a mouse spermatogonial stem cell line as an in vitro model to assess the nanotoxicity of silver. Concentrations of silver nanoparticles (SN) between 5 μg/mL and 10 μg/mL induced necrosis or apoptosis of mouse spermatogonial stem cells [20]. Moreover, heavy metal accumulation in the environment has been mentioned in the US Agency for Toxic Substances and Disease Registry (ATSDR) Comprehensive Environmental Response, Compensation, and Liability Act 2007 Priority List [http://www.atsdr.cdc.gov/cercla/] as well as by the European Commission on heavy metals waste [21]. Nevertheless, silver has not been cited amongst the most prevalent heavy metals in the priority list of hazardous substances to public health [21].

Despite this, as the use of silver and the number of available silver-based products has increased, it is becoming important to clarify the efficacy of silver against different microorganisms and biofilms. It is also essential to answer questions related to the mechanisms of the various silver forms as antimicrobial agents as well as the dissociation of SI and the resulting antimicrobial efficacy. Thus, this literature review was carried out using references from the last 35 years regarding silver as an antimicrobial agent, specifically silver zeolite (SZ) and SN, SI release and biofilm formation.

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2. Antimicrobial properties

Medical devices such as endotracheal tubes, vascular and urinary catheters, and hip prosthetics are responsible for over one-half of nosocomial infections in the USA [22,23]. To create surfaces resistant to bacterial adhesion and colonisation, several methods of incorporating silver on medical devices have been described [3,22,23]. Silver has been used in ionised and elementary forms, as SZ or as nanoparticles.

2.1. Silver zeolite

Silver exhibits a strong affinity for zeolite, a porous crystalline material of hydrated sodium aluminosilicate, and can electrostatically bind this ion up to ca. 40% (w/w) of its framework [24]. In the dental field, SZ has been incorporated in tissue conditioners, acrylic resins and mouthrinses [24–27].

In an in vitro study [25], tissue conditioners containing SZ showed antimicrobial effects for 4 weeks against *Candida albicans*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Moreover, 10 of 11 subjects showed a reduced plaque score after using a SZ mouthrinse for 5 days [26]. This could be explained by gradually releasing SI from SZ, since human saliva contains several kinds of cations and SZ does not dissolve in water.

Kawahara et al. [24] suggested that SZ could be beneficial for use in the dental field as it inhibited the growth of several oral bacteria under anaerobic conditions. Approximately 75% of SI contained in SZ were released into a brain–heart infusion broth after 24 h, since SI have a strong affinity for sulphur-containing amino acids in broth. However, the release of detectable silver in water did not occur after 24 h. Thus, since the SI released from SZ are influenced by the liquid environment, SZ can exhibit slow and gradual release of SI in solutions with low ionic strength, resulting in long-term antibacterial activity.

Casemiro et al. [27] found that addition of 2.5%, 5.0%, 7.5% and 10% silver zinc zeolite in denture base resins resulted in antimicrobial activity against *C. albicans* and *Streptococcus mutans*. However, the addition of zeolite in percentages >2.5% resulted in a significant decrease in mechanical properties. For these authors, the addition of 2.5% zeolite to these materials produced a less significant impact on mechanical properties than its potential antimicrobial activity, being of basic importance for patients who do not show appropriate denture cleaning.

2.2. Silver nanoparticles

Nanoparticles are insoluble particles that are smaller than 100 nm in size [28]. SN can be prepared based on the Turkevich method [29] by reduction of $AgNO_3$ with citrate. To prepare SN, $AgNO_3$ is dissolved in water in a tri-neck flask. The solution is brought to boiling and an aqueous solution of sodium citrate is added after 2 min of boiling. The solution increasingly turns yellow in a few minutes, indicating the formation of SN. It is kept boiling for 6 min and the solution is then allowed to cool (Figs. 1 and 2).

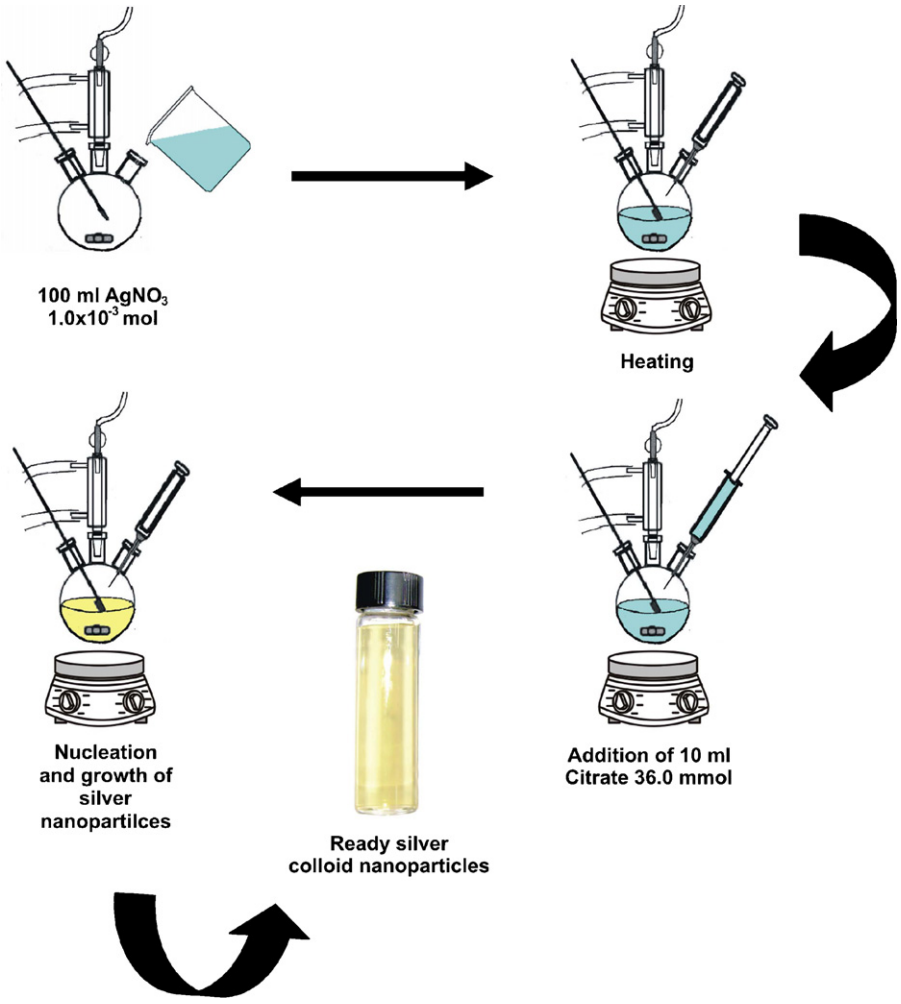

Fig. 1. Schematic illustration of the synthesis of silver nanoparticles by reducing silver nitrate with citrate.

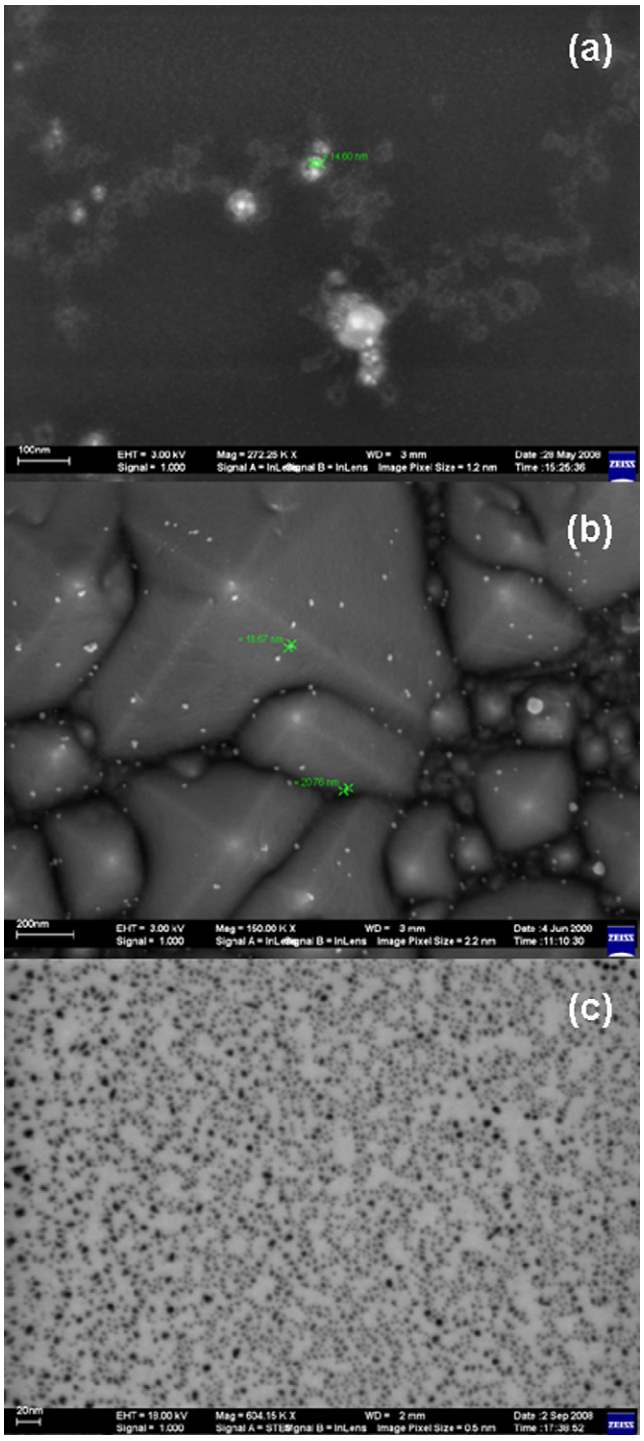

Fig. 2. (a and b) Scanning electron micrograph and (c) transmission electron micrograph images of silver colloid nanoparticles synthesised via reduction of $AgNO_3$ by citrate. (a) A drop of silver colloid was deposited on a silicious substrate and dried to obtain the image. White dots are the agglomerated silver nanoparticles (SN) involved with the citrate salt (the bar indicates nanoparticles size, ca. 14 nm). (b) Silicious substrate was degraded by KOH for 2 h and silver colloid was deposited and then dried to obtain the image. This technique illustrates more clearly the shape and size of the SN (white particles ca. 10–20 nm; dark, citrate salt precipitated to the deeper part of the silicious substrate). (c) Image of silver colloid nanoparticles. Note the extremely small size of the silver particles. Magnification: (a) 272.26×; (b) 150.00×; and (c) 604.15×.

Studies have focused on the potential antimicrobial activity possessed by SN [7,30,31]. Baker et al. [30] found that smaller particles with a larger surface-to-volume ratio provided a more efficient means of antibacterial activity and that surfaces effectively cytotoxic to *Escherichia coli* can be obtained at Ag concentrations as low as 8 $\mu g/cm^2$. The decrease of particle size is in agreement with Panácek et al. [7] who reported that smaller particles with a larger surface area available for interaction will give more bactericidal effects than larger particles.

The shape of SN may interfere with their antimicrobial effect. Pal et al. [31] found that triangular SN displayed greater biocidal action against *E. coli* than rod or spherical nanoparticles. The differences can be explained by the percent active facets present in nanoparticles of different shapes. An oriented particulate monolayer X-ray diffraction pattern indicated that triangular nanoparticles have more high-atom-density facets than other shapes, favouring the reactivity of silver.

Another factor that can interfere with the effectiveness of the antimicrobial activity is the formation of stable dispersions of SN in the acrylic formulation. SN protected by polymers have been reported as being more important than the size of nanoparticles, increasing their stability against aggregation and their biocompatibility [32]. Three techniques have been developed for the preparation of nanocomposites [32]: (i) nanoparticles are mixed with the polymer or the nanoparticles are distributed in the matrix of the host polymer; (ii) nanoparticles are generated during polymerisation, a technique requiring the use of electronically active polymers that are able to reduce silver salts in SN; and (iii) dispersion of the nanoparticles in the monomer, in this case the polymerisation is initiated in the presence of nanoparticles that are simultaneously trapped in the polymer network.

Sondi and Salopek-Sondi [33] investigated the application of SN as an antimicrobial agent by growing *E. coli* on agar plaques and in liquid Luria–Bertani (LB) medium. Nanoparticles at a concentration of 50–60 $\mu g/cm^3$ presented to ca. 10^5 colony-forming units (CFU) LB agar plates completely inhibited bacterial growth. The inhibition depended on the concentration of the SN and on the CFU of the bacterial strain cultured on the agar plates. SN in liquid medium caused only growth delay of *E. coli*. In this case, the concentration of the nanoparticles gradually decreased, allowing growth of the bacteria. The authors concluded that SN can have a limited use as biocidal materials in liquid systems because of their low colloidal stability.

Djokic and Burrell [34] examined the antimicrobial effect of silver films of various origin, e.g. physical vapour deposed (PVD), electrodeposited, electroless deposited and metallurgical. These films were immersed in physiological saline solution and calf serum, and only PVD films showed silver oxides that exhibited inhibition of zone growth for *E. coli*, *S. aureus* and *P. aeruginosa*. This could be attributed to the dissolution of Ag_2O from the silver material and the formation of SI that became antimicrobially active.

The antibacterial properties of SN synthesised by borohydride reduction methods were tested by Lok et al. [35]. For the preparation of partially oxidised SN, a portion of the reduced SN was bubbled with oxygen for 30 min to oxidise the nanoparticles. *Escherichia coli* bacteria colony formation was not affected by treatment with reduced SN, whereas oxidised SN showed significant decreases in colony formation. Taken together, the antibacterial activities of SN are critically dependent on surface oxidation and optimal particle dispersion.

The surface modifications that incorporate SI would be effective in reducing bacterial colonisation to medical devices. Poly(vinyl chloride) (PVC) used in endotracheal tubes, when chemically modified using NaOH and $AgNO_3$ wet treatments, completely inhibited bacterial adhesion of *P. aeruginosa* and efficiently prevented colonisation over 72 h [3]. Also, a facemask coated with a mixture of

$AgNO_3$ and TiO_2 produced 100% reduction in viable *E. coli* and *S. aureus* after 48 h [36]. After wearing these facemasks, 20 volunteers were examined and no signs of local skin inflammation were found.

Additionally, Kong and Jang [37] compared the antibacterial properties of poly(methyl methacrylate) (PMMA) nanofibre containing SN with silver sulfadiazine and $AgNO_3$ at the same silver concentration against *E. coli* and *S. aureus*. The silver/PMMA nanofibre had a faster kill rate than silver sulfadiazine and $AgNO_3$. $AgNO_3$ and silver sulfadiazine had an antimicrobial property by releasing SI. When the SI contacted bacteria, black precipitates were formed via ion reduction or salt formation and the precipitates deteriorated the antimicrobial ability of $AgNO_3$ and silver sulfadiazine. On the other hand, the silver/PMMA nanofibre released SN with a 7 nm diameter and contacted bacteria without direct precipitation. For these reasons, an effective concentration of SN could be much lower than that of SI, and SN have an enhanced biocidal ability than that of SI at the same concentration. However, the mechanism of bactericidal action of SN is still not well understood. Pal et al. [31] speculated that the action of SN is broadly similar to that of SI. Sulphur-containing proteins in the membrane or inside the cells as well as phosphorus-containing elements, such as DNA, are likely to be the preferential sites for SN binding [31].

Different results related to the release of SI from nanoparticles are found in the literature. Damm and Münstedt [38] detected SI released from the polyamide/silver nanocomposites. However, using a rhodanine test Kong and Jang [37] detected that most of the silver released from the silver/PMMA nanofibre was as SN.

Publications have highlighted the necessity of developing a strategy to reduce bacterial adhesion to dental materials [27,39]. In this direction, SN could be added to PMMA acrylic resin used in the construction of removable dentures, preventing denture stomatitis infection in denture wearers. Fig. 3 illustrates a scanning electron micrograph of a PMMA acrylic resin/Ag nanocomposite.

The proper amount of SN added to polymer materials may be relevant in not producing an adverse effect on their physical properties. Ahn et al. [39] incorporated silica nanofillers and SN in orthodontic adhesives. Even with an increased surface roughness due to the incorporation of SN, the adhesives produced a significant reduction in the adhesion of cariogenic streptococci, regardless of the silver added (250 ppm and 500 ppm). The bond strength of the orthodontic adhesives was not affected and, because the antimicrobial effect was maintained after saliva coating, the silver was able to penetrate the saliva coating, which could bring beneficial clinical implications.

To many authors [4,7,18,24,25,28,40], the antimicrobial activity of silver is dependent on SI, which binds strongly to electron donor groups in biological molecules containing sulphur, oxygen or nitrogen. This may result in defects in the bacteria cell wall so that cell contents are lost [40]. A complex formation between SI and proteins may disturb the metabolism of bacterial cells and their power functions, such as permeability and respiration [7,40]. Both effects lead to death of the bacterial cells. Furthermore, SI can interact with the DNA of bacteria, preventing cell reproduction [40].

Differences among bacterial species may influence their susceptibility to antibacterial agents. The cell walls of Gram-positive species contain 3–20 times more peptidoglycan than Gram-negative bacteria [24]. Since peptidoglycans are negatively charged, they probably bind some portion of SI in the broth. Consequently, Gram-positive bacteria are generally less susceptible to antibacterial agents containing SI than Gram-negative species [24].

2.3. Silver release

An alternative to reducing bacterial adhesion on medical devices is to focus on materials that release antimicrobial agents.

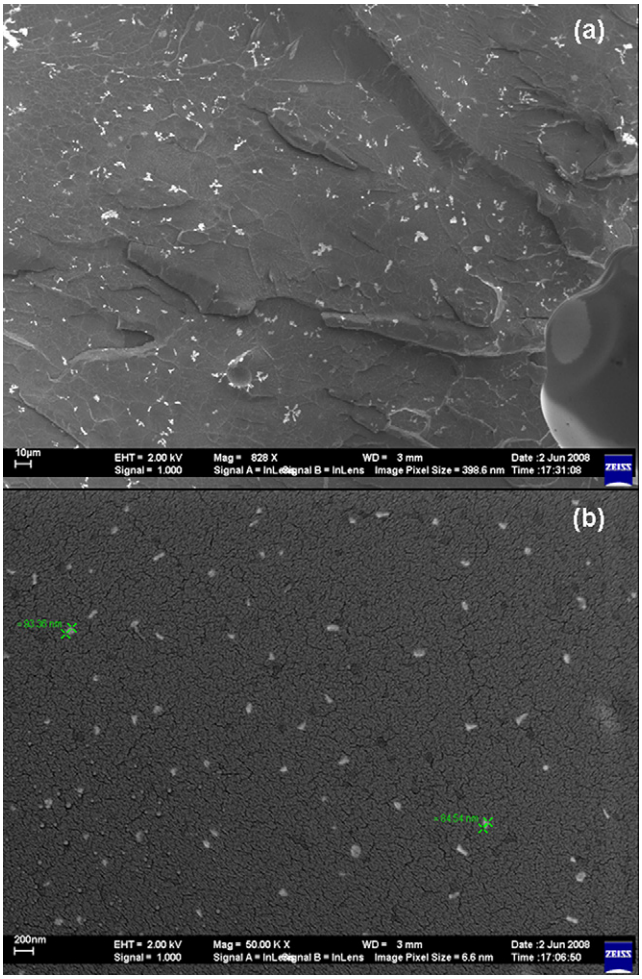

Fig. 3. Scanning electron micrograph of a fractured poly(methyl methacrylate) PMMA/Ag nanocomposite containing ca. 0.04 wt% silver. Micrographs show the fine distribution of silver particles in the PMMA acrylic resin matrix. (a) White areas are agglomerated silver nanoparticles (SN) distributed in the PMMA (magnification 828×). (b) SN (white dots) with size ca. 88 nm dispersed in the PMMA matrix (magnification 50.00×).

Coatings that incorporate agents with direct antibacterial activity were effective at reducing bacterial adhesion in vitro and, in some cases, lessening the effects of implant-associated infection in vivo [41]. The antimicrobial properties of silver are related to its oxidised form, a form of silver that is not necessarily present at the surface coated with metallic silver [41]. Polymers that release silver in the oxidised form have shown strong antibacterial activity and would act as reservoirs of silver and be capable of releasing SI for extended periods [41].

Silver is known for having a high affinity for protein, and the presence of a protein conditioning film has been responsible for inactivating any SI released [42]. Furno et al. [18] demonstrated that SI penetrated the protein conditioning film. There was a storable effect and a diffusion pressure available to push the SI through the film. Consequently, there must be enough SI available over a sufficient period of time to exceed those lost to protein binding. The avidity of silver for protein could explain the results found by the authors, where a greater quantity of SI was released into the plasma samples during 5 days whereas very few SI were released into the water samples.

SI release in an aqueous environment may also have shown different results. For SI release, water molecules are required to enter the polymer to oxidise the metallic silver powder [43]. Kumar et al.

[43] found a marginal increase over 4 days for silver-based antimicrobial fillers in polyamide (PA). Afterwards, the release attained an almost constant value followed by an abrupt rise after 1 week, especially for composites containing greater silver concentrations. During the first few days, the release occurred at the expense of the silver particles limited to the surface layers. The increase after 7 days was attributed to plasticisation of the polymer after this period of continuous diffusion. They associated the absence of antimicrobial properties against *S. aureus*, *E. coil* and *C. albicans* in the first week to the poor SI release by the specimens during this period.

The flux of SI from the PA polymer was also affected by polymer crystallinity [44]. Decreased polymer matrix crystallinity resulted in greater levels of SI release [44]. The crystallinity affected the water uptake, which in turn controlled the release of SI. Kumar and Münstedt [44] showed that the rates of SI released for both the specimens with low and high crystallinities increased after the 7th day of immersion in water and this increase was more significant for the specimens with lower crystallinities. The results showed a lack of antimicrobial efficacy of the PA/Ag composites against *E. coli* and *S. aureus* within <7 days, which indicated poor SI release. However, between 7 and 28 days the specimens had a good efficacy against the microorganisms, especially those with lower crystallinities.

Silver ionisation and release are dependent on water uptake [1]. Kumar et al. [43] tested the antimicrobial PA and attributed the release longevity to the interruption of intermolecular hydrogen bonding within the existing PA matrix after extended water absorption, resulting in increased mobility of SI through the plasticised matrix medium. Significant levels of SI were released from the coatings after 3-month soaking periods. The good antimicrobial efficacy of the composite containing 8% silver powder against *E. coli* and *S. aureus*, found especially after 28 days, could be related to the late SI release.

Transportation of SI from the bulk to the surface becomes more relevant with increasing immersion time, whereas the contribution of silver particles in the surface to the release of SI decreases because these particles are consumed faster than particles situated in the bulk of the material [38]. SI release becomes controlled by diffusion, as most of the SI must move from the interior to the surface to be released [38]. The transport processes through the matrix are influenced by the polymer properties and when shown with longer immersion times [38]. Therefore, SI release is expected to increase with a growing water content of the polymer [38].

The SI release rate may still be controlled by the morphology of the silver particles. Damm et al. [40] found that, for a fixed filler content, the SI release from nanocomposites is much more effective than that from microcomposites owing to the much larger specific surface area of the nanoparticles. A SI release rate of ca. 9.5×10^{-4} mg/L/cm^2/day killed all *E. coli* within 24 h, with this value reached for polyamide 6 filled with 0.06 wt% of SN. However, in the presence of microcomposites, not all of the bacteria were killed within 24 h, even with the highest filler content (1.9 wt% silver) 20% of the bacteria survived. The much better antimicrobial efficacy of the nanocomposites is explained by their more efficient silver release, which goes back to the much larger specific surface area of the SN.

Finally, SI release can be proportional to the concentration gradient between the SI in the polymer and in the immersion liquid [40]. In the polymers there is a chemical equilibrium between silver atoms on the surface of the particles, water and SI [40]. Therefore, the equilibrium concentration of SI in the polymers is a function of the total surface area of the silver particles used as fillers [40]. This explains the much more efficient SI release from the nanocomposites.

3. Biofilm formation

Biofilms are defined as communities of bacteria that colonise surfaces in an aqueous environment [28]. Biofilm formation occurs as a result of a sequence of events: microbial surface attachment, cell proliferation, matrix production and detachment [45].

The conditioning film, formed by a layer of organic molecules adhered to the surface, is considered a precursor for the initial attachment of planktonic cells [28]. Once the microorganisms reach critical proximity to the surface, the determination of adhesion depends on the net sum of attractive or repulsive forces generated between the two surfaces [46]. The adhesion of microorganisms then occurs on the surface, which is subsequently facilitated by bacterial signalling [28]. A mature biofilm is characterised when adherent bacteria produce extracellular polymeric substances (EPSs) that aid in trapping nutrients from the surrounding environment [28,47]. The final stage is the release of microorganisms back into their surroundings, where they return to their free-living state [28].

Biofilms create an environment that enhances antimicrobial resistance [48,49]. The EPSs of biofilms contain considerable amounts of polysaccharides, proteins, nucleic acids and lipids [50], which are responsible for maintaining the structural integrity of the biofilm and providing an ideal matrix for bacterial cell growth [49]. Intermolecular interactions between the functional groups within these macromolecules serve to strengthen the overall mechanical stability of the EPSs and the survivability of the microorganisms [49]. The increased tolerance can, at least in part, be attributed to the early stationary-phase physiology detected within in vitro biofilms compared with the physiology seen in exponentially growing planktonic cells [51,52].

Biofilm formation is also critical in the development of denture stomatitis, affecting ca. 65% of edentulous individuals [53,54]. Despite the use of antifungal drugs to treat denture stomatitis, infection is often re-established soon after treatment [54]. Likewise, candidiasis is associated with indwelling medical devices on which a layer of organic molecules adheres to their surfaces, contributing to biofilm development [54].

For *C. albicans*, biofilm formation is a process that occurs in three stages: (i) an early phase characterised by adhesion of blastospores to the surface; (ii) an intermediate phase where yeast cells proliferate to cover a large surface area and have begun to produce extracellular polymers; and (iii) a maturation phase [55]. Mature *C. albicans* biofilms are matrix entrenched and arranged into layers, with yeast cells attached to the surface with hyphae on the outer surface of the biofilm [54,55].

Using the PMMA biofilm model, Chandra et al. [54] showed that grown biofilm *C. albicans* cells are highly resistant to antifungal agents such as fluconazole, nystatin, amphotericin B and chlorhexidine. The progression of drug resistance was associated with the concomitant increase in metabolic activity of developing biofilms. This indicated that the observed increase in drug resistance was not simply a reflection of lower metabolic activity of cells in maturing biofilms but that drug resistance develops over time, coinciding with biofilm maturation.

There are still few effective control strategies and they are poorly understood in many contexts. Many antimicrobial agents that are effective against planktonic cells turn out to be ineffective against the same bacteria growing in a biofilm [56,57]. Combined application of multiple antimicrobial agents with different chemistries and modes of action may be a strategy to improve the performance of these antimicrobial agents and circumvent bacterial adaptation [57].

Biofilms can remove minerals and metals from the liquid phase that they are in contact with [58]. In particular, the exopolysaccharides of Gram-negative bacteria play an important role in

metal biosorption [58]. The binding affinity depends on the cation size/charge ratio, the bacterial polysaccharide charge, the pH and the physical state of the biofilm [59]. Thus, silver would inhibit biofilm development because the biofilm absorption capacity should be exceeded with higher silver concentrations or longer exposure times [58].

Harrison et al. [55] examined how metal ions may affect cellular differentiation in *C. albicans* and *Candida tropicalis* biofilms. They found that subinhibitory concentrations of metal ions (CrO_4^{2-}, Co^{2+}, Cu^{2+}, Ag^{+}, Zn^{2+}, Cd^{2+}, Hg^{2+}, Pb^{2+}, AsO_2^{-} and SeO_3^{2-}) caused changes in biofilm structure by blocking or eliciting the transition between yeast and hyphal cell types. In the case of Ag^{+}, morphological changes occurred in *C. albicans* biofilms around 0.04 mM, whereas for Pb^{2+} there was no observed change in cell morphology at concentrations of 24 mM. To these authors, certain yeasts have the potential to overwhelm some bacterial species normally present in sites that have been polluted with metals. In principle, this may occur because bacterial biofilms are killed with comparatively low concentrations of metal ions, whereas biofilms of yeasts, such as *Candida* spp., may continue growing.

Biofilm formation by *Candida* spp. is a process that normally involves differentiation, and community maturation, concomitantly arising with drug resistance [55]. To Harrison et al. [55], this cumulative evidence led them to hypothesise that metal ions may influence cellular differentiation, community structure and antifungal resistance of mature biofilms. Cellular polymorphism in *Candida* populations may be significant, as mature biofilms are more resistant to antifungal agents than those at an earlier stage of development [54]. The polymorphic character of *Candida* spp. can act in pathogenic biofilm formation in some plants and animals, as hyphae may help the invasive penetration of physical barriers [55,60].

The cells of biofilm can be more resistant to the metal action than the planktonic cells of *Candida* spp. Harrison et al. [61] observed that biofilm *C. tropicalis* were up to 65 times more tolerant to death by metals than corresponding planktonic cultures. Ag^{+} was highly toxic to planktonic cells, inhibiting growth and killing this form of *Candida* at concentrations of ca. 0.5–1.0 mM and 20–25 mM, respectively. However, biofilms were highly tolerant to Ag^{+} and were not killed at the highest concentration added in vitro (150 mM). Of the majority of the metals tested, only very high concentrations of CrO_4^{2-} (100 mM) and Cu^{2+} (64 mM) killed surface-adherent *Candida*. Hg^{2+} was the most toxic metal tested, inhibiting yeast growth and killing both planktonic cells and biofilms over a concentration of 0.5–2.0 mM. *Candida* biofilms may adsorb metal cations from their surroundings and sequestration in the extracellular matrix may contribute to resistance [61]. Therefore, *Candida* spp. may survive bactericidal concentrations of these compounds and may continue to grow as biofilms [61].

Bjarnsholt et al. [51] studied the action of silver on mature in vitro biofilms of *P. aeruginosa*. Concentration of 5–10 μg/mL silver sulfadiazine eradicated the biofilm, whereas a lower concentration (1 μg/mL) had no effect. The bactericidal concentration of silver required to eradicate the biofilm was 10–100 times higher than that used to eradicate planktonic bacteria. This indicates that the concentration of silver in currently available wound dressings is too low for treatment of chronic biofilm wounds. It is suggested that clinicians and manufacturers of wound dressings consider whether they are treating wounds primarily colonised either by biofilm-forming or planktonic bacteria.

SI at 50 ppb concentrations are effective antimicrobial agents against *Staphylococcus epidermidis* planktonic cells [49]. However, SI have been shown to be ineffective against cells within the biofilm [49]. SI are active only at the periphery of the biofilm and therefore are ineffective at penetrating the deeper core of the biofilm where the bulk of the cells are usually present [62]. This suggested that a small dosage of SI is insufficient for releasing excess unbound SI for antimicrobial action and is unsuitable for the treatment of biofilm infections [49].

The use of combinations of agents that have similar antimicrobial behaviours might be an effective strategy for preventing microbial adaptation and facilitating the antimicrobial actions of the agents. Kim et al. [57] found that the combination and sequential treatments with silver and tobramycin showed an enhanced antimicrobial efficiency of more than 200% on *P. aeruginosa* biofilm.

The antimicrobial action of the silver against the formation of biofilm is also a time-dependent process. Stobie et al. [2] determined that the release of SI from silver-doped phenyltriethoxysilane sol–gel coating reduced the adhesion and prevented formation of a *S. epidermidis* biofilm over 10 days. Silver-doped coatings also exhibited significant antibacterial activity against planktonic *S. epidermidis*.

Phosphate-based glass disks containing 5 mol%, 10 mol% and 15 mol% silver significantly reduced the number of viable *Streptococcus sanguis* over 24 h, and after 48 h this number increased [63]. This reduction and then recovery pattern is due to the silver initially killing the bacteria, and the recovery is due to the dead bacteria forming a layer through which the ions must diffuse [63]. Thus, the amount of silver released is reduced and the bacteria can keep growing.

Valappil et al. [45] examined the effect of increasing silver contents (10 mol%, 15 mol% or 20 mol%) in phosphate-based glasses to prevent the formation of *S. aureus* biofilms. Silver was an effective bactericidal agent against *S. aureus* biofilms and the rates of silver ion release were 0.083 ppm/h, 0.055 ppm/h and 0.064 ppm/h for the Ag10, Ag15 and Ag20 glasses, respectively.

Saravanapavan et al. [64] demonstrated the minimum bactericidal concentration of silver was 0.1 ppm and that the cytotoxic concentration was 1.6 ppm for human cells. To investigate its bioactivity, they immersed gel-glass foams used for orthopaedic and craniomaxillofacial tissue in simulated body fluid at different times. Cellular responses were assessed by seeding primary human osteoblasts on the surface of silver-doped S70C30 foam substrates. Nevertheless, Valappil et al. [45] called attention to the fact that it was unclear whether these levels of 0.1 ppm and 1.6 ppm were total values or whether they were rates in hours, days, etc.

According to the previous authors [45], the amount of silver released was below the levels that were cytotoxic for human cells [64]. Despite this, if high concentrations of free SI are needed for a bactericidal effect against biofilms, it is crucial not to sacrifice any cyto/biocompatibility aspects of the material while maintaining an effective antimicrobial action [45].

4. Conclusion

Silver antimicrobial agents have been pursued as an alternative strategy for reducing bacterial adhesion and to prevent biofilm formation. Antibacterial experiments demonstrated that silver is effective against a broad range of bacterial cells and mature biofilms, however the concentration is an important factor. The current review suggests that elementary silver, SZ and SN in polymers can constitute effective antimicrobial biomaterials for a variety of promising applications. Owing to the much larger specific surface area, SN can be used in lower concentrations without reducing the material's mechanical properties.

SI release depends on the nature and concentration of the silver antimicrobial material as well as the polymer matrix. In future, other studies should be directed towards programming the silver release in accordance with the necessity of each biomaterial. Moreover, studies regarding mechanisms of binding between silver and polymer matrix to create biocide materials would also be truly opportune.

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