Journal of Optometry

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ORIGINAL ARTICLE

Ability of silver-impregnated contact lenses to control microbial growth and colonisation

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Abstract

Purpose: To examine the ability of silver nano-particles to prevent the growth of *Pseudomonas aeruginosa* and *Staphylococcus aureus* in solution or when adsorbed into contact lenses. To examine the ability of silver nano-particles to prevent the growth of *Acanthamoeba castellanii*.
Methods: Etafilcon A lenses were soaked in various concentrations of silver nano-particles. Bacterial cells were then exposed to these lenses, and numbers of viable cells on lens surface or in solution compared to etafilcon A lenses not soaked in silver. *Acanthamoeba* trophozoites were exposed to silver nano-particles and their ability to form tracks was examined.
Results: Silver nano-particle containing lenses reduced bacterial viability and adhesion. There was a dose-dependent response curve, with 10 ppm or 20 ppm silver showing > 5 log reduction in bacterial viability in solution or on the lens surface. For *Acanthamoeba*, 20 ppm silver reduced the ability to form tracks by approximately 1 log unit.
Conclusions: Silver nanoparticles are effective antimicrobial agents, and can reduce the ability of viable bacterial cells to colonise contact lenses once incorporated into the lens.
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Capacidad de las lentes de contacto impregnadas con plata para controlar el crecimiento y colonización microbiana

Resumen

Objetivos: Examinar la capacidad de las nanopartículas de plata para prevenir el crecimiento de *Pseudomonas aeruginosa* y *Staphylococcus aureus* en soluciones para lentes de contacto o cuando éstas las adsorben. Examinar la capacidad de las nanopartículas de plata para prevenir el crecimiento de *Acanthamoeba castellanii*.

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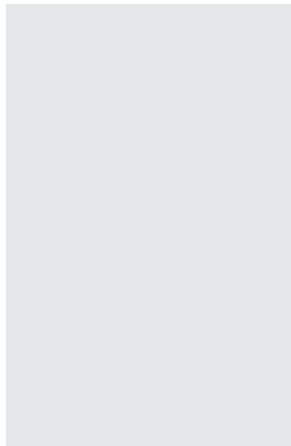
Métodos: Se sumergieron lentes etafilcon A en diversas concentraciones de nanopartículas de plata. Las células bacterianas fueron posteriormente expuestas a dichas lentes, y se compararon cantidades de células viables en la superficie de la lente o en la solución con las presentes en lentes etafilcon A que no habían sido sumergidas en plata. Trofozoítos de *Acanthamoeba* fueron expuestos a nanopartículas de plata y se examinó su capacidad para formar quistes.

Resultados: Las lentes que contienen nanopartículas de plata redujeron la viabilidad bacteriana y la adhesión. Hubo una curva de respuesta dependiente de la dosis, en la que 10 ppm o 20 ppm de plata mostró una reducción logarítmica > 5 en la viabilidad bacteriana tanto en la solución como en la superficie de la lente. Para *Acanthamoeba*, 20 ppm de plata redujeron la capacidad de formar quistes en aproximadamente 1 unidad logarítmica.

Conclusiones: Las nanopartículas de plata son agentes antimicrobianos eficaces y pueden reducir la capacidad de células bacterianas viables para colonizar las lentes de contacto una vez que se han incorporado en la lente.

Introduction

Contact lenses, whilst being an excellent form of vision correction, are a significant risk factor for keratitis. The most serious form of keratitis, microbial keratitis, is a frank infection of the cornea. Epidemiological studies of contact lens wear over the past 20 years have shown that there remains an almost constant rate of microbial keratitis associated with wear; 2-4/10,000 wearers per year if lenses are worn on a daily wear schedule and 20-26/10,000 wearers per year if worn on an extended wear (i.e. sleep in lenses) schedule.[1-3] Furthermore, contact lens wear is associated with other non-infectious forms of keratitis including contact lens induced acute red eye (CLARE) and contact lens induced peripheral ulcers (CLPU).[4-8] The rates of the non-infectious keratitis events is higher than that for MK; around 10/100 wearers per year.[9] With the advent of silicone hydrogel lenses, and their frequent prescription for new fits,[10] studies have shown that the rate of MK is approximately the same in extended wear but higher in daily wear[3] whereas the rate of many of the non-infectious keratitis conditions is approximately twice as high with the silicone hydrogel lenses as with non-silicone hydrogel soft lenses.[11] Bacteria are the most common cause of MK (especially *Pseudomonas aeruginosa*), CLARE and CLPU, with any lens type.[5-8,12] However, in rare cases *Acanthamoeba* and fungi can also cause MK.[13-16]

The rate of these microbially-driven adverse responses have lead researchers and the contact lens industry to examine ways of controlling the events, and the development of antimicrobial surface for contact lenses or contact lens storage cases has been proposed.[17,18] Mathews et al.[19] investigated selenium covalently bonded to silicone hydrogel contact lenses in a rabbit model. The selenium-coated lenses reduced the colonization of *P. aeruginosa in vitro* and were safe on animals eyes up to 2 months of extended wear. Willcox et al.[20] and Cole et al.[21] have shown that a contact lens coated with a cationic peptide has broad spectrum antimicrobial activity, and can prevent the development of CLARE and CLPU in animal models. Zhu et al.[22] have shown that contact lenses coated with fimbrolides (bacterial quorum-sensing inhibitors) can also reduce colonisation by bacteria (and *Acanthamoeba* sp.) and are safe to wear in a short term clinical trial.

Silver is a well known antimicrobial agent and has been used to coat catheters to provide antimicrobial surface for a number of years.[23,24] Silver-coated contact lenses have been tested in the laboratory and shown to be effective at reducing the colonisation by *Pseudomonas aeruginosa* but not as effective against *Staphylococcus aureus*.[25] Furthermore, silver has been incorporated into contact lens storage cases by several manufacturers (CIBA Vision Corp., GA, USA; Sauflon Pharmaceuticals, Twickenham, UK; Marietta Vision, GA, USA). A laboratory and clinical study has shown that silver is very effective at preventing adhesion of a range of Gram-negative bacteria to cases, as well as reducing the colonisation of lens cases during use in the clinical trial.[26] The aim of the present investigation was to determine whether colloidal silver nanoparticles could kill microbes in solution, and prevent the colonisation of lenses by bacteria after soaking in colloidal silver nanoparticles.

Methods

Contact lenses

Contact lenses made from etafilcon A (Johnson and Johnson Vision Care, Jacksonville, FL, USA) were removed from their packaging, rinsed three times in phosphate buffered saline and then soaked a colloidal silver nano-particle suspension (NP-Ag; Health Beacons Inc, Seattle, USA), with average particle size of < 1nm suspended in phosphate buffered saline (pH 7.2). Soaking solutions contained either 0.5, 10 or 20 ppm of silver nanoparticles. Lenses were allowed to equilibrate in the NP-Ag suspension for 5 days on average. Control lenses were removed from their package and stored in phosphate buffered saline only. Nano-particulate silver was chosen as it helps stabilise the silver and control the release of silver particles/ions, the antimicrobial form of silver.
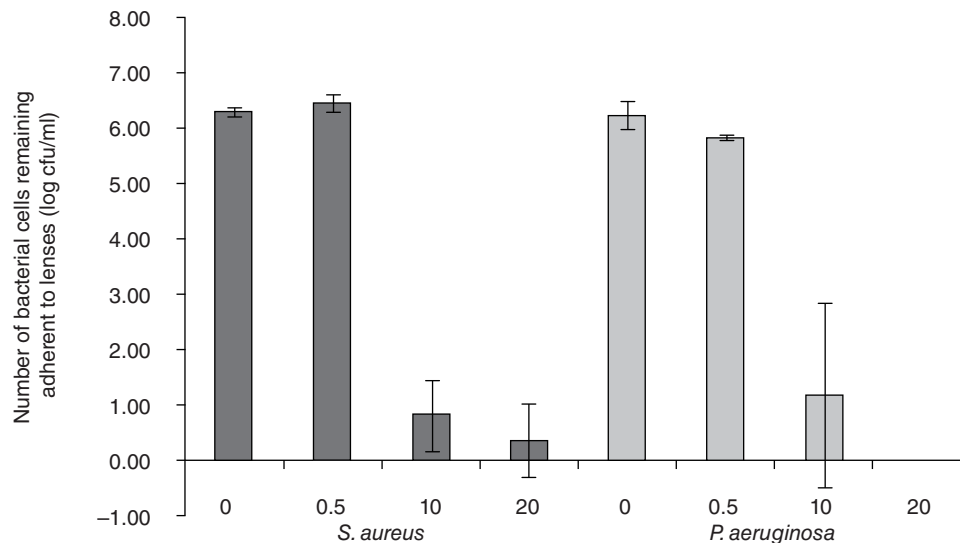
Figure 1 Reduction of number of viable cells of *S. aureus* or *P. aeruginosa* adhered to lenses containing silver.

Bacterial strains and culture conditions

Pseudomonas aeruginosa 6294 (Paer6294, isolated from microbial keratitis) and *Staphylococcus aureus* 31 (Saur31, isolated from contact lens induced peripheral ulcer) were used in the study. Bacterial strains were inoculated from $-80\,^{\circ}$C storage into 10 ml of tryptone soy broth (TSB; Difco laboratories, Sparks, MI, USA) and incubated at $37\,^{\circ}$C overnight. After centrifugation at 3,000 rpm for 10 minutes, bacterial cells were washed once in phosphate buffered saline (PBS) and re-suspended in 1/1000 TSB/PBS for Paer6294 and in 1/50 TSB/PBS for Saur31 to OD_{660nm} 0.1 (equivalent to 10^8 CFU/ml). The bacterial cell suspensions were then serially diluted (1/10) to 10^3 CFU/ml and used for adhesion assay.

Bacterial adhesion

All lenses were washed twice with 1 ml PBS prior to the assay. The lenses were then transferred into 1 ml of bacterial suspension (prepared above) in 24-well tissue culture plates and incubated at $37\,^{\circ}$C for 24 hours. After washing three times in 1 ml PBS (each time shaking for 30 seconds) to remove loosely bound bacteria, contact lens was transferred into a test tube containing 2 ml of PBS and a small stirring bar. The test tube was then vortexed for 1 min at a maximum speed to allow bacterial cells to detach. Following log serial dilution in Dey-Engley neutralising broth (Difco laboratories) which has been used previously to neutralize silver[27], $3 \times 50\,\mu$l of each dilution were plated on a nutrient agar plate for the bacterial counts. After incubation at $37\,^{\circ}$C overnight, colony forming units (CFU) on the plate were counted and converted to CFU/lens by multiplying with the appropriate dilution factor. The bacterial adhesion on test lenses was compared with that on the control lenses, and the reduction of bacterial adhesion was calculated accordingly. Three lenses each from test and control groups were included in each experiment and the experiment was repeated twice (n = 6 lenses for test or control).

Inhibition of bacterial growth

Following the bacterial adhesion assay, bacterial growth in the culture solutions from each test or control lens (i.e. 6 of each) were examined by plating out and enumerating the remaining culture solutions after log serial dilution.

Effect on *Acanthamoeba*

Acanthamoeba polyphaga MCC 3315 trophozoites were produced according to Zhu et al.[22] After growth, the trophozoites were resuspended in PBS to $0.5-1.0 \times 10^7$ Track Forming Units (TFU)/ml. An aliquot (50 μl) was incubated in 5 ml of silver solutions (5 ppm, 10 ppm or 20 ppm) or control PBS for 6 hours at $25\,^{\circ}$C. After incubation, samples were serially diluted 10 fold in D/E neutralizing broth, $4 \times 100\,\mu$l of each dilution were plated on non-nutrient agar plates pre-seeded with *Escherichia coli*. Following 7 days incubation at $32\,^{\circ}$C, the plates were examined for track forming units under a microscope and the number of survivors (TFU/ml) estimated using Reed and Muench computation. The number of survivors for each silver concentration was compared to that of the control sample.

Statistical analysis

The data were log transformed prior to data analysis. Differences between the groups were analyzed using linear mixed model ANOVA, which adjusts the correlation due to repeated observations. Post hoc multiple comparisons were done using Bonferroni correction. Statistical significance was set at 5%.

Results

Figure 1 shows the effect on bacteria adhered to NP-Ag-containing etafilcon A lenses. Adhesion, as measured by the number of viable cells that could be cultured after release from the surface of lenses, was reduced for both
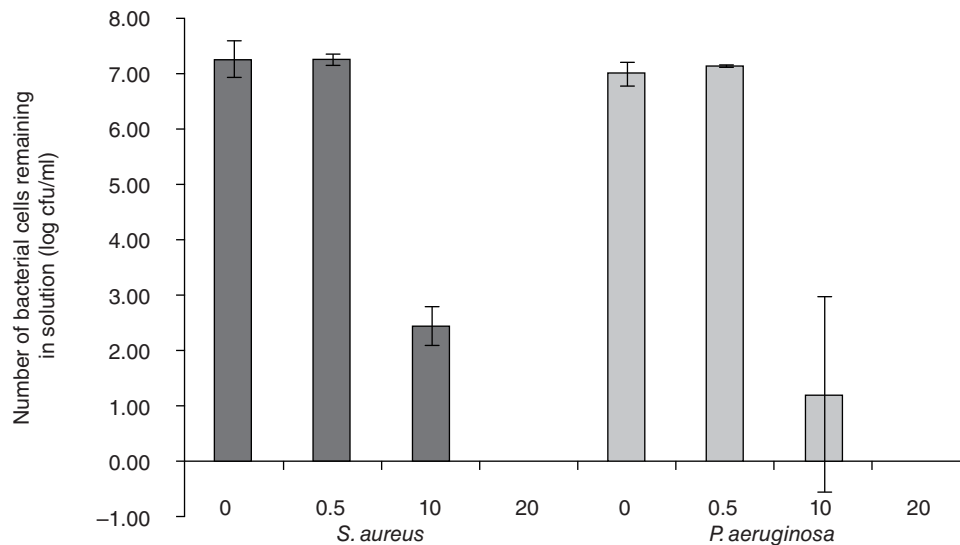
Figure 2   Reduction in bacteria cell numbers in the solution surrounding lenses.
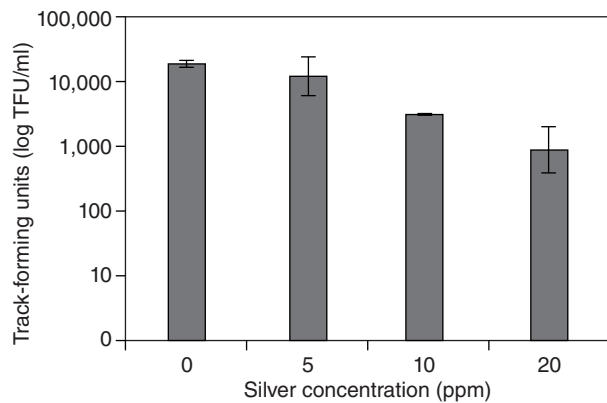

Figure 3   Effect of silver on the number of track-forming units of *Acanthamoeba*.

number of viable cells in suspension after exposure to lenses soaked in 10 or 20 ppm silver and cells in suspension after exposure to lenses soaked in 0.5 ppm silver or not soaked in silver (p < 0.01).

There were no statistically significant differences in the ability of silver-soaked lenses to kill adherent cells or cells in suspension between the two bacterial types, *P. aeruginosa* or *S. aureus.*

The number of track forming units of *Acanthamoeba castellanii* in silver-containing solutions was also examined. Figure 3 demonstrates the reduction seen. Solutions containing both 10 and 20 ppm showed a large reduction in the number of track forming units, with 20 ppm showing an approximate 1 log reduction, indicating that the viability of the exposed trophozoites was reduced in the presence of silver.

types of bacteria. The 1×10^3 bacterial cells/ml initially added to control lenses had grown to *S. aureus* 6.49 ± 0.15 log cfu/lens and *P. aeruginosa* 6.18 ± 0.13 log cfu/lens on the lenses. As can be seen in Figure 1, there was almost a total killing of bacterial cells of either type when adhered to lenses containing 20 ppm silver. There was a dose-dependent response to the amount of silver associated with the lenses, with 0.5 ppm showing almost no activity. For both bacterial types there was a significant difference between adhesion of cells to lenses soaked in 10 or 20 ppm silver and lenses soaked in 0.5 ppm silver or not soaked in silver (p < 0.01).

The number of bacteria in the PBS surrounding the lenses during incubation was also examined (Figure 2). The total number of cfu in the 1 ml of PBS surrounding each control lens after 24 hours incubation was 7.28 ± 0.35 log cfu for *S. aureus* and 7.04 ± 0.38 log cfu. Lenses with 20 ppm showed almost total kill of cells of either bacteria in the PBS (i.e. not associated with the lens surface) and there was a dose-dependent response. This indicates that the silver was being released into the solution surrounding the lenses during incubation, and was active against the bacteria. For both bacterial types there was a significant difference between

Discussion

This research has demonstrated the ability of silver in solution to control the growth of *S. aureus*, *P. aeruginosa* and *Acanthamoeba castellanii*. In addition, silver, presumably leached from the soaked lens, could reduce the adhesion to a contact lens surface by *P. aeruginosa* and *S. aureus*. Unlike a previous report[25] we found no evidence of more activity against *P. aeruginosa* compared with *S. aureus.* Silver has been shown to be effective as an anti-bacterial agent when associated with various biomaterials. An endotracheal tube coated with silver could reduce adhesion of several strains of *P. aeruginosa* by at least 90% (i.e. greater than 1 log unit reduction),[28] and silver nano-particles were effective in killing *S. aureus* and *P. aeruginosa* when incorporated into poly(vinyl alcohol)-b-poly(acrylonitrile) micelles.[29] Similarly, silver coated onto perfluoropolyether-urethane is active against *P. aeruginosa* and *S. aureus*.[30] Effects of silver on *Acanthamoeba* sp. has not been studied in great detail to date. Sökmen et al.[31] have shown that silver may promote the anti-amoebal effects of UV and titanium oxide. Schuster and Jacob[32] demonstrated that silver increased

the anti-amoebal effects of the cationic peptide magainin against *Acanthamoeba polyphaga*.

Comparisons can be made with the anti-bacterial anti-adhesion activity of other coatings for contact lenses. Zhu et al.[22] demonstrated that fimbrolides reduced the adhesion of *P. aeruginosa* by 67% and of *S. aureus* by 87%. Willcox et al.[20] reported that the cationic peptide Melimine could reduce the adhesion of *P. aeruginosa* by 92% and of *S. aureus* by 76%. Both of these studies report values that are well below the > 5 log reduction in the present study for lenses soaked in 10 ppm silver. If the degree of bacterial adhesion is associated with the rate of adverse events, then those substances that reduce adhesion the most might be supposed to have the greatest effect on the incidence of adverse events. The study by Mathews et al.[19] that examined the effect of selenium-coating of contact lenses did not give values for the reduction in adhesion of *P. aeruginosa* so no direct comparison can be made with this study.

In *in vivo* studies, silver-coated endotracheal tubes were associated with reduced mortality in patients with ventilator-associated pneumonia (silver vs control, 5/37 [14%] vs 20/56 [36%]; p = 0.03).[32] Silver catheters were found to significantly reduce the incidence of asymptomatic bacteriuria in hospitalized adults catheterized for < 1 week (RR 0.54, 95% CI 0.43-0.67) or > 1 week (RR 0.64, 95% CI 0.51-0.80), and may be more beneficial than antibiotic-coated catheters when used > 1 week.[24] Chlorhexidine-silver sulfadiazine catheters reduce colonisation (odds ratio [OR] 0.51 [95% CI 0.42-0.61]) and catheter-related bloodstream infection (OR 0.68 [0.47-0.98]).[34] However, other reports have shown no benefit for use of silver-containing catheters in controlling blood stream infections.[35] The publication by Amos and George[26] on the effectiveness of silver-containing contact lens cases demonstrated that these cases had significant reductions in colonisation rates during use, in particular associated with reductions in contamination by Gram-negative bacteria.

Resistance to silver seems to be very rare and difficult for bacteria.[36] Genes that mediate resistance to silver are known to occur in bacteria,[37] although these genes have only been found infrequently and even when present a silver-containing wound dressing was able to reduce bacterial growth after 24h exposure.[38,39] The presence of silver may even reduce resistance to conventional antibiotics.[40] Another potential issue with the use of silver in the eye is the production of ocular argyrosis. Ocular argyrosis has also been reported following accidental exposure to high levels of silver solder, with the level of argyrosis being associated with duration of exposure to the silver solder.[41,42] In another report, a person wearing soft lens containing 20% (100,000 ppm) silver nitrate in water for 17 years for the management of diplopia developed argyrosis.[43] As the lenses in the current study had excellent antimicrobial effects at 20 ppm, it is unlikely that argyrosis would occur in lens wearers, even, perhaps, if they wore the lenses on a continuous basis (24h/day) for many years.

In conclusion, this preliminary investigation of the effectiveness of silver incorporated into contact lenses has shown that the silver can prevent colonisation of the lens surface by a strain of *P. aeruginosa* or *S. aureus*. In addition, we demonstrated activity of silver against *Acanthamoeba*. The present research, whilst conducted in soft etafilcon A contact lenses, may have applicability to silicone hydrogel lenses as well, although further research to determine the most appropriate silver loading concentration would be required as the silicone hydrogel lenses tend to have lower water contents than the etafilcon A material. These findings, taken with the overall reports of benefits of using silver-coated biomaterials, highlights the potential of silver-containing contact lenses in reducing the incidence of microbially-driven adverse events.

Financial disclosure

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