Application of silver antibacterial nanolayers for hard contact lenses coating

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Abstract. The antibacterial action of Ag-doped Al2O3 nanolayers, deposited by RF reactive magnetron sputtering on hard contact lenses, was established. Synthesis of Ag/Al2O3 protective coatings is necessary for suppressing the infections caused by pathogenic microorganisms following the placement of hard contact lenses. The chemical composition, morphology and optical properties of the coatings were studied. Microbiological studies conducted of the nanocomposite Ag/Al2O3 layers against Gram-positive and Gram-negative bacteria (Pseudomonas aeruginosa, Escherichia coli, and Staphylococcus aureus) proved their antibacterial effects. Intense action of the layers was found against Pseudomonas aeruginosa – full inactivation after 2 hours; Escherichia coli - full inactivation after 5 hours; Staphylococcus aureus – full inactivation after 24 hours. Our experimental findings suggest a very promising application of such antibacterial Ag/Al2O3 nanolayers regarding the reduction of eye infections when the hard contact lenses are used.

1. Introduction
Contact lenses are widely used to treat myopia, hyperopia, astigmatism, and presbyopia. Different types of lenses have been applied in the practice: rigid or “hard” contacts - made from polymethyl methacrylate (PMMA); rigid gas-permeable lenses (RGP's) - made from plastics combined with other materials, which allow oxygen in the air to pass directly through the lens; soft contact lenses - made from hydrated polymer, hydroxyl-ethyl methacrylate (HEMA) [1].

Developing eye infections is a potential danger for people wearing contact lenses [2]. Concerning the eye infections, the contact lens are associated with several risk factors such as microbial keratitis and conjunctivitis [2].

Previous studies suggested that patients who do not follow the contact lens hygiene recommendations run greater risk for corneal inflammatory events [3-5].

Although, the hard contact lenses have been used less than the soft lenses, they are the most appropriate choice for some eye diseases like astigmatism and keratokonus. While, the rigid gas permeability lenses have been used to perform ortho-k, a non-surgical procedure designed to improve vision.

It is well known that silver (Ag) particles possess antibacterial properties [5-8]. They act as antioxidants, blocking cellular processes and leading to bacterial cell death. Already, the antibacterial activity of Ag
nanoparticles has been determined against Gram-positive Staphylococcus aureus and Gram-negative Escherichia coli and P. aeruginosa bacteria and Candida albicans by many authors [9-11].

The silver nanocomposite layers are a promising tools for preventing eye infection. As a broad-spectrum agent, it kills a wide range of microorganisms in low levels without associated toxicity [11,12]. The antibacterial power of silver nanoparticles has been widely documented [13-20], although the exact mechanism causing it is not known well. It is believed that the silver provokes inactivation of enzymes essential for the respiratory chain of the pathogens, or generates hydroxyl radicals [15,16] that, in turn, would cause damage to the pathogen.

The purpose of this study was to synthesize Ag/Al$_2$O$_3$ nanocomposite layers on hard contact lenses with a broad-spectrum antibacterial effect on Gram-positive and Gram-negative bacteria; and to investigate the chemical composition, optical and morphological properties of the layers as well as their antibacterial activity.

2. Experimental

2.1. Material synthesis and Analytical methods

The nanocomposite thin layers of Ag/Al$_2$O$_3$ were synthesized on hard contact lenses and on glass substrates (to facilitate the analytical analyses) by RF magnetron co-sputtering of Ag and Al under the following conditions: frequency 14 MHz; working pressure in the vacuum system was fixed at $\sim$ 5.5 Pa with optimal mixture of Ar and O$_2$ gasses. The optimal concentration of Ag in the Al$_2$O$_3$ matrix was obtained by precise control of the process parameters during the deposition.

The surface elemental composition of the nanolayers on the glass samples was investigated by X-ray photoelectron spectroscopy (XPS) on an ESCALAB MkII (VG Scientific) electron spectrometer at a base pressure in the analysis chamber of $5 \times 10^{-8}$ Pa (during the measurement $1 \times 10^{-6}$ Pa), using an AlK$\alpha$ X-ray source (excitation energy of 1486.6 eV). The thickness and the optical transmission of the layers in UV-Visible spectrum were determined by using a Woollam M2000D rotating compensator spectroscopic ellipsometer and an Ocean Optics HR 4000 spectrophotometer, respectively. Scanning electron microscopy (SEM) (SEM/FIB Lyra/Tescan dual beam system) was used to investigate the coatings’ surface morphology.

2.2. Antibacterial activity assay

Microbiological assay was carried out to examine the antibacterial properties of the coatings in regard to deactivation of Gram-positive and Gram-negative bacteria. The strains used in these experiments were: Staphylococcus aureus strain 29213 and Escherichia coli strain 35218 of the American Collection of Cell Cultures (ATCC); and Pseudomonas aeruginosa strain 1390 of the collection of “Stefan Angeloff” Institute of Microbiology, Bulgarian Academy of Sciences.

500 $\mu$L of microbiological suspension of the respective microorganisms in saline with concentration of $1 \times 10^6$ CFU/mL (colony-forming units per mL) was plated in 24 wells plate for cell culture. Uncoated hard contact lenses were placed in the control wells and the hard contacts lenses covered with the test coating of Ag/Al$_2$O$_3$ were placed in the sample wells.

Samples and controls were incubated at a temperature of 37 °C and shaken continuously. At predefined intervals of 0, 2, 5, and 24 hours, the samples were taken to determine the number of viable microorganisms. This was performed by seeding ten-fold diluted solutions of the incubation mixtures and plating in 25 $\mu$L nutrient medium of Tryptic Soy Agar for microbiology (Sigma-Aldrich). After 24 hours of incubation at 37 °C, the number of bacterial colonies emerged. All the results were expressed in CFU/mL.

2.2.1. Statistics

Each experiment was performed in triplicate and the data were presented as a mean ± standard deviation (SD). The difference between two means was compared by a two-tailed unpaired Student’s test. The values of P<0.05 were considered as significant.
3. Results

3.1 Analytical characterisations

The thickness measured of the Ag/Al₂O₃ layers synthesized and deposited on the substrates was about 18 nm was. All layers were highly transparent in the visible light spectrum with transmittance of about 95%. The optical transparency is great advantage, since it makes the as-obtained layers suitable for application like transparent coatings on eye lenses. The SEM imaging (figure 1) showed a uniform surface morphology without cracks, which determines the possibility for successful applications as coatings on eye lenses. Quasi spherical nanoparticles with average size distribution about 100 nm were formed. This leads to an increase of the active surface area of the layers thus further promoting their antibacterial function as protective coating.

![SEM image](image)

Figure 1. SEM image of the surface morphology of the Ag/Al₂O₃ nanocomposite layer

XPS measurements were performed to assess the surface chemical composition and the corresponding concentration of the Ag in the layers synthesized. The binding energies of peaks in the high resolution XPS spectra are assigned as Ag3d, Al2p, O1s and Si2p shown in Table 1 [21,22]. Based on these values the atomic concentrations are calculated and shown in the Table 1 as well.

| Photoelectron peaks | O1s  | Al2p | Si2p | Ag3d |
|---------------------|------|------|------|------|
| Concentration [at.%] | 33.20| 29.29| 28.13| 9.38 |
| Binding Energy [eV]  | 531.7| 74.4 | 103.6| 368.7|

3.2 Antibacterial test

The antibacterial activity of the layers deposited on hard contact lenses was examined by microbiological tests as described above.

The most effective activity of the layers was found against *Pseudomonas aeruginosa*. Complete elimination of pseudomonads occurred within two hours (figure 2). Reduction of 4 log in bacterial count
of *Escherichia coli* was observed at the 2\textsuperscript{nd} hour. The bacteria were eliminated completely after the fifth hour (figure 3). A ten-fold reduction of the *Staphylococcus aureus* bacterial count was observed at the fifth hour, and complete inactivation of the bacteria was established at the 24\textsuperscript{th} hour (figure 4).

**Figure 2.** Number of viable *Pseudomonas aeruginosa* cells incubated for 24 hours at temperature of 37 °C with pristine eye lenses (control) and Ag/Al\textsubscript{2}O\textsubscript{3} layers deposited on eye lenses (sample). Complete inactivation at the 2\textsuperscript{nd} hour

**Figure 3.** Number of viable *Escherichia coli* cells incubated for 24 hours at temperature of 37 °C with pristine eye lenses (control) and Ag/Al\textsubscript{2}O\textsubscript{3} layers deposited on eye lenses (sample). Complete inactivation at the 5\textsuperscript{th} hour
Table 1. Number of viable S. aureus cells (CFU/ml)

| Time  | Control | Sample |
|-------|---------|--------|
| 0 h   | 1000000 | 100000 |
| 2 h   | 10000   | 100    |
| 5 h   | 1000    | 0.1    |
| 24 h  | 100     | 0.1    |

Figure 4. Number of viable Staphylococcus aureus cells incubated for 24 hours at temperature of 37 °C with pristine eye lenses (control) and Ag/Al₂O₃ layers deposited on eye lenses (sample). Complete inactivation at the 24th hour.

4. Discussion
The antimicrobial efficiency of silver nanoparticles was reported by Salomoni R. et al. [17,18] on microbial keratitis causing microorganisms. Authors Maryam Shayani et al. [19] explored the effect of monomer composition on silver nanoparticles' (SNPs) binding capacity of hydrogels and evaluated their antibacterial efficacy. They established that SNP-loaded hydrogels demonstrate excellent antimicrobial effects against P. aeruginosa and S. epidermidis after soaking in 10 and 20 ppm SNP suspensions. In their work the SEM images revealed inhibitory effect of SNPs against biofilm formation on the surface of the hydrogels. We obtained similar results for Ag/Al₂O₃ coatings on hard lens surface.

Our research found that the Ag/Al₂O₃ coatings exhibited antibacterial action against Gram-positive and Gram-negative bacteria (Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus).

5. Conclusions
Optically transparent antibacterial Ag/Al₂O₃ nanocomposite layers were synthesized. The Ag/Al₂O₃ coating were deposited by using RF magnetron sputtering on the hard contact lenses. Antibacterial effects against Pseudomonus aerogginosa, E. Coli, and Staphylocococcus aeroginosa were established. The antimicrobial properties of the surface of Ag doped Al₂O₃ synthesized nanocomposite products are very promising for many biomedical applications.

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