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# Application of Silver Antibacterial and Antifungal Nanolayers for Ocular Prostheses Coating

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**The paper reports on antibacterial and antifungal properties of Ag-doped $Al_2O_3$ nanolayers deposited by RF reactive magnetron sputtering on glass ocular prostheses. The study is provoked by the need of suppressing the infections caused by pathogenic microorganisms following the placement of ocular prostheses. This brings about the idea of forming protective coatings with antibacterial and antifungal properties on the prostheses. The surface elemental composition and the morphological characteristics of the coatings are investigated by XPS and SEM measurements. Further, the microbiological studies are conducted to establish the antibacterial and antifungal action of the nanocomposite $Ag/Al_2O_3$ layers against Gram-positive and -negative bacteria, and *Candida albicans*. The strongest action of the layers is found against *Pseudomonas aeruginosa* – full inactivation after 2 h; *Escherichia coli* and *Candida albicans* – full inactivation after 5 h; *Staphylococcus aureus* – full inactivation after 24 h. The experimental findings suggest a very promising application of such antibacterial and antifungal $Ag/Al_2O_3$ nanolayers regarding the reduction of eye infections when implanting ocular prostheses.**

## 1. Introduction

A still unsolved problem concerning the various types of ocular prostheses are the intra-orbital infections and conjunctivitis caused by microorganisms, such as Gram-positive and -negative bacteria and fungi. Ocular prostheses are used following eye enucleation due to tumors or eye injury, in cases of phthisis following eye inflammation, after eyeball atrophy, in congenital microphthalmia, leucoma, abulbia, etc.[1] The multipurpose ocular prosthesis (see **Figure 1**) consists of a dense body of biocompatible polymer or glass with shape and size of a whole eye. Placing ocular prostheses after eye enucleation is often associated with tissue infections.

It is well known that silver (Ag) particles have antibacterial activity.[2–4] They act as antioxidants, blocking cellular processes, and leading to bacterial cell death. The antibacterial properties of Ag nanoparticles have already been determined against Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli* and *Pseudomonas aeruginosa* bacteria and *Candida albicans*.[5–7] Ocular prostheses have been used,[8] made entirely of polymer (polymethyl methacrylate – PMMA) mixed with silver (300–700 ppm), to achieve antibacterial and antifungal properties, but they lack the necessary efficiency.

In the case considered here, the ocular prosthesis was coated with a *protective* $Ag/Al_2O_3$ nano-layer with antibacterial and antifungal activity. The coating could also be deposited on partial ocular prostheses, as frontal part of the eye, eye shell, or implants – hard or flexible, round or oval of various size, on keratoprostheses, etc. To the best of our knowledge, so far no ocular prostheses have been manufactured with a coating of $Ag/Al_2O_3$ for antibacterial and antifungal activity applications.

This study was focused on: (i) deposition of $Ag/Al_2O_3$ nanolayers on ocular prostheses by RF reactive magnetron sputtering; (ii) investigation of their composition, morphologic and optical characteristics; and (iii) investigation of the antibacterial and antifungal properties, and the cytotoxicity of the coatings.

## 2. Experimental Section

### 2.1. Deposition and Measurements

In this work, in order to investigate the properties, for easy of use the coatings were deposited on glass 15 × 15 mm samples by RF magnetron co-sputtering of Ag and Al under the following conditions: frequency 13.5 MHz; working pressure in the vacuum system ≈5.99 Pa; mixture of Ar as a sputtering gas and $O_2$ as a reactive gas.

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**Figure 1.** Ocular prosthesis.

The surface elemental composition and the corresponding chemical state of the nanolayers was investigated by X-ray photoelectron spectroscopy (XPS). The XPS measurements were carried out using 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 Al $K_\alpha$ X-ray source (excitation energy $h = 1486.6$ eV). The thickness of the nano-layers was determined by ellipsometry measurements performed by using a Woollam M2000D rotating compensator spectroscopic ellipsometer in the wavelength range from 193 nm to 1000 nm in the reflection mode. Optical transmission of the layers in the UV–visible range of spectra was measured by Ocean Optics HR 4000 spectrophotometer. Scanning electron microscopy (SEM) (SEM/FIB Lyra/Tescan dual beam system) was used to investigate the coatings' surface morphology.

### 2.2. Microbiological Activity Analysis

We further carried out microbiological experiments to determine the antibacterial and antifungal properties of the coating considered in suppressing Gram-positive and -negative bacteria, and *C. albicans*. The following strains were used: *S. aureus* strain 29213 and *E. coli* strain 35218 of the American Collection of Cell Cultures (ATCC); *P. aeruginosa* strain 1390 and *C. albicans* strain 74 of the collection of "Stefan Angelov" Institute of Microbiology, Bulgarian Academy of Sciences.

In these experiments, we used glass lamellas covered with Ag/$Al_2O_3$ nanolayers. The lamellas were placed in 12-well polystyrene plates and covered by 800-μL suspension of the respective microorganisms in a concentration (colonies-forming units per mL) of $1 \times 10^6$ CFU mL$^{-1}$. The plates were shaken continuously and sampled at pre-defined intervals (0, 2, 5, and 24 h) to determine the number of viable microorganisms. The latter was carried out by seeding 10-fold diluted solutions of the incubation mixtures in a nutrient medium (Tryptic soy agar, Oxoid) and counting the colonies-forming units (CFUs) after culturing for 24 h at 37 °C.

#### 2.2.1. Statistics

Each experiment was carried out 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 *t*-test. The values of $p < 0.05$ were considered as significant.

### 2.3. Cytotoxicity Test

The in vitro cytotoxicity of Ag/$Al_2O_3$ covered glasses was determined on mouse fibroblasts (L-929 cells – NCTC clone 929: CCL 1, American Type Culture Collection-ATCC) according to ISO 10993-5:2009(E), Annex C (1).[9] The cells were maintained and incubated for 48 h with Ag/$Al_2O_3$ covered glasses following the instructions of Annex C. The test was performed after the cells reached 85–90% of confluence in order to resemble the natural conditions of the tissue environment. Since the population doubling time of this cell line is approximately 48 h, an incubation period of 48 h was chosen for evaluation of the cell viability. The absorbance of viable cells was measured at $\lambda = 550$ nm (the reference wavelength of 690 nm). The fraction of survived cells was calculated as percentage of the untreated controls.

## 3. Results

### 3.1. Physical and Chemical Properties – Characterization

Very thin layers of Ag/$Al_2O_3$ with thickness of about 25 nm were deposited. All layers were highly transparent in the visible light spectrum with transmittance measured around ≈93%. This allows deposition of the layers to the various types of ocular prostheses (listed above) without disturbing the aesthetics of the prosthesis design. SEM investigation revealed uniform surface morphology of the layers, without cracks (**Figure 2**), which is very suitable in terms of their application as ocular prosthesis' coatings. It is seen, that nearly spherically shaped nanoclusters with average size about 100 nm were formed and distributed homogeneously on the surface. This leads to significant increasing of the active surface area of the coatings.

Since it is well-known that the antibacterial effect is due to the presence of silver on the specimen's surface,[2–7] we investigated the elemental composition of the layers by XPS. The results are shown in **Figure 3**. Bearing in mind the binding energy value (368.7 eV) of Ag3d (Figure 3a), the shape of the Auger peak (AgMNN), the kinetic energy value (Figure 3b), and that of the modified Auger parameter ($\alpha' = 725.3$)[10,11] we assumed that on the surface the silver is in silver oxide ($Ag_xO$) and $Al_2O_3$ environment. It is worth noting that $Ag_2O$, as a p-typed semiconductor possessing catalytic properties, has been found to be a highly efficient visible light photocatalyst[12] with key role in the bacterial inactivation of *E. coli*.[13] The values of the content

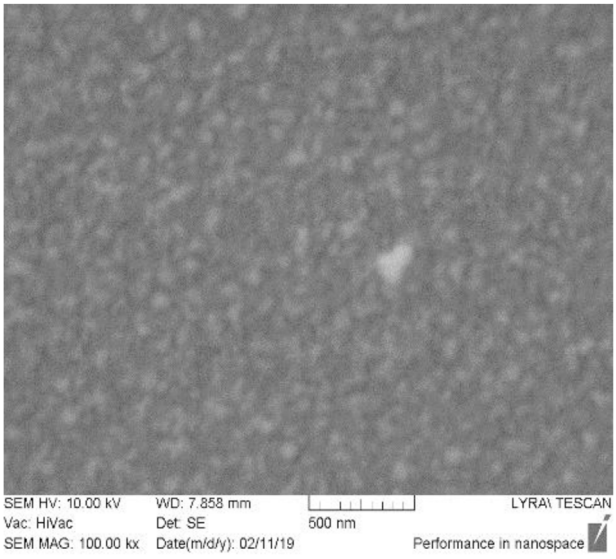


**Figure 2.** SEM image of the surface morphology of the $Ag/Al_2O_3$ nanolayer.

of the elements on the specimen's surface and the $Al_2O_3$ stoichiometry are quoted in **Table 1**.

### 3.2. Microbiological Activity – Characterization

The microbiological studies performed established the antibacterial and antifungal action of the coatings formed on ocular prostheses. The coating was most effective against *P. aeruginosa* – a complete inactivation of the bacteria took place as early as the second hour (**Figure 4**). The coating was also particularly effective against *E. coli* – full inactivation was seen after the 5th hour (**Figure 5**). An effective antifungal action was also observed against *C. albicans* fungi, which were completely inactivated at the 5th hour (**Figure 6**). The count at the 24th hour confirmed the complete inactivation of the aforementioned microorganisms. Regarding to the *S. aureus*, the coating reduced their number by 1.5 logs at the fifth hour and inactivated them completely at the 24th hour (**Figure 7**).

**Table 1.** Surface atomic concentrations of the elements measured on the surface of the glass substrate and $Ag/Al_2O_3$ layers.

| Photoelectron peaks | O1s | Al2p | Si2p | Ag3d | Na1s |
|---|---|---|---|---|---|
| Glass Substrate Concentration [at%] | 67.47 | – | 30.73 | – | 1.8 |
| $Ag/Al_2O_3$ layers Concentration [at%] | 33.11 | 29.38 | 28.03 | 9.48 | – |

### 3.3. Cytotoxicity Test – Characterization

The knowledge of the eventual cytotoxic effect of the nanocomposite $Ag/Al_2O_3$ layers is necessary in terms of their new application like protective coatings on ocular prostheses with antibacterial and antifungal action. For safe application of this nanocomposite layer, like implantable interface with direct contact with soft tissues their cytotoxicity on mouse fibroblasts was determined. In the **Table 2**, the absorbance values corresponding to the controls and to the viable cells are presented, which were used to define the fraction in percentage of the cells survived in comparison with the untreated ones. It was found that the viability of the mouse fibroblasts decreased with approx. 5% after 48 h of $Ag/Al_2O_3$ layers exposure, which is in the range of the standard deviation of the control sample.

## 4. Discussion

The long-term use of ocular prostheses may cause in a number of adverse effects. The contact with foreign bodies often causes a secondary infection, a serious complication which is not easy to control once the ocular prostheses are inserted. This is why patients often find it difficult to wear the prostheses. What is more, the ocular prostheses may alter the normal bacterial flora

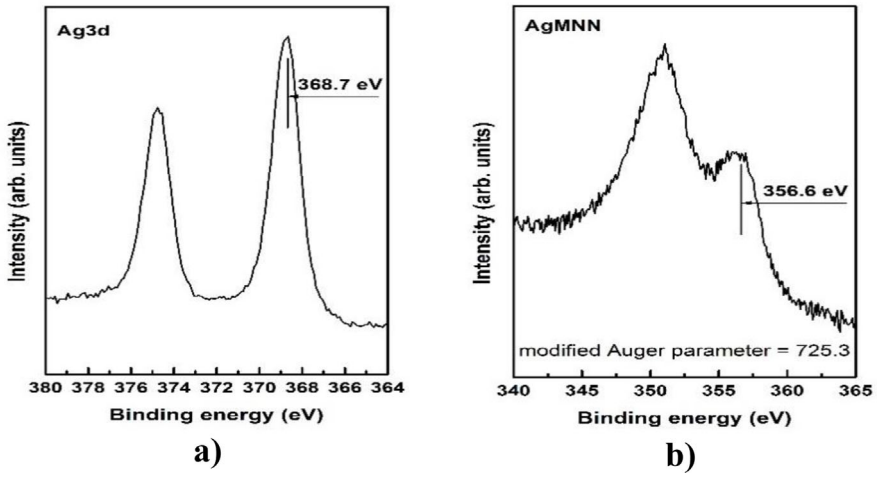


**Figure 3.** XPS analysis: a) Photoelectron line – Ag 3d; b) Auger electron line – Ag MNN.

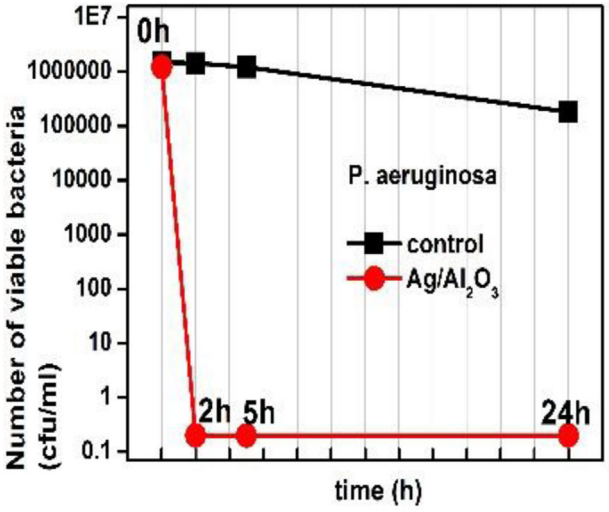


**Figure 4.** Antibacterial action of $Ag/Al_2O_3$ layers against *Pseudomonas aeruginosa* – complete inactivation of the bacteria at the 2nd hour.

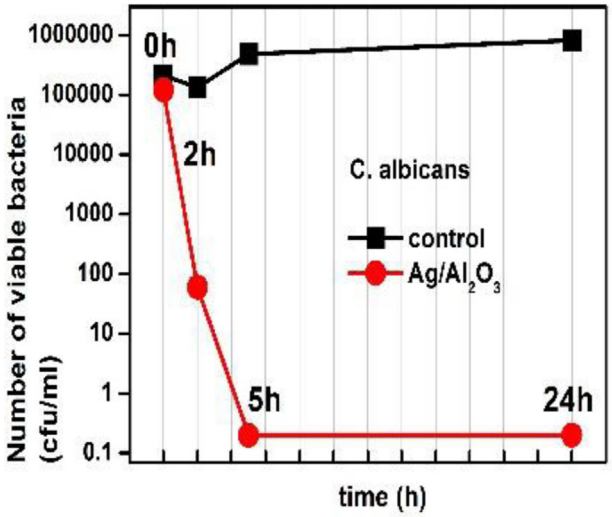


**Figure 6.** Antifungal action of $Ag/Al_2O_3$ layers against *Candida albicans* – complete inactivation of the fungi at the 5th hour.

in the patient's conjunctiva. One way of overcoming such postoperative infections is improving the antimicrobial properties of the prosthetic surface.

The ocular prosthesis proposed by us is with enhanced functionality and prevents the orbital content and the ocular appendages from inflammation processes. It is convenient and comfortable and with high biocompatibility.

Silver has already proved itself as a powerful inorganic compound, and can, if used correctly, prevent infection. As a broad-spectrum agent, it kills a wide range of microorganisms in low levels without associated toxicity.[6,14] The antibacterial power of silver nanoparticles has been widely documented,[15,16] although the exact mechanism causing it is not known. It is believed that it may derive from the inactivation of essential enzymes for the respiratory chain of the pathogen or by the generation of hydroxyl radicals[17,18] that, in turn, would cause damage to the pathogen. With regard to the above, Yang et al.[8] included silver particles in amounts of 300–700 ppm in a PMMA resin used to make an ocular prosthesis. Then, they assessed and compared the growth of different microorganisms (*Streptococcus pneumoniae*, *S. aureus*, *P. aeruginosa*, and *E. coli*) on the surface of materials containing these particles and the surface of others that did not (controls). The results showed antimicrobial activity between 4.8 and 6.2 times higher in materials that contained silver particles compared to controls, with a reduction of up to 99.9% of the bacterial load of the forms. The comparative analysis that we conducted with the work cited showed that the ocular prosthesis coatings of $Ag/Al_2O_3$ proposed by us exhibits a faster antimicrobial and antifungal effect.

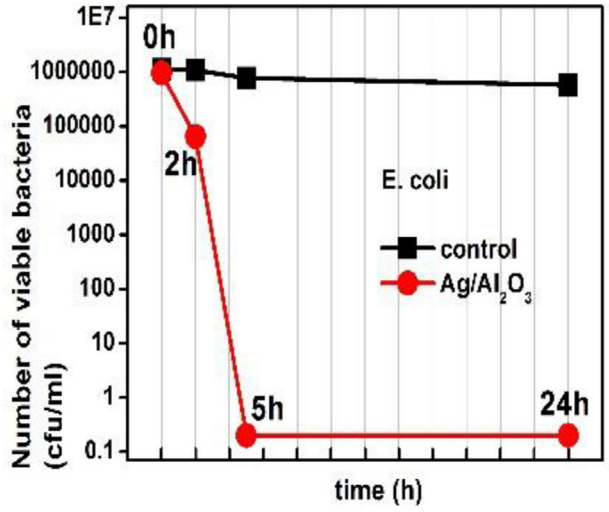


**Figure 5.** Antibacterial action of $Ag/Al_2O_3$ layers against *Escherichia coli* – complete inactivation of the bacteria at the 5th hour.

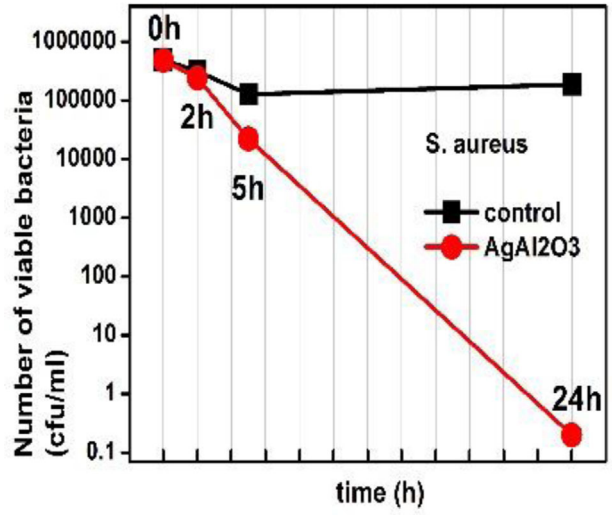


**Figure 7.** Antibacterial action of $Ag/Al_2O_3$ layers against *Staphylococcus aureus* – reduction of the viable bacteria's number by 1.5 logs at the 5th hour and complete inactivation at the 24th hour.

**Table 2.** Cytotoxicity of $Ag/Al_2O_3$ layers covered on mouse fibroblasts after 48 h of incubation.

| | Absorbance $\lambda = 550$ nm/ref 690 nm | [%] |
|---|---|---|
| Control | $0.700 \pm 0.046$ | $100.00 \pm 6.597$ |
| Sample | $0.667 \pm 0.027$ | $95.169 \pm 3.829$ |

Also, it is worth to note that Liu et al.[13] investigated the photocatalytic and antibacterial properties of series of $TiO_2/Ag_2O$ heterostructures with different mass ratio, synthesized by a facile chemical precipitation method. These structures revealed effective visible light-driven photocatalytic properties for inactivation of *E. coli*. However, the structures still have nanoparticulate nature, but not the layer character, as it is in our experimental findings. Moreover, their effective antibacterial action was induced by irradiation with visible light. In contrast, in our experiments the antibacterial action of the $Ag/Al_2O_3$ nanolayers was induced without light irradiation, the process just occurred without the need of any additional stimulation.

Based on the in vitro cytotoxicity results it could be assumed that nanocomposite $Ag/Al_2O_3$ layers are not cytotoxic for the cell line recommended for this assay by ISO 10993-5:2009(E). In confirmation of our results achieved at optimal concentration, other authors also reported the low cytotoxicity of silver.[5,7,19]

## 5. Conclusion

To the best of our knowledge, optically transparent $Ag/Al_2O_3$ nano-layers for application as ocular prosthesis's protective coatings against bacteria and fungi have been obtained by using RF magnetron sputtering. The coatings exhibited strong antibacterial and antifungal effects without requiring any additional stimulation of the process.

The antimicrobial and antifungal properties of the surface of Ag doped Al oxide coated products are very promising for many biomedical applications.

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The results presented are under patenting process.

## Conflict of Interest

The authors declare no conflict of interest.

## Keywords

$Ag/Al_2O_3$ nanolayers, antibacterial and antifungal action, ocular prostheses coating, RF reactive magnetron sputtering

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