Antibacterial silver nanolayers for coatings on surgical and microsurgical instruments

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Abstract. In this paper, we describe the antibacterial action of Ag-doped Al₂O₃ nanolayers deposited by RF reactive magnetron sputtering on stainless-steel surgical and microsurgical instruments. Synthesizing Ag/Al₂O₃ protective coatings is necessary for suppressing the infections caused by pathogenic microorganisms following the application of surgical instruments. We further conducted microbiological studies on the action of the nanocomposite Ag/Al₂O₃ layers against Gram-positive and Gram-negative bacteria (Staphylococcus aureus and Escherichia coli). The microbiological studies conducted proved the antibacterial effect of the nanocomposite Ag/Al₂O₃ layers, the strongest effect against Escherichia coli and Staphylococcus aureus being observed after 48 hours of exposure. Also, the Ag/Al₂O₃ nanolayers showed no cytotoxic effect. Our experimental findings suggest a very promising application of such antibacterial Ag/Al₂O₃ nanolayers regarding the reduction of infections when stainless-steel surgical and microsurgical instruments are used.

1. Introduction

Applications of nanotechnologies in medicine and in the manufacture of surgical instruments are continuously being developed and improved [1]. The surgical instruments are usually made of stainless steel, titanium, tungsten, etc., stainless steel for long being a standard material for this purpose [2]. Despite the good sterilization of the surgical instruments, the danger is always present of microbial infection during surgery, which necessitates the search for new methods of reducing the operative risks.

Several authors have reported the antibacterial action of silver [3-8]. Also, electrochemical methods have been used to deposit silver on stainless steel [2]. The studies on the antimicrobial properties of silver coatings against P. aeruginosa showed such an effect after 24 hours [2]. P. aeruginosa was chosen because over 80 % of infections with metal implants are caused by Gram negative bacteria. In addition, experimental studies have shown that silver coatings are non-toxic [9].

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We set out to investigate the antimicrobial properties of the nanocomposite Ag/Al₂O₃ layers deposited on stainless-steel surgical and microsurgical instruments.

2. Experiments

2.1. Material synthesis and analytical methods
The nanocomposite thin layers of Ag/Al₂O₃ were synthesized on medical surgical and microsurgical instruments of stainless steel (to facilitate the analytical analyses) by RF magnetron co-sputtering (figure 1) of Ag and Al under the following conditions: frequency 15 MHz; working pressure in the vacuum system fixed at ~5.6 Pa with optimal mixture of Ar and O₂ gasses (50%/50%). The forward power of magnetron discharge was 96 W, with a reflective power of 42 W and DC bias of -134 V. The thin film so deposited was amorphous. The thickness of the layers was determined to be 40 nm by using a Woollam M2000D rotating-compensator spectroscopic ellipsometer.

2.2. Antibacterial activity assay
A microbiological assay was carried out to examine the antibacterial properties of the coatings with 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).

The pincette examined (about 8 cm in length) was inserted in a 15-mL tube with a microbial suspension (10⁶ microbial cells/mL) (figure 2). The tube (left at room temperature) was shaken periodically. Samples were taken at 24 hours and at 48 hours, serially diluted (10-fold) and seeded (50 µL) on Tryptic Soy Agar medium (Sigma-Aldrich). After 24-hour incubation at 37 °C, the number of microbial colonies was counted. The results are expressed in CFU/mL (colony forming units/ml). A microbial suspension only was placed in the control tube and was also sampled at 24 hours and at 48 hours and processed and examined as above.

![Figure 1. Microsurgical instruments of stainless steel covered with Ag/Al₂O₃ coating by RF magnetron co-sputtering.](image1)

![Figure 2. Medical surgical and microsurgical instruments of stainless steel in tubes with nutrient solution.](image2)

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 and discussion
The number of survivors of *E. coli* in the 24th hour decreased about 550 times. After 48 hours of contact of the *E. coli* suspension with the treated tweezers, single *E. coli* (30 cells) survived, which is a reduction of 460 times from the 24th to the 48th hour of the sample, and $2.6 \times 10^5$ times relative to the control.

The microbiological studies conducted proved the antibacterial effect of the nanocomposite Ag/Al$_2$O$_3$ layers, the strongest effect against *Escherichia coli* and *Staphylococcus aureus* being observed after 48 hours of exposure. The Ag/Al$_2$O$_3$ nanolayers showed no cytotoxic effect.

Silver’s antimicrobial properties are utilized in medical devices, wound dressings, door handles, etc. [1, 2, 10]. For bactericidal effects, a minimum concentration of 2 – 20 μg/mL is required [12]. So far, the development of bacterial resistance to silver has been observed very rarely [11].

In [2], the authors described the antimicrobial properties of silver which proved to be quite effective against *p. Aeruginosa*. A 13-fold decrease in the number of colony forming units was observed in only 24 hours. In our research, we registered the antimicrobial action of the silver nanolayer on stainless steel surgical instruments. We observed complete inactivation of *Escherichia coli* (figure 3) and *Staphylococcus aureus* (figure 4) after 48 hours. Similar effects have been described by other authors who have used silver as an antimicrobial agent [6, 7, 13]. There are several hypotheses concerning the antimicrobial properties of Ag$^+$ coatings. In general, the Ag$^+$ ions are very efficient in inactivating bacteria [14-15]. One possible explanation is that when the Ag$^+$ ion enters the cell, the DNA changes to a condensed form and loses its replication capacity. These effects were observed in both Gram positive and Gram negative bacteria. Another way in which silver can kill bacteria is by interacting with ribosomes, thereby inhibiting the expression of enzymes and proteins required for the production of ATP. Moreover, the antibacterial property of silver upon reacting with compounds containing sulfur and phosphorus is well known. Bacterial membranes include proteins containing sulfur, so silver probably binds to them. This could explain the changes observed in the cell membrane morphology and the disruption of the cellular respiration. Unlike other authors who used electrochemical methods for silver plating, we employed RF magnetron co-deposition of Ag/Al$_2$O$_3$ nanolayers under certain conditions of frequency and operating pressure in the vacuum system.

4. Conclusions
Antibacterial Ag/Al$_2$O$_3$ nanolayers were deposited on stainless-steel surgical and microsurgical instruments by means of RF magnetron sputtering. Antibacterial action against *Escherichia coli* and *Staphylococcus aureus* was established, a very promising effect in view of biomedical applications.
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