THE ANTIMICROBIAL ACTIVITY OF MAGNETRON SPUTTERED Ag DOPED ALUMINUM OXIDE COATINGS IN VITRO

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The effect of Ag-doped aluminium oxide coatings deposited by magnetron sputtering method on the antibacterial efficiency against Gram-positive, Gram-negative bacteria and fungi has been investigated. The structure and composition of coatings were analysed by means of scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDS), photoelectron spectroscopy (XPS) methods. The results of the study suggest that Ag-doped aluminium oxide coatings demonstrate improved bactericidal effect and have great potential in various medical applications.

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INTRODUCTION

The standard methods for sterilization of medical instruments and products require time, intensive treatment, and aggressive chemicals using. The antimicrobial coatings formation on the surface of metal and plastic medical products is a possible alternative and effective way to improve the quality of modern prosthesis, stents, and optical lenses. Nanocomposite coatings exhibit unique properties [1, 2], which are very important for next implant and tissue engineering applications. Recently, it has been reported that Ag ions could be effective against more than 16 species of bacteria [3]. To increase the antibacterial properties of coatings based on Al, Zr, Ti oxides, nitrides and oxinitrides against different bacterial strains, silver dopes with bactericidal effect as antibiotics were added during deposition process [4-9]. The antimicrobial properties of Ag doped aluminum oxide coatings deposited by magnetron sputtering method against representative strains of microorganisms associated with hospital-acquired infections such as Gram-positive Staphylococcus aureus, and Gram-negative Escherichia coli, Pseudomonas aeruginosa bacterium and fungi Candida albicans were analyzed in vitro study.

1. MATERIALS AND METHODS

Alumina coatings were deposited in a high vacuum pumping system with a base pressure of about 10^{-2} Pa by ion source-assisted magnetron sputtering [2]. A combined aluminum/silver target with a different areas ratio was used.

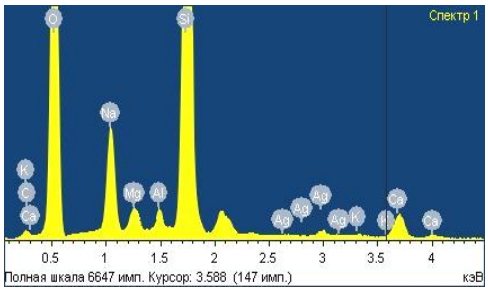
The microstructure and morphology of the coatings were studied by JEM-700F scanning electron microscope. The Energy-dispersive X-ray spectroscopy (EDS) method with a high-energy electron beam was used for determination of the elements and the Ag distribution in the Al_2O_3-Ag samples. X-ray photoelectron spectroscopy was carried out using ESCALAB MkII (VG Scientific) electron spectrometer using AlKalpha X-ray source (excitation energy hν=1486.6 eV).

The following microbial strains and microorganisms were selected: bacteria Gram-positive Staphylococcus aureus, methicillin resistant strain (MRSA), Gram-negative Escherichia coli, Pseudomonas aeruginosa bacterium and fungi Candida albicans from the Collection of Institute of Microbiology, BAS. The Ag-doped aluminum oxide coatings deposited by magnetron sputtering method were testd. The bacterial suspensions were put on the coated and uncoated surfaces of glass substrates. 1: 1 to 1: 1000 – the dilutions of the bacterial suspension that had been in contact with the silver doped coatings and control for a respective time period of 0, 1, 2, 5, and 24 hours and from which 25 µl was plated on Tryptic Soy agar medium Agar for microbiology (Sigma-Aldrich). After 24 hours of incubation at 37 °C, the number of bacterial colonies emerged. The direct microscopic counting of viable cells by haemocytometer was made. The results were expressed as CFU/ml (colony-forming units per milliliter). The statistical correlation of the results of antibacterial activity tests between the coated samples and control was determined. Difference was considered significant at the $p < 0.05$.

2. RESULTS AND DISCUSSION

Fig. 1 shows SEM images of Ag-doped Al_2O_3 coatings deposited by magnetron sputtering method. The coatings surface was smooth and uniform, without cracks and delamination. EDS spectra reveal the presents of main characteristic elements such as aluminum (Al), oxygen (O), silver (Ag). The presence of silicon (Si), calcium (Ca), sodium (Na) elements results from the small thickness (0.1 µm) of coating on the glass substrate.

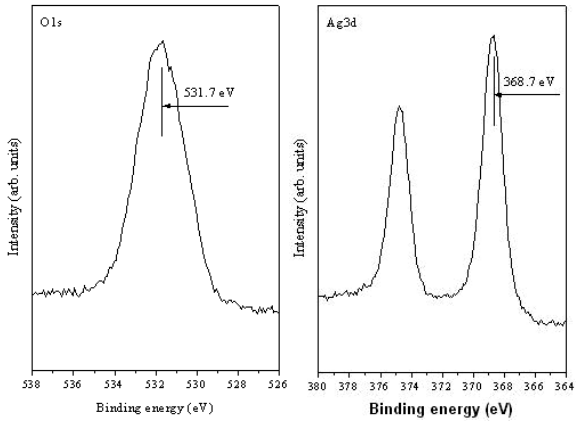
a



b

Fig. 1. SEM images (a) and EDS spectra (b) of Ag-doped aluminium oxide coating deposited by magnetron sputtering method on the glass substrates

The bonding states of the deposited Ag-doped Al_2O_3-coatings were characterized by XPS. The high-resolution photoelectron spectra of Al2p, O1s, Ag3d were observed. The chemical composition was found to be close to stoichiometric composition [10]. Fig. 2 shows O1s and Ag3d XPS spectra of the deposited Al_2O_3-Ag coating. The O1s high-resolution spectra demonstrate the peak at binding energy position E = 531.7 eV, associated with Al-O chemical bond. The Al2p peak with binding energy position E=75 eV, which is corresponded to the Al_2O_3 composition was detected.



a b

Fig. 2. The high-resolution photoelectron spectra of O1s (a), Ag3d (b) of the Ag-doped aluminium oxide coating deposited by magnetron sputtering method on the glass substrates

The characteristic Ag3d3/2 and Ag3d5/2 spectra (see Fig. 2,b) are detected at 374.4 and 368.7 eV, correspondently. Ag3d5/2 spectrum shows broad peak which can be deconvoluted into three components corresponding to an Ag peak at 368.2 eV, an Ag_2O-bond peak at 367.7 eV, and a silver oxide (AgO)-bond peak at 367.1 eV [7].

The bacterial-viability tests in Fig. 3 demonstrate strong bactericidal activity of Ag-doped aluminium oxide coating deposited by magnetron sputtering method against Gram-negative Escherichia coli, Pseudomonas aeruginosa bacterium.

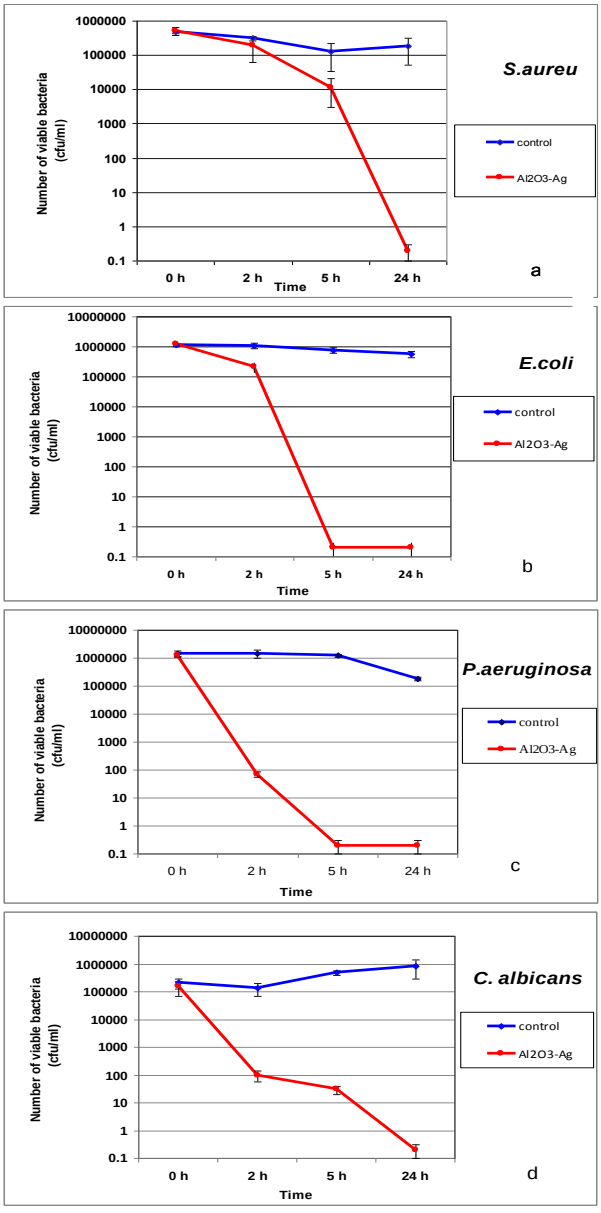


Fig. 3. Bacterial-viability tests of Ag-doped aluminium oxide coating deposited by magnetron sputtering method on the glass substrates: a) Staphylococcus aureus, strain 29213 (ATCC), b) Escherichia coli strain 35218 (ATCC), c) Pseudomonas aeruginosa, strain 1390 (ATCC), d) Candida albicans strain 74 (ATCC)

After 5 hours incubation all bacterium were died in comparison with control samples. In the contrary, the coatings influence on the Gram-positive Staphylococcus aureus and fungi Candida albicans was less effective. This fact confirms that the application of Ag-doped Al_2O_3 coatings significantly improved the antibacterial properties of coatings against Gram-negative bacterium, as it was previously reported in the studies [3, 4].

CONCLUSIONS

The results demonstrate the principal possibility to increase the antimicrobial activity of the Ag-doped aluminum oxide coatings against representative strains of microorganisms. The effect of the bactericidal action of coated surfaces results in the lethality of all studied bacterial strains and fungi during 24 hours of testing period. The activation of surface antimicrobial properties of various medical products is very challenging for many biomedical applications.

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АНТИМИКРОБНАЯ АКТИВНОСТЬ ПОКРЫТИЯ ОКСИДА АЛЮМИНИЯ С СЕРЕБРОМ, НАНЕСЕННОГО МЕТОДОМ МАГНЕТРОННОГО РАСПЫЛЕНИЯ В ТЕСТАХ IN VITRO

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Исследовано влияние покрытий оксида алюминия с серебром, нанесенных методом магнетронного напыления на антибактериальную эффективность против грамположительных, грамотрицательных бактерий и грибков. Структура и состав покрытий были проанализированы с помощью сканирующей электронной микроскопии (SEM), энергодисперсионной рентгеновской спектроскопии (EDS), методов фотоэлектронной спектроскопии (XPS). Результаты исследования позволяют утверждать, что покрытия оксида алюминия с серебром демонстрируют улучшенный бактерицидный эффект и имеют большой потенциал применения в различных областях медицины.

АНТИМІКРОБНА АКТИВНІСТЬ ПОКРИТТЯ ОКСИДУ АЛЮМІНІЮ ЗІ СРІБЛОМ, НАНЕСЕНИМ МАГНЕТРОННИМ РОЗПИЛЕННЯМ У ТЕСТАХ IN VITRO

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Досліджено вплив покриттів оксиду алюмінію зі сріблом, нанесених методом магнетронного напилення на антибактеріальну ефективність проти грампозитивних, грамнегативних бактерій та грибків. Структура та склад покриттів були проаналізовані за допомогою скануючої електронної мікроскопії (SEM), енергодисперсійної рентгенівської спектроскопії (EDS), методів фотоелектронної спектроскопії (XPS). Результати дослідження дозволяють стверджувати, що покриття оксиду алюмінію зі сріблом демонструють поліпшений бактерицидний ефект і мають великий потенціал використання в різних галузях медицини.