Effect of copper and silver doping on the antibacterial properties of magnetron-sputtered aluminium oxide coatings

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Abstract. Ag- and Cu- doped aluminium oxide coatings were deposited by magnetron sputtering and their structure and composition were analyzed by means of scanning electron microscopy (SEM) and photoelectron spectroscopy (XPS). The coatings’ antimicrobial properties and efficiency against representative bacterial strains of Gram-positive, Gram-negative bacteria and fungi were analyzed by in vitro tests. The results of the study suggest that Ag and Cu doped aluminium oxide coatings possess a combined bactericidal effect and demonstrate a promising potential for various medical applications.

1. Introduction
Depositing antimicrobial coatings on the surface of metal and plastic medical products is a possible and effective alternative way of improving the quality of modern prostheses, stents, and optical lenses. The novel antibacterial coatings should combine high adhesion strength, good mechanical properties and corrosion and wear resistance with efficient antibacterial activity and biocompatibility. The coatings should prevent undesirable reactions and bacterial infections on the surface of modern medical products. The adhesion of various bacteria is affected by the surface characteristics of the specific underlying material, such as the surface morphology, chemical composition, surface charge, and wettability [1].

To increase the antibacterial surface properties of coatings based on Al, Zr, Ti, Ta oxides, nitrides and oxinitrides against different bacterial strains, silver and copper additives have been added during the deposition process thus producing a bactericidal effect comparable to that of antibiotics [2-4]. Recently, it was reported that Ag ions have strong bactericidal effects on more than 16 species of bacteria, while Cu ions can be effective against other groups of bacteria [5]. Ag- and Cu-doped inorganic antibacterial coatings have demonstrated a wider spectrum of bactericidal effects [6]. The deposition of TaN-(Ag,Cu) coatings resulted in a combined effect, namely, TaN-(Ag, Cu) films show...
a better antibacterial behavior than either TaN-Ag or TaN-Cu separately. It was further found that Ag is not very effective against Gram-positive bacterial strains, while Cu is less active against Gram-negative bacteria [7]. In this paper, we describe an analysis of the antimicrobial properties of Ag- and Cu-doped aluminium oxide coatings deposited by magnetron sputtering against representative strains of microorganisms associated with widespread hospital-acquired infections, such as Gram-positive Staphylococcus aureus, Gram-negative Escherichia coli and Pseudomonas aeruginosa bacteria and Candida albicans yeast.

2. Materials and methods
Alumina (Al₂O₃) coatings were deposited in a high-vacuum system with a base pressure of 6.6 Pa by high-frequency (13.56 MHz) magnetron sputtering [8] of combined aluminium/silver/copper targets with different area ratios at a distance of about 3 cm to a glass substrate. Argon as a sputtering gas and oxygen as a reactive gas were used at flows regulated by mass flow controllers operated by two-channel process control unit. The forward magnetron discharge power was 80 W, the reflected power 42 W, and the DC bias, -163 V. The deposition rate was 0.2 μm/hour. The films’ thickness was about 50 nm, as determined by a Woollam M2000D rotating-compensator spectroscopic ellipsometer.

The microstructure and morphology of the coatings were studied by a JEM-700F scanning electron microscope (SEM). X-ray photoelectron spectroscopy (XPS) was used to determine the bonding states of Ag and Cu elements in the samples by means of an ESCALAB MkII (VG Scientific) electron spectrometer with an Al Kα X-ray source (excitation energy 1486.6 eV).

The following microbial strains and microorganisms were selected: Gram-positive Staphylococcus aureus, methicillin resistant strain (MRSA); Gram-negative Escherichia coli and Pseudomonas aeruginosa; and Candida albicans fungi, from the Collection of Institute of Microbiology, Bulgarian Academy of Sciences. The bacterial suspensions were placed on coated and uncoated surfaces of glass substrates. Diluted bacterial suspension (25 μl, 1:1 to 1:1000) that had been in contact with the silver/copper-doped coatings for periods of 0, 1, 2, 5, and 24 hours and a control were placed on Tryptic Soy Agar medium (Sigma-Aldrich). After 24 hours of incubation at 37 °C, the number of viable cells was counted by a hemocytometer; the results were expressed as CFU/mL (colony-forming units per milliliter). The statistical correlation was determined of the results of antibacterial activity tests between the coated samples and the control; a difference was considered significant for p < 0.05.

3. Results and discussion
Figure 1 shows SEM images of Ag- and Cu doped Al₂O₃ coatings deposited by magnetron sputtering. The coatings surface was smooth and uniform, without cracks and delamination. A rough surface generally increases the possibility of bacterial contact and promotes colonization by bacteria. As was shown in [9], a smooth surface has a low bacterial adherence, which results in a low probability of infection.

Figure 1. SEM images with different magnification (a) and (b) of Ag- and Cu-doped aluminium oxide coating deposited by magnetron sputtering on a glass substrate.
The bonding states of the coatings were characterized by high-resolution XPS. The photoelectron spectra observed (figure 2) of Al2p, O1s, Ag3d, and Cu2p proved a chemical composition close to the stoichiometric one [10]. The O1s spectrum has a peak at a binding energy position of 531.7 eV, associated with the Al-O bond. The Al2p peak at 74.4 eV corresponds to the Al2O3 composition. The characteristic Ag3d3/2 and Ag3d5/2 peaks (figure 2c) are seen at 374.4 eV and 368.7 eV, correspondingly. The Ag3d5/2 spectrum exhibits a broad peak that can be deconvoluted into three components corresponding to a Ag peak at 368.2 eV, a Ag2O-bond peak at 367.7 eV, and a silver oxide (AgO)-bond peak at 367.1 eV [11]. The Cu2p3/2 spectrum (figure 2d) of the deposited Al2O3 coatings doped by Ag and Cu also demonstrates a broad peak at 934.6 eV, which can be deconvoluted into two components corresponding to a Cu peak at 932.5 eV and a small CuO bond peak at 933.8 eV [12]. These results show that Ag and Cu were incorporated into the coatings, but their low content did not affect the principal aluminium oxide structure.

Table 1 summarizes the XPS results concerning the content of the main characteristic elements, namely, aluminium (Al), oxygen (O), silver (Ag), cooper (Cu). The presence of silicon (Si) arises from the small thickness of the coatings on glass substrates.

|          | O1s | Al2p | Si2p | Ag3d | Cu2p3/2 |
|----------|-----|------|------|------|---------|
| Surface atomic concentrations, at % | 31  | 28   | 27   | 9    | 5       |
| Binding energy, eV                  | 531.7 | 74.4 | 103.6 | 368.7 | 934.6   |
| Al-O:                                |      |      |      |      |         |
| Si-O:                                |      |      |      |      |         |
| Cu2p3/2:                             |      |      |      |      |         |

Figure 2. High-resolution photoelectron spectra of Al2p (a), O1s (b), Ag3d (c), and Cu2p (d) of Ag- and Cu-doped aluminium oxide coating deposited by magnetron sputtering on glass substrates.
The results of the bacterial viability tests illustrated in figure 3 demonstrate the strong bactericidal activity of the Ag- and Cu-doped aluminium oxide coating deposited by magnetron sputtering against the bacterial strains quoted above.

In a previous research [8], we studied the effect of Ag-doped aluminium oxide coatings deposited by magnetron sputtering against Gram-negative Escherichia coli and Pseudomonas aeruginosa bacteria. After 5-hour incubation, all Gram-negative bacterial strains were not viable, unlike the control samples. In contrast, the coatings were less effective against the Gram-positive Staphylococcus aureus and the Candida albicans fungi. At the present study, the effect of adding Cu was shortening the bactericidal action time from 24 to 5 hours incubation for Staphylococcus aureus and Candida albicans fungi, and from 5 to 2 hours for Pseudomonas aeruginosa. Previous studies on the antimicrobial and antifungal activities of Cu nanoparticles (NPs) against methicillin-resistant Staphylococcus aureus, Candida albicans, Pseudomonas aeruginosa and Salmonella choleraesuis have confirmed that the antibacterial activity of Cu NPs is considerably weaker than that of Ag NPs.[13,14]. In the present study, the application of Ag- and Cu-doped Al₂O₃ coatings significantly improved the coatings’ antimicrobial properties against both Gram-negative and Gram-positive bacteria, as previously reported in [2, 5].

![Bacterial-viability tests](image)

**Figure 3.** Bacterial-viability tests of Ag- and Cu-doped aluminium oxide coating deposited by magnetron sputtering on 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).

4. Conclusions
Bacterial viability tests demonstrated the strong bactericidal activity of aluminium oxide coatings with copper and silver additives deposited by magnetron sputtering, their effect being a complete loss of viability of all bacterial strains and fungi studied within a five-hour testing period. The results suggest that Ag- and Cu-doped aluminium oxide coatings combine their bactericidal effects, thus pointing to a possibility of increasing the antimicrobial activity of medical products against representative strains of microorganisms. Activating the antimicrobial surface properties of various medical products is a pressing and challenging problem in many biomedical applications.
Acknowledgment
The research was supported by the international scientific cooperation program between the Bulgarian Academy of Sciences and the National Academy of Sciences of Ukraine, and the international scientific cooperation project # 17-03-18 between the National Academy of Sciences of Ukraine and the National Academy of Sciences of Belarus.

References
[1] Barth E, Myrvik Q M, Wagner W and Gristina A G 1989 Biomaterials 10 325
[2] Hsieh J H, Chiu C H, Li C and Wu W 2013 Surf. Coat. Technol. 233 159
[3] Tian X B, Wang Z M and Yang S Q 2007 Surf. Coat. Technol. 201 8606
[4] Chang Y Y, Huang H L, Chen Y C, Weng J C and Lai C H 2013 Surf. Coat. Technol. 231 224
[5] Hsieh J H, Yeh T H, Hung S Y, Chang S Y, Wu W and Li C 2012 Mater. Res. Bulletin 47 2999
[6] Huang H L, Chang Y Y, Weng J C, Chen Y C, Lai C H and Shieg T M 2013 Thin Solid Films 528 151
[7] Liu P C, Hsieh J H, Li C, Chiu C H and Huang C T 2013 Vacuum 87 160
[8] Safonov V, Donkov N, Zykova A, Avramov I, Dudin S, Yakovin S, Naidenski H and Avramova I 2019 Prob. At. Sci. Technol. 1 187
[9] Wang X, Wang G, Liang J, Cheng J, Ma W and Zhao Y 2009 Surf. Coat. Technol. 203 3454
[10] Zykova A, Safonov V, Goltsev A, Dubrava T, Rossokha I, Smolik J, Rogovska R, Yakovin S, Kolesnikov D, Sudzhanskaya I and Goncharov I 2016 Surf. Coat. Technol. 301 114
[11] Huang H L, Chang Y Y, Chen Y C, Lai C H and Chen Y C 2013 Thin Solid Films 549 108
[12] Svintsitskiy D A, Stadnichenko A I, Demidov D V, Koscheev S V and Boronin A J 2011 J. Appl. Surf. Sci. 257 8542
[13] Zhang X, Huang X, Ma Y, Lin N, Fan A and Tang B 2012 J. Appl. Surf. Sci. 258 10058
[14] Yao X, Zhang X, Wu H, Tian L, Ma Y and Tang B 2014 J. Appl. Surf. Sci. 292 944