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Silver nanocluster/silica composite coatings obtained by sputtering for antibacterial applications

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Abstract. Silver nanocluster silica composite coatings were deposited by radio frequency co-sputtering technique on several substrates. This versatile method allows tailoring of silver content and antibacterial behaviour of coatings deposited on glasses, ceramics, metals and polymers for several applications. Coating morphology and composition as well as nanocluster size were analyzed by means of UV-Visible absorption, X-ray diffraction (XRD), Field Emission Scanning Electron Microscopy (FESEM), electron dispersive spectroscopy (EDS), X-ray Photoelectron Spectroscopy (XPS) and Atomic Force Microscopy (AFM). The antibacterial effect was verified through the inhibition halo test against standard bacterial strain, *Staphylococcus aureus,* before and after sterilization process. Tape test demonstrated a good adhesion of the coatings to the substrates.

1. Introduction

Materials with antibacterial properties are widely requested in several fields where the risk of microbial contamination is considered a relevant issue, such as biomedical implants, agricultural/food industry, facilities in crowded places (bus, telephones), personnel protective systems and also space structures [1-3].

Silver is the most known and documented antimicrobial agent and its powerful action can be expressed in several forms as metallic, nanoparticles and ions [4, 5]. Silver antibacterial properties can be conferred to glasses, ceramics, metals and polymers by means of several techniques as ions-exchange, sol-gel method and sputtering [6–10]. Sputtering is one of the most versatile coating methods, suitable for most of substrates, because it does not reach high temperatures which could compromise the mechanical and thermal properties of materials to be coated (e.g. polymers) [10].

Preparation and characterization of materials with antibacterial properties are topics well covered by the authors' research group [11–17].

In this paper, antibacterial silver nanocluster silica composite coating were deposited on several substrates, using radio frequency (RF) co-sputtering technique [15–17] and setting several process parameters as a function of the substrate. Silica was chosen as a matrix for the composite coating because it provides good mechanical and thermal resistance, whereas silver nanoclusters confer antibacterial activity. In addition, silver nanoclusters embedded in a silica matrix allows the reduction of silver amount, if compared with pure silver coatings, with advantages in terms of toxicity [18, 19].

A characterization of the composition and morphology of the coating was also reported. The antimicrobial behavior was analyzed through the evaluation of the inhibition halo against standard bacterial strain, *Staphylococcus aureus* (ATCC29213) [20], before and after sterilization processes (gamma rays and EtO). Adhesion of the coatings was evaluated through standard tape test [21].

2. Materials and methods

RF co-sputtering (Microcoat™ MS450) was the technique used for the deposition of a silver nanocluster silica composite coating on several substrates. Silver (Sigma–Aldrich™ 99.99 %) and silica (Franco Corradi s.r.l. 99.9 %) were used as targets.

The morphology, composition and structure of coatings were evaluated by means Field Emission Scanning Electron Microscope (FESEM, SUPRATM 40, Zeiss™), UV-Visible absorption spectra (UV-Vis, Varian™ Cary 300 Bio), X-Ray Diffraction (XRD, X'Pert Philips™ diffractometer equipped with X'Pert High Score™ software analysis), Electron Dispersive Spectroscopy (EDS, SEM-FEI- Quanta™ Inspect 200 and EDAX PV 9900), X-ray Photoelectron Spectroscopy (XPS, VSW TA10 non-monochromatic Al Kα (1486.6 eV) X-ray source) and Atomic Force Microscopy (AFM, XE-100 with XEI 1.8.0 software).

The antibacterial potential of the silver nanocluster silica composite coatings on different substrates was determined against the standard bacterial strain, *Staphylococcus aureus*, by the inhibition zone evaluation in accordance to National Committee for Clinical Laboratory Standards (NCCLS) [20]. Several samples were tested before and after a sterilization treatments (gamma rays and EtO).

Tape test was performed on the coated samples according to the ASTM D3359 standard [21] in order to evaluate the adhesion of the coatings to the several substrates.

3. Results and discussion

Silver nanocluster/silica composite coatings were deposited on several substrates as glasses (silica, soda-lime), metals (aluminium, steel) and polymers (polypropylene, polyurethane, aramidic fibers and a commercial multilayer polymer composed of ethylene vinyl alcohol (EVOH), polyamide and polyethylene as the outer layer) by means of RF co-sputtering technique. These substrates were chosen among materials available for different applications in food industry, biomedical implants, space structures, personal protective systems. The main process parameters, optimized after a series of preliminary depositions and fixed for all the substrates, are reported in Table 1.

Table1: Sputtering Process Parameters

Sputtering Process Parameters	Set Values
Pre-Deposition Pressure	70 µPa
Working Pressure	5.5dPa
Power on silica target (6 inch)	200 W RF
Power on silver target (1 inch)	1 W DC

On the contrary, the deposition time was changed between 8 and 80 minutes depending on the substrates and the final application. In particular, the increment of deposition time allows the increment of coating thickness (from 15 to 300 nm), total silver content in the coating and consequently antibacterial effect. Even if the temperature reached in sputtering chamber is not so high (about 80 °C), the maintenance of this temperature for long time can damage thermo-sensitive substrates, such as polymers. For these materials, short sputtering treatments (8-15 min) have been selected. In addition, the silver amount has to be strictly optimized for materials in direct contact with human organs and tissues in the biomedical field in order to obtain an antibacterial activity without inducing cytotoxic effects. The correlation between deposition time and coating thickness and main properties is schematized in figure 1.

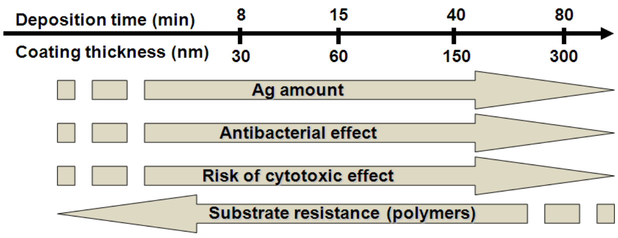

Fig. 1 : Schematic representation of the influence of deposition time on silver amount and its effects.

The coating morphology was observed by means FESEM (Fig. 2). The cross-section of a silica substrate coated with the antibacterial layer is reported in Fig. 2a. Silver nanoclusters are well visible as bright dots. The magnification in Fig. 2b shows the typical porous structure of the sputtered silica with embedded silver nanoclusters. EDS analyses showed the presence of silver, silicon and oxygen confirming coating presence on all tested samples.

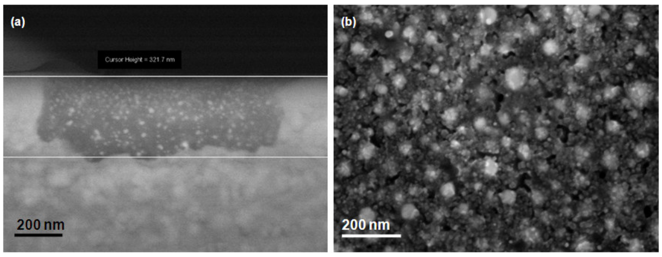

Fig. 2 : FESEM images relative to coating morphology: (a) cross-section of a coated silica substrate and (b) a micrograph of silver nanoclusters embedded into a silica matrix.

The UV–VIS absorption spectra relative to coatings with different thicknesses for comparison are reported in Fig. 3. In this case, the multilayer polymer was used as control. A large absorption with the maximum peak at about 396 – 398 nm was detected in all coated samples even if it is barely visible between 335 and 500 nm in the thinner coating (30 nm). The absorption could be ascribable to the localized surface plasmon resonance (L–SPR) typical of silver metal nanoparticles with dimensions of about 20 nm [22]. The coating thickness increment increases the maximum peak intensity, but it does not affect silver nanoclusters dimensions which depend on the position of the maximum peak (almost equal for all the coated samples).

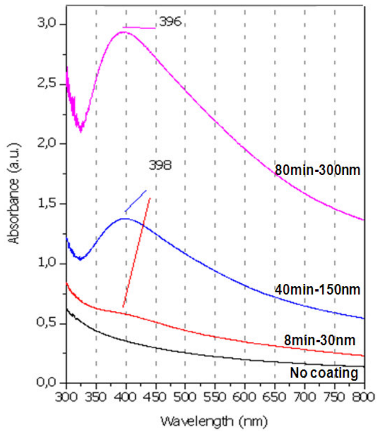

Fig.3: UV-Vis analysis on coatings with different thickness (30, 150 and 300 nm) compared with uncoated multilayer polymer used as substrate.

XPS analysis of the silver nanocluster/silica composite coatings, with two different thicknesses (300 nm vs 60 nm) and deposited on soda-lime and silica glass substrates, respectively, is reported in Fig. 4. In both the spectra, the principal peaks of silver (doublet Ag 3d), oxygen (O 1s), silicon (Si 2p) and carbon (C 1s) are revealed. The carbon is mainly due to surface contamination. Other secondary peaks (Si 2s and Auger peaks of Ag and O) are indicated in the graphs. The ratio Ag/Si remained equal to 0.1 for both the samples, thereby indicating that the thickness and the substrate material do not influence the coating composition.

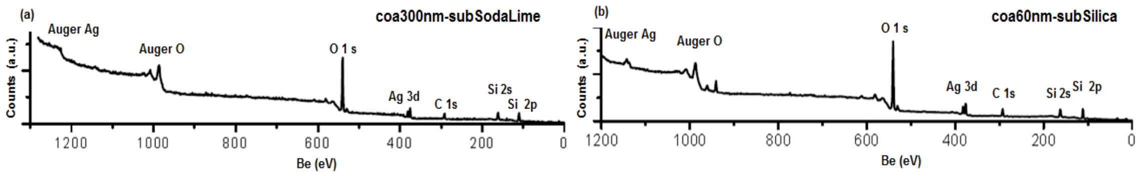

Fig. 4 : XPS analysis relative to (a) 300nm-thick coating on soda-lime glass substrate and (b) 60nm-thick coating on silica glass substrate.

AFM image of a deposited sputtered layer on a silicon wafer substrate, having a presumed thickness of 300 nm, is shown in Fig. 5. The root mean square roughness (RMS) value of about 6 nm and the roughness average (R_a) value of about 5 nm confirmed the nano-structured nature of the analysed layer; the overall uniformity and flatness of the image established that the sputtering technique allows a homogeneous growth of the coating and the uniformity of its surface morphology.

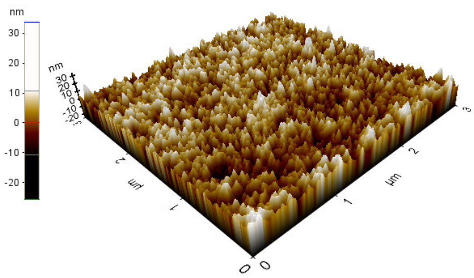

Fig. 5: AFM topography signal 3D image of an as deposited sputtered layer on a silicon wafer substrate

XRD analysis in Fig. 6 shows the presence of a wide peak around 20° not completely reported here: its nature can be connected with the amorphous silica present in the matrix and in the glass substrate; two low intensity peaks due to metallic silver are visible at 38 and 45° .

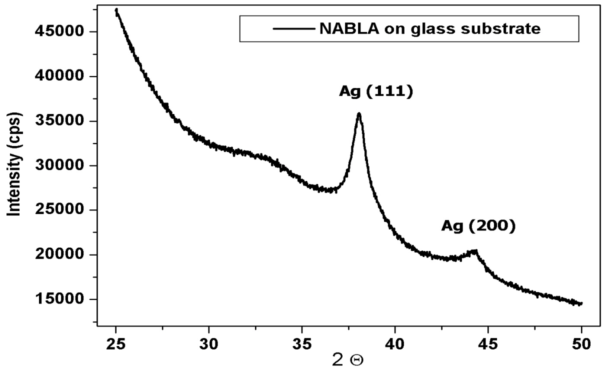

Fig. 6: XRD analysis of a 300nm-thick coating on glass substrate (constant grazing incidence angle: 2°)

The antibacterial activity against *S. aureus* of the silver nanocluster/silica composite coatings deposited on different substrates was verified by the observation of inhibition halo development. Fig. 7 reports some examples of the test performed with several substrates. A well visible halo of about 2-3 mm formed around all tested samples demonstrating that the antimicrobial behaviour can be conferred to all the material classes by means of the prepared sputtered silver nanocluster/silica composite coating. The same antibacterial effect was observed also for samples after sterilization by EtO or gamma rays.

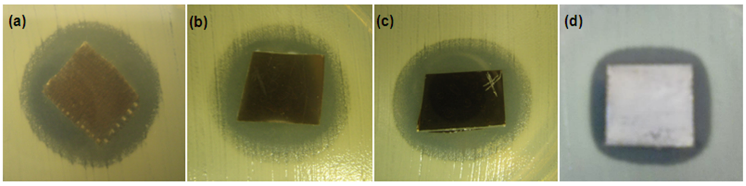

Fig. 7: Inhibition halo test with S. aureus strain for (a) fabric, (b) multilayer polymer, (c) silica and (d) steel

After the tape test (ASTM D3359 – 97 standard [21]), the silver nanocluster silica composite coating resulted well adherent and no sign of coating damage on the sample or coating detachment from the substrate on the tape was visible on the tape (not reported here).

4. Conclusions

The co-sputtering technique allowed the successful deposition of a coating composed of silver nanoclusters embedded in a silica matrix on glasses, ceramics, metals and polymers. The process parameters were set as a function of substrate material and final application. In particular, coating thickness and silver amount can be modulated by variation of the deposition time. Silver nanoclusters were observed well embedded in the silica matrix and XRD signal confirmed their presence. The coating thickness seems not to influence the nanoclusters dimensions and the Ag/Si ratio. The coating surface morphology shows a typical nano-structure. The coating antibacterial activity against *S. aureus* was verified with the formation of a well visible inhibition halo and it is not affected by the substrate and the sterilization procedure. The coating resulted well adherent to all the analysed substrate.

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