Applied Surface Science 371 (2016) 1–8

Contents lists available at ScienceDirect

Applied Surface Science

journal homepage: www.elsevier.com/locate/apsusc

Morphology and oxygen incorporation effect on antimicrobial activity of silver thin films

Rita Rebelo[a,b,c,*], N.K. Manninen[b,d], Luísa Fialho[b], Mariana Henriques[c], Sandra Carvalho[b,d]

[a] 2C2T, University of Minho, Campus de Azurém, 4800-058 Guimarães, Portugal
[b] GRF-CFUM, University of Minho, Campus de Azurém, 4800-058 Guimarães, Portugal
[c] CEB, Center for Biological Engineering, LIBRO—Laboratório de Biofilmes Rosário Oliveira, University of Minho, Campus de Gualtar, 4710-335 Braga, Portugal
[d] SEG-CEMUC, University of Coimbra, 3030-788 Coimbra, Portugal

ARTICLE INFO

Article history:
Received 15 July 2015
Received in revised form 12 February 2016
Accepted 15 February 2016
Available online 18 February 2016

Keywords:
Silver nanoparticles
Silver oxide thin films
Sputtering
Microstructure
Halo tests

ABSTRACT

Ag and Ag_xO thin films were deposited by non-reactive and reactive pulsed DC magnetron sputtering, respectively, with the final propose of functionalizing the SS316L substrate with antibacterial properties. The coatings were characterized chemically, physically and structurally. The coatings nanostructure was assessed by X-ray diffraction (XRD) and X-ray photoelectron spectroscopy (XPS), while the coatings morphology was determined by scanning electron microscopy (SEM). The XRD and XPS analyses suggested that Ag thin film is composed by metallic Ag, which crystallizes in fcc-Ag phase, while the Ag_xO thin film showed both metallic Ag and Ag—O bonds, which crystalize in fcc-Ag and silver oxide phases. The SEM results revealed that Ag thin film formed a continuous layer, while Ag_xO layer was composed of islands with hundreds of nanometers surrounded by small nanoparticles with tens of nanometers. The surface wettability and surface tension parameters were determined by contact angle measurements, being found that Ag and Ag_xO surfaces showed very similar behavior, with all the surfaces showing a hydrophobic character.

In order to verify the antibacterial behavior of the coatings, halo inhibition zone tests were realized for *Staphylococcus epidermidis* and *Staphylococcus aureus*. Ag coatings did not show antibacterial behavior, contrarily to Ag_xO coating, which presented antibacterial properties against the studied bacteria. The presence of silver oxide phase along with the development of different morphology was pointed as the main factors in the origin of the antibacterial effect found in Ag_xO thin film. The present study demonstrated that Ag_xO coating presented antibacterial behavior and its application in cardiovascular stents is promising.

1. Introduction

Cardiovascular diseases (CVD) are the leading cause of death worldwide. In Europe, CVD are responsible for 40% of deaths and thereis an estimated annual cost of 196 billion euros spent between health care, informal care and loss of productivity related to these diseases [1]. In this context, cardiovascular stents assume a great value in the treatment of vascular obstructions. These devices are inserted in blood vessels to keep them open, using mechanical pressure until blood circulation normalizes [2]. Despite the great innovations in the development of new stents with improved lifetime, namely the use of fibrous materials instead of metal, in order to avoid corrosion, stents, independently of the material, present some disadvantages like bacterial colonization, which leads to the occurrence of infections. Usually, these infections result in prolonged hospitalization with consequent high costs and, in extreme cases, death of the patient (especially immunocompromised ones) [3]. Infection of medical devices can arise due to several reasons and from different sources; some of the most common sources of infection are: contaminated device surfaces, hands of medical staff and patients own skin or mucus membrane, among others. Many of these risk factors can be easily avoided, but, infections are impossible to evade completely [4]. One of the most common bacteria in nosocomial sepsis is *Staphylococcus epidermidis*, which is a

* Corresponding author at: 2C2T, University of Minho, Campus de Azurém, 4800-058 Guimarães, Portugal.
E-mail address: ritarebelo@det.uminho.pt (R. Rebelo).

http://dx.doi.org/10.1016/j.apsusc.2016.02.148
0169-4332/

gram-positive, coagulase-negative *Staphylococcus* that is a part of our normal flora and colonizes the skin and mucous membranes of the human body [5]. Another bacterium associated with coronary diseases is *Staphylococcus aureus*, which is a gram-positive bacterium that can be found in the human respiratory tract and skin [6,7]. Thus, in order to prolong the stent lifetime and patientís safety the development of new stent materials with antibacterial properties is of great importance.

In the past years, silver has been studied as an antimicrobial agent [8–10]. The antimicrobial effect provided by silver depends on the amount of silver and the rate of Ag^+ released along time. Silver can exist in diverse forms: as metal, as a compound or as a free dissolved ion. The exact mechanism of the antimicrobial action of silver is not fully understood yet, still several theories have been pointed to explain its bactericidal effect, namely: (i) release of Ag^+ ions; (ii) direct interaction between Ag nanoparticles and bacteria and (iii) formation of reactive oxygen species; among them the Ag^+ are claimed to be the most effective bactericidal agent [11,12,9]. In respect to silver ions, their antimicrobial activity is attributed to the structural and morphological changes induced in bacteria. Silver ions can penetrate inside bacterial cells, inhibiting the DNA replication and, consequently, causing cell lysis. Moreover, silver ions have a far lower propensity than classic antibiotics to induce high-level, single-step resistance mutations [9,13].

The antibacterial efficiency of bulk Ag and Ag thin films is quite poor due to the high stability of Ag, which turns its ionization and solubility in water very low. In fact, any perfectly formed metallic surface in contact with an electrolyte will stabilize quickly; thus if a continuous release of Ag^+ ions is required, a driving force must exist to provide silver ionization. In order to achieve a continuous ion release researchers have attempted many novel activation processes, such as: thermal, mechanical, chemical and electrical stimulation [14–17], still, in a practical point of view the application of these external driving forces are quite complex. Sant et al. [12] claimed that the presence of heterogeneities in Ag based coatings enable to develop bioactive films. In fact, those authors found that the presence of fine grain size, incorporation of oxygen species and presence of lattice defects allowed to improve the Ag^+ dissolution rate, which is attributed to the high energy associated with these structural defects. Another adopted approach is the incorporation of Ag nanoparticles in different matrix coatings or polymers, which are claimed to present an effective bactericidal effect, due to their higher reactivity, promoted by the high surface to volume ratio [12].

Thus, the main objective of this work is the modification of stainless steel (SS316L) surfaces (the most common material used in cardiovascular stents) with silver and silver oxide thin films deposited by pulsed DC reactive magnetron sputtering, for cardiovascular stents application, with the aim of providing antibacterial properties. The antibacterial activity was determined by halo inhibition zone tests against *S. epidermidis* and *S. aureus*. The coatings structure, morphology and surface characteristics were evaluated in order to understand the influence of these parameters on the biological response.

2. Experimental details

2.1. Sample preparation

Ag and Ag_xO coatings were deposited onto 316L stainless steel (20×20 mm) and silicon substrates (10×10 mm) by reactive pulsed DC magnetron sputtering. Stainless steel 316L substrates were used in all characterization techniques that evaluate the functional properties, while silicon substrates were used in characterization techniques that evaluate the coatingís morphology and structure. Thus, silicon substrates were used in SEM analysis, once these substrates are easily cut, while in XRD analysis, silicon avoids the appearance of substrate's peaks. In XPS, since silicon's surface is smoother than stainless steel 316L, the analysis is more reliable. Before deposition the substrates were ultrasonically cleaned with distillate water, ethanol and acetone during 10 min in each solution. In order to minimize the contamination and remove impurities, before each deposition, the silver target and the substrates were cleaned by an argon plasma etching process during 5 min. The etching process was performed in an argon atmosphere (Ar flow of 80 sccm) and by applying a pulsed DC power supply with a current density of 400 mA and a reverse time and frequency of 1536 ns and 200 kHz to the substrate holder. Simultaneously, the Ag target was connected to a DC power supply, and a current density of 0.5 mA/cm^2 was applied. The silver based coatings were deposited from an Ag target (purity of 99.99%) with 200×100 mm^2. During deposition, the sputtering atmosphere consisted in a constant argon flow (60 sccm) for both coatings and for oxide coating oxygen was introduced with a constant flow of 15 sccm. Samples were placed in a substrate holder that rotated at a constant velocity of 7 rpm, 70 mm away from the target. The current density applied from a pulsed DC power supply to the Ag target was 2.5 mA/cm^2 and 1 mA/cm^2, for the deposition of Ag and Ag_xO coatings, respectively. The reverse time and frequency were kept at 1536 ns and 200 kHz, respectively.

2.2. Surface characterization

XPS analysis was performed in order to obtain information about the coatings binding state. The analysis was performed on silicon samples coated with Ag and Ag_xO in a Kratos AXIS Ultra HSA equipment, with VISION software for data acquisition and CASAXPS software for data analysis. The analysis was carried out with a monochromatic Al Kα X-ray source (1486.7 eV), operating at 15 kV (90 W), in FAT mode (Fixed Analyser Transmission), with a pass energy of 40 eV and a pass of 0.1 eV for regions ROI and 80 eV of pass energy and 1.0 eV of step for survey. Data acquisition was performed with a pressure lower than 1×10^{-6} Pa, and a charge neutralization system was used. The effect of the electric charge was corrected by the reference of the carbon peak (285 eV). The samples were sputter-cleaned in situ using a broad 2 keV Ar + beam for 5 min.

The morphology of Ag and Ag_xO coatings deposited in SS316L and the thickness of coatings deposited on silicon substrates were evaluated by scanning electron microscopy (SEM) analysis in an EDAXNova nano-SEM200 equipment, being analyzed 3 different regions and presented a representative micrograph.

X-ray diffraction (XRD) analysis was carried out in order to understand the structure and phase distribution of the coatings, in a PANalytical X'Pert PRO MPD system using CuKα radiation (45 kV and 40 mA) with a parallel beam configuration. The analysis was performed in grazing incidence mode with an angle of incidence of 5°. The X-rays reach the entire sample area, thus only one sample was analyzed, which is the common procedure in this technique. The grain size was determined by Scherrer formula using the (1 1 1) peak in case of Ag thin film. The XRD peak was fitted with pseudo-voigt function, which allowed us to calculate either the full-width at half-maximum (FWHM) and the peak position (2θ). Silicon substrates were used for XRD analysis.

The coatings hydrophobicity was evaluated through contact angle measurements, using the van Oss approach in coatings deposited onto SS316L substrate. For the contact angle analysis, 8 measurements were made, with three different liquids: water, formamide and α-bromonaphtalene, with different values of surface free energy, Y_l^{Tot}; apolar Lifshitz–van der Waals surface free energy component, Y_l^{lw}; electron acceptor surface free energy compo-