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Abstract

Diamond-like carbon (DLC) coatings have been extensively studied over the last two decades in the field of materials engineering. The addition of effective bactericidal agents like silver suggests the potential application of silver-doped DLC coatings in the biomedical sector. In this paper, DLC coatings containing about 2 at. % Ag were grown on CoCrMo substrates by pulsed filtered cathodic arc co-deposition using pure graphite and silver targets. Morphology, structure and chemical composition of the coatings were characterized by SEM, XPS, XRD, Raman and HRTEM. Wettability and surface free energy were also measured. Mechanical properties were analyzed by means of nanoindentation and Rockwell-C adhesion tests. Corrosion resistance of coated and uncoated samples was determined by potentio-dynamic polarization tests in Hank's solution. Cytotoxicity and antibacterial activity against S. aureus and P. aeruginosa were also studied. For comparative purposes, nitrogen-doped DLC coatings obtained by RF magnetron sputtering were also prepared and characterized. Well-adhered and corrosion resistant Ag-DLC nanostructured coatings of up to 1.5 microns thick and 21 GPa in hardness were obtained. The doping concentration of silver studied in this work seems to be optimal for the preparation of non-cytotoxic coatings with a significant bactericidal activity.

Keywords: Diamond-like carbon; filtered cathodic arc; bactericidal activity; silver doping; P. aeruginosa; S. aureus.

1. Introduction

In the last two decades, amorphous diamond-like carbon (DLC) coatings have received a great interest due to its exceptional properties such as high hardness, low friction, chemical inertness, corrosion protection, biocompatibility, optical transparency in the IR spectral range and tunable electrical resistivity [1, 2]. Nowadays, DLC coatings have been industrially implemented in many engineering applications where excellent tribological properties are required. Additionally, the outstanding biocompatibility properties of these coatings offer a wide range of potential biomedical applications for the improvement of the mechanical, chemical and biological response of prostheses and implants [3]. Specifically, the possibility of doping DLC coatings with bactericidal elements such as silver has resulted in an intensive research in this field.

Silver has long been known to exhibit strong antibacterial activity to a wide range of microorganisms, a low susceptibility to bacterial resistance and the ability to inhibit polymicrobial colonization. The main bactericidal effect of silver is conducted through the release of Ag^+ ions via an oxidative reaction in aqueous solution or biological medium [4]. The kinetics of this dissolution process, which is related to the duration of the antibacterial effect, can be exponentially increased if silver is used in the form of nanoparticles due to its higher surface to volume ratio. Furthermore, the stability and the long-term antibacterial effect of Ag-doped DLC coatings will be determined by different factors: surface energy, roughness, micro- and nanostructure of the coating, as well as the concentration and distribution of silver across the thickness of the coating (dispersed or agglomerated in the form of nanoparticles).

Various methods have been reported to prepare Ag-DLC coatings with different silver concentrations addressed to biomedical applications: RF or DC reactive magnetron sputtering of silver target in a hydrocarbon atmosphere [5-13], DC magnetron sputtering of silver and graphite targets [14-16], hybrid RF/magnetron sputtering plasma assisted chemical vapour deposition (RF/MS PACVD) [17-18], dip coating of a PVP polymer film with a colloidal dispersion of stabilized silver nanoparticles transformed to DLC by ion implantation [19-21], polyethylene transformed to DLC by silver implantation [22-23], thermionic vacuum arc [24], silver nanoparticle solution combined with a DLC coating obtained by PACVD [25], cathodic arc deposition [26-33] and pulsed laser deposition [34-38]. Most of these works demonstrate a good antibacterial efficacy of these coatings against E. coli, S. aureus and S. epidermis for silver concentrations higher than about 2 at.%. In addition, Ag-DLC coatings offer an antibacterial activity over a wide spectrum of other bacterial species: C. jejuni and L. monocytogenes [12]; A. israelii, S. sanguinis, F. nucleatum, C. rectus, E. corrodens, P. micra, P. intermedia, A. actinomycetemcomitans and P. gingivalis [14]; S. warneri [35], and P. aeruginosa, E. faecalis and C. albicans [38]. On the other hand, good hemocompatibility for DLC coatings with a silver concentration of 9.7% [5] and between 70-90% [29] has been demonstrated. Furthermore, M. Chekan et al. have shown that DLC coatings doped with a silver concentration of 3.5-6.5% have an inhibiting effect on the growth of some tumor cells [30].

Despite the good antibacterial efficacy shown by Ag-DLC coatings, the main concern related to its applicability in prostheses and implants lies in the adjustment of the optimal concentration of silver in order to obtain a significant antibacterial activity without cytotoxicity. In this sense, coatings with a silver concentration as low as 1.6 and 2.1% have shown a significant level of cytotoxicity [11]. Other studies point to a silver limit concentration of 4.5% [21] or 5.4% [17], above which the coating becomes cytotoxic. In this regard, excellent non-cytotoxic properties have been reported for DLC coatings with silver concentrations of 2.0% [18] and 3.1% [13]. On the contrary, non-cytotoxic coatings have been obtained for silver concentrations of 5.6% [26] and 6% [14]. More detailed studies demonstrate that the adequate concentration of silver, in terms of physical-chemical properties, an efficient protection against microbial colonization and a non-cytotoxic behavior, ranges between 2 and 7% [36] or around 3.6% [38].

The disparity of these results reveals that the antibacterial activity and cytotoxicity of this type of coatings not only depend on the concentration of silver, but also, as mentioned above, there are other factors that determine the kinetics of the release of Ag^+ ions. However, in view of the published results, a conservative value of the concentration of silver that guarantees an antibacterial effect without causing cytotoxicity would be around 2%.

In this work, hard and well-adhered Ag-DLC coatings with a silver concentration of 2% were obtained on CoCrMo substrates by means of pulsed filtered cathodic arc co-deposition using independent silver and graphite cathodes. For comparative purposes, nitrogen-doped DLC (N-DLC) coatings obtained by RF magnetron sputtering were also processed. The surface morphology, the microstructure, as well as the chemical and mechanical properties of the coatings were investigated. The antibacterial activity was tested against Pseudomonas aeruginosa and Staphylococcus aureus. It is worth noting that P. aeruginosa is an opportunistic multi-resistant bacteria pathogen with recognized high adaptability that can rapidly develop resistance to different types of broad-spectrum antibiotics such as carbapenems [39-40] fluoroquinolones such as norfloxain and ciprofloxacib [41-42] and beta-lactam and aminoglycoside antibiotics [43], among others. The aforementioned multi drug-resistance of P. aeruginosa, and its ability to form biofilms on implanted and indwelling medical devices, encourage the development of new effective, safe, and broad-spectrum antimicrobial agent alternatives, such as Ag nanoparticles, to prevent and treat P. aeruginosa infection as shown in recent studies [44].

To our knowledge, this is the first report of the use of this specific arc-based deposition technique for the preparation of silver-doped DLC coatings. In previous works, J.L. Endrino et al. used alternating arc pulses from graphite and silver in a dual cathodic arc plasma source

[26-28], S.C.H. Kwok et al. employed a pulsed filtered cathodic vacuum arc equipped with a co-axial Ag–C target [29], and N.M. Chekan et al. proceeded to the alternating layer deposition of carbon and silver using a pulsed and a DC cathodic arc source, respectively [30]. More recently, composite graphite/silver targets [31, 32] and independent graphite and silver cathodes [33] have been used for the preparation of Ag-DLC coatings by means of unfiltered cathodic arc deposition technique. Conversely, the coating process developed in this study, consisting in the continuous co-evaporation of independent carbon and silver targets by means of filtered cathodic arc sources, allows the preparation of microparticle-free coatings, as well as a precise control of the silver concentration, being a more feasible deposition technique to be implemented in the biomedical sector on an industrial scale.

2. Experimental procedure

2.1 Coating preparation

Ag-DLC coatings were grown on CoCrMo mirror-polished substrates using the pulsed filtered cathodic vacuum arc PFCVA-450 system (Plasma Technology Limited, Hong Kong) shown in Fig. 1. In order to reduce the incorporation of detrimental microparticles in the growing films, the system was provided with two pulsed filtered cathodic arc sources, where the generated arc plasmas were guided into the vacuum chamber by an electromagnetic field applied to the curved ducts. The samples were positioned in the center of the chamber (240 mm away from the duct exit) and were negatively biased to different voltages as described below. More details of this equipment can be found elsewhere [45].

Table 1 summarizes the experimental parameters of the different deposition stages used for Ag-DLC coatings preparation. The temperature was measured using a floating K-type thermocouple placed 18 cm from the substrate (Fig. 1). The base pressure in the vacuum chamber was 4×10^{-3} Pa. Prior to the carbon coating deposition, a chromium ion etching was applied to the substrates at 150ºC for 2.5 minutes with a DC bias of −600 V. An additional

DC filtered cathodic arc source provided with a pure chromium cathode (99.5%) was used for this purpose. After the substrate temperature dropped to ~ 60ºC, a non-doped amorphous carbon was deposited by using a single cathodic arc source supplied by a pure graphite cathode (99.997%). The cathode was ignited using a pulse duration of 3 ms at a frequency of 8 Hz. An argon flow of 10.6 sccm was introduced through the pulsed arc sources. The substrate was biased to a pulsed potential of −400 V (100 kHz, 20% duty cycle). The thickness of this carbon layer was about 0.5 μm, and the deposition rate was 12 nm/min. Subsequently, a silver-doped carbon layer of about 70 nm in thickness was deposited during 30 min by co-evaporation of graphite and silver cathodes. The substrate bias, the argon flow and the evaporation conditions of carbon remained the same as those used in the previous layer, whereas a pure silver cathode (99.95%) was ignited using a pulse duration of 2.25 ms at a frequency of 8 Hz. During this stage, the samples were continuously rotated at 1.4 rpm. A silver concentration of about 2 at. % in the surface of the coatings was obtained by keeping the ratio of the mean arc current I_{Carbon}/I_{Silver} at a constant value of 1.19.

In order to properly characterize the hardness and adhesion, thicker Ag-DLC coatings (1.0-1.5 μm) were deposited by increasing proportionally the duration of the second and third stages. Complementarily, N-doped DLC coatings were deposited by reactive RF magnetron sputtering technique with the deposition system described elsewhere [46]. Nitrogen doping of DLC improves its mechanical properties and reduces stress, which contributes to the deposition of relatively thick coatings without compromising adhesion to the substrate. Moreover, they are not expected to have bactericidal properties, so they can serve as a reference to evaluate bacterial activity of the Ag-DLC coatings. In order to improve the adherence of the coating, a gradient concentration CrC_x interlayer was previously deposited in a linear magnetic filtered arc deposition system using a Cr target in an acetylene/argon atmosphere, with an increasing carbon concentration varying from x = 0 to x = 0.3. The base pressure was always less than 10^{-4} Pa. Pre-sputtering for 10 min was performed in a pure Ar atmosphere as a last step in the cleaning substrate process. The N-DLC layer (N content 10 at. %, determined by X-ray Photoelectron Spectroscopy) was deposited by sputtering using a 3″ diameter pure graphite target (99.99% purity) using a RF input power of 350 W and a target-substrate distance of 10 cm. The working pressure was 3.6×10^{-3} mbar, with an Ar partial pressure of 3.0×10^{-3} mbar and a N_2 partial pressure of 0.6×10^{-3} mbar. The substrate temperature was 100°C, the negative bias voltage was 100 V and the deposition time was 2h in order to obtain film thickness of 1 μm.

2.2 Morphological, chemical and structural characterization

The surface morphology of the coatings was studied by field emission scanning electron microscope (FESEM) using a Carl Zeiss Neon 40 system (Zeiss, Germany) equipped with an Energy Dispersive X-ray Spectroscopy probe (EDS).

High resolution transmission electron microscopy (HRTEM) and high-angle annular dark field scanning transmission electron microscopy (HAADF-STEM) images were acquired using a FEI Tecnai G2 F20 (FEI, OR, USA) microscope operated at 200 kV. Sample cross-sections were prepared by focused ion beam (FIB) using a Zeiss Neon 40 Cross Beam system (Zeiss). Before ion milling, a thin protective platinum layer was deposited onto the surface of the sample.

The surface texture height parameters S_a (arithmetical mean height) and S_q (root mean square height) were measured with a confocal laser scanning microscope (CLSM) Sensofar Plμ 2300 (Sensofar, Spain) with a 50x objective lens. The area of analysis was 483 x 191 μm^2. Data analysis was performed using MountainsMap 5.1 software (Digital Surf).

Static water contact angle measurement was estimated by sessile drop method using a contact angle system OCA15 plus (DataPhysics, Germany) to assess the surface wettability of CoCrMo samples before and after DLC coating. MQ water and N,N-dimethylformamide

(DMF) were used to calculate the superficial free energy (SFE) (polar and non-polar component) based on Owens-Wendt-Rabel-Kaelble (OWRK) equation [47].

X-ray Photoelectron Spectroscopy (XPS) experiments were performed in a PHI 5500 Multitechnique System (Physical Electronics, MN, USA) with monochromatic X-ray source (Aluminium Kalfa line of 1486.6 eV energy and 350 W), placed perpendicularly to the analyzer axis and calibrated using the $3d_{5/2}$ line of Ag with full width at half maximum (FWHM) of 0.8 eV. The analyzed area was a circle of 0.8 mm diameter. The selected resolution was 187.85 eV of pass energy and 0.8 eV/step for the general spectra and 58.7 eV of pass energy and 0.25 eV/step for the spectra of the different elements. The Ar $2p_{3/2}$ line was used as reference to compensate for any surface charging. In-depth measurements for composition depth profiles were obtained by sputtering the surface with an Ar^+ ion source (4 keV energy). The sputtering ratio was about 5-6 nm/min for a Si_3N_4 film grown on silicon.

The bonding structure of the coatings was studied by using a Horiba Jobin Yvon LabRam HR 800 micro-Raman (Horiba, Japan) spectrometer coupled to a BXFM microscope (Olympus). Unpolarized visible Raman spectra were recorded in backscattering geometry with a solid state laser emitting at 532 nm wavelength in the visible range (VIS). The power used was 0.5 mW with a 100x objective (spot diameter 0.7 μm) and a 600 grooves/mm grating. The detector used was a CCD cooled at -70 ºC.

Grazing incidence X-ray diffraction measurements (GIXRD) were performed in a PANalytical X'Pert PRO MRD (Malvern Panalytical Ltd, UK) diffractometer system with a parabolic mirror in the primary beam and flat graphite secondary monochromator in the secondary beam, using Cu K-α radiation and a fixed divergence slit of 0.1 mm.

2.3 Mechanical characterization

The Daimler-Benz Rockwell-C adhesion test was used to qualitatively assess the coating adhesion. Coatings nanohardness (H) was measured using a NanoIndenter XP (MTS, MN, USA) system fitted with a Berkovich diamond tip. The tip was calibrated using a fused silica sample following the Oliver and Pharr method [48]. The evaluation of H was conducted as a function of depth using the continuous stiffness measurement (CSM) operation mode, where the harmonic displacement amplitude was fixed to 2 nm at 45 Hz. The samples were allowed to thermally equilibrate with the instrument until the drift rate was below 0.06 nm/s. The indentations were carried out at a constant strain rate of 0.050 s^{-1} up to a maximum penetration of 6000 nm. In order to avoid the substrate mechanical influence, the intrinsic coating hardness was determined from depth-hardness curves at indentation depth equal to or less than 10% of the coating thickness. A Poisson's ratio of 0.25 was assumed for all samples. H values were averaged over 25 indents for each sample.

2.4 Electrochemical corrosion analysis

Corrosion tests were performed on both uncoated and coated CoCrMo samples. All specimens were cleaned with ethanol prior to corrosion test. Electrochemical experiments were performed using a Gamry Reference 600 potentiostat (Gamry, PA, USA) in Hank's Balance Salt Solution (pH 6.8) at 37.2 ± 0.2°C. The corrosion cell was set up with three electrodes: a Ag/AgCl reference electrode, a Pt counter electrode and the sample as working electrode. The experiments were carried out in two subsequent tests: (i) recording of the free corrosion potential (E_{0C}) for 15 min; (ii) and potentio-dynamic polarization scan ranging from E_{0C}-1.5 V to E_{0C}+1.5 V, with a scan rate of 0.16 mV/s. The corrosion rate was determined using the Tafel slope obtained from the polarization curve.

2.5 Biological evaluation

2.5.1 Cell culture

Human osteoblast-like SaOS-2 cells (ATCC, USA) were cultured in McCoy's 5A medium (Sigma-Aldrich, USA) supplemented with 10% foetal bovine serum (FBS), 2 mM L-glutamine, 20 mM HEPES buffer and penicillin/streptomycin (50 U/mL and 50 µg/mL, respectively), all from Invitrogen (USA), in a humidified atmosphere at 37ºC and 5% CO_2. Sub confluent cultures were passaged after detaching cells using TrypLE (Invitrogen).

2.5.2 Cytotoxicity

Samples were incubated in complete McCoy's 5A medium during 72 h following the guidelines from ISO 10993.5 and ISO 10993-12 standards. Then, supernatants were serially diluted using complete medium (1:0, 1:1, 1:10, 1:100 and 1:1000) and placed into new microplate wells containing pre-seeded SaOS-2 cells (10.000 cells/well). Cells were incubated in these extracts for 24 h, rinsed with phosphate-buffered saline (PBS) and lysed with 100 µL of Mammalian Protein Extraction Reagent (M-PER, Thermo Scientific, USA). The number of viable cells in each extract dilution was quantified measuring the enzymatic activity of lactate dehydrogenase (LDH) using the Cytotoxicity Detection KitPLUS LDH (Roche Applied Sciences, Germany). The percentage of cell viability was calculated according to the guidelines of the kit after measuring spectrophotometrically at 492 nm the LDH activity in a Synergy HTX multi-mode reader (BioTek, USA).

2.5.3 Bacterial assays

Bacterial culture. Bacterial experiments were performed using Staphylococcus aureus subsp. aureus (CCUG 15915) and Pseudomonas aeruginosa (CECT 110), which were streaked in Brain Heart Infusion (BHI) agar (Scharlab SL, Spain). Colonies were picked and inoculated overnight at 37ºC into BHI broth. The optical density was adjusted to 0.2 units with BHI broth at 600 nm, which corresponds to approximately 1 x 10^8 colony forming units (CFU)/ml.

Bacterial adhesion. Samples were immersed in 1 ml of bacterial suspension for 4 h at 37ºC. Then, medium was aspirated and 3 rinses with PBS were performed to remove non-adhered bacteria. Bacteria were detached from samples in 1 ml PBS by sonication in a Xuba1 Ultrasonic Bath (Grant Instruments, UK) during 1 min and then serial diluted in PBS (1:10, 1:100, 1:000, 1:10.000). Dilutions were cultured in triplicate on BHI agar plates and CFUs were counted after incubating the plates overnight at 37ºC.

Evaluation of biofilm formation. Cultures at 0.2 optical density units were diluted 10 times and 1 ml was placed on each sample. Bacteria were allowed to grow for 24 h and were sonicated as explained above. Then, bacteria were serial diluted (1:10.000, 1:100.000, 1:1.000.000) in PBS and cultured on BHI agar plates. Plates were incubated overnight at 37ºC and the resulting CFUs were counted.

Viability of bacteria. Samples prepared for bacterial adhesion at 4 h were rinsed thrice in PBS and fixed with 2.5% glutaraldehyde solution in PBS. Samples were then incubated with the LIVE/DEAD BackLight bacterial viability kit following the manufacturer's instructions in the dark for 15 min. Samples were rinsed in PBS and visualized using an LSM 800 confocal laser scanning microscope (Carl Zeiss). Images were analysed by ImageJ and the percentage of dead cells was calculated as a ratio of red fluorescent cells (dead cells) versus red and green (dead and live cells, respectively) fluorescent cells.

3. Results and discussion

The cathodic arc co-deposition technique used in this work allows a very precise control of the silver doping concentration by adjusting the arc current of the silver cathode. Fig. 2 shows the linear variation of the silver concentration (determined by XPS) of the Ag-DLC top layer as a function of the average arc current generated in the silver cathode. The doped DLC samples studied in this work were processed at an arc current of 1.7 A, thus obtaining a silver concentration of about 2 at. % on the surface of the coatings.

Fig. 3(a) shows the surface morphology of the Ag-DLC coatings, which is characterized by the presence of some defects that can be attributed to the small fraction of microdroplets that, through multiple collisions, have escaped from the magnetic filter. Chemical analysis performed by EDS indicates that these microdroplets have been originated by the arc discharge of the graphite cathode. In contrast, at this scale of analysis, silver microdroplets have not been observed on the surface of the coatings. On the other hand, Fig. 3(b) shows a STEM cross-section image, where the two layers forming the coating can be observed: a 540 nm thick DLC sublayer, and a thinner Ag-DLC top layer of about 70 nm in thickness. It is worth noting that the interface between these layers is sharp, with no evidence of atomic interdiffusion. This observation has also been confirmed by the HRTEM analysis. Furthermore, no carbon or silver microparticles have been observed either at the substrate interface or within the coating. The carbon microparticles generated by the arc discharge on the graphite cathode that have escaped the action of the magnetic filter have been segregated towards the surface during the growth of the coating (Fig. 3(a)). A similar phenomenon was already observed in a previous work related to the growth of copper-doped DLC coatings using the same experimental setup [45]. The absence of microparticles at the substrate interface is essential to ensure good corrosion resistance. Since the microparticles do not have good adhesion, they can be easily detached, giving rise to the appearance of defects and pinholes that can initiate or accelerate the corrosion mechanisms of the substrate.

Roughness parameters (S_a and S_q) values are shown in Table 2. As it can be seen, the surface roughness increases slightly when DLC coatings are deposited. The roughness is known to play a significant role in the attachment process, when the surface irregularities are comparable to the size of the bacteria, but it is not an important factor in nanostructured surfaces [49]. Considering that the average roughness of the Ag-DLC and N-DLC coatings is in the range of ten nanometers, the roughness would not be expected to influence the initial bacterial attachment.

Wettability measurements (Table 2) show that Ag and N-doped DLC coatings have similar contact angle and surface free energy values. Moreover, when the CoCrMo substrate surface is coated with Ag and N-doped DLC, water contact angle increases from 50 to 75-76º, so the surface becomes more hydrophobic. DMF contact angle also increases from 21 to 53º. This result is associated with a pronounced decrease in surface polar energy component, from 21 to 7-8 mJ/m^2. Nevertheless, no significant differences in the dispersive energy component are observed. As the result, in Ag-DLC and N-DLC coatings, SFE decreases with respect to the CoCrMo substrate (from 55 to 39 mJ/m^2). As reported in the literature, antibacterial behavior is favored by the low surface free energy and hydrophobicity of DLC films [7, 37].

XPS analysis of Ag-DLC coatings confirms the existence of silver in the carbon matrix. Oxygen and nitrogen were also detected as contaminant elements, showing an atomic concentration across the analyzed depth below 1% and 2%, respectively. Concerning the bonding nature of the coatings, the high-resolution spectrum of C1s (Fig. 4(a)) can be properly fitted using 3 Gaussians centered at a binding energy of 284.4, 285.3 and 286.5 eV that can be assigned to sp^2 carbon, sp^3 carbon and carbon-oxygen bonds, respectively. Regarding the chemical state of silver embedded in the coating, the high-resolution spectrum of Ag 3d (not shown) displays both the Ag 3d$_{3/2}$ (~ 374 eV) and Ag 3d$_{5/2}$ (~ 368 eV) peaks, confirming that Ag ions exist in the metallic state and does not create chemical bonds with carbon. Fig. 4(b) corresponds to the XPS depth profile showing the atomic percentages of carbon and silver on the first 30 minutes of sputtering with argon. As it can be seen, after 1 minute of sputtering, the concentration of silver is about 2 at.%, which keeps nearly stable along the doped region and decreases gradually to zero once the interface with the undoped amorphous carbon layer is reached.

Fig. 5(a) shows a HRTEM image corresponding to the undoped layer of the Ag-DLC coating. The structure of this layer appears mostly as amorphous, although a detailed observation reveals the existence of some small regions showing short-range bond order in the form of fullerene-like structures or carbon nano-onions. This fact that can be assigned to curved carbon basal planes, and attributed to a relaxation of the stress induced by the relatively high intensity of the ion bombardment during the film growth inherent to the deposition process [50]. The doped layer of the coating (Fig. 5(b)) shows a matrix with a similar structure than the undoped layer (brighter region) with darker spots that correspond to metallic Ag clusters. Ag nanoparticles with a size of about 2 nm are uniformly distributed in the film. On the other hand, it has been observed that the morphology and composition of the Ag-DLC layer is stable overtime, remaining unaltered for at least one year. The formation of these clusters may be associated with the low solubility of silver with carbon and its inability to form carbides. Similar results have been reported by other authors for amorphous carbon coatings doped with low concentrations of silver [5-6, 51-54]. Interestingly, Choi et al. demonstrated that, for a concentration of 0.1 at. %, silver atoms are fully dispersed in the amorphous carbon matrix, whereas for 1.7 at. % silver forms crystalline nanoparticles with a size of 2 nm [5].

Raman spectroscopy is a useful tool frequently used in DLC based materials to obtain information on the bonding structure inside the amorphous material. The experimental Raman spectra of Ag-DLC and N-DLC coatings are shown in Fig. 6. It is worth noting that the spectrum of Ag-DLC coating is practically identical to that obtained for a non-silver doped DLC sample grown under the same deposition conditions (not shown). For the Ag-DLC coating, the result of the conventional analysis using two Gaussian peaks (G and D) in the region 900-1900 cm^{-1} results in position of the peaks at 1378 cm^{-1} (D) and 1550 cm^{-1} (G), peak-widths of 320 cm^{-1} (D) and 155 cm^{-1} (G) and relative areas $I_D/I_G = 1.1$. This fitting is relatively good with a correlation coefficient of $R^2 = 0.997$, and the fitting values corresponding to our 2% silver content coatings are in good agreement with the study of Meskinis et al. [55] on the dependence of the G peak position and the D/G ratio on the silver concentration for different values of the excitation wavelength. However, a small but clear deviation from the experimental spectrum is observed in the regions 940-1150 cm^{-1} and 1335-1420 cm^{-1}. A much better fit is obtained using four Gaussian peaks, with no noticeable deviation from the experimental spectrum and with a correlation coefficient of $R^2 = 0.9997$. The parameters of the fit are shown in Table 3. These results are similar to the ones obtained by other authors (see for example [56] and [57]). Although the origin of the observed peaks is not fully understood, it is usually attributed as follows: D2 peak: nanocrystalline diamond; D1 peak: microcrystalline or nanocrystalline graphite; G1 peak: disordered graphite; G2 peak: ordered graphite. However, it has been suggested [58] that the D2 peak together with the presence of the observed two extra-peaks at ~400 cm^{-1} and ~700 cm^{-1} might also reflect the formation of fullerene-like structures or carbon nano-onions inside the amorphous matrix, as can be observed in the HRTEM images of our samples. On the other hand, the Ag and N-doped DLC samples show very similar Raman spectra, revealing analogous structure in both materials. The only somewhat significant difference appears in the position and intensity of the D2 peak, which can be attributed to the fact that the distribution or size of the fullerene-like structures inside the amorphous matrix is slightly different.

GIXRD measurements were performed to confirm the presence of Ag nanoclusters in Ag-DLC coatings. Fig. 7 shows the GIXRD diffractograms corresponding to three different incident angles (ω) of 0.1°, 0.3° and 2.0° measured with a scan step size of 0.05° and a time of 30 s per step. A value of ≈ 0.2° has been reported [50] for the critical angle under which X-ray are totally reflected in DLC thin films. For angles of incidence higher than the critical angle (ω = 0.3° and ω = 2°), peaks corresponding to the CoCrMo substrate can be clearly seen in the diffractograms, indicating that a significant part of the radiation penetrates into the substrate. The intensities of these peaks decrease as the angle of incidence decreases because of a lower penetration depth. For ω = 0.1°, a weak peak is observed at 38.2°, which could be attributed to the (111) peak of silver, which is the most intense peak in the Ag powder pattern. The presence of residual substrate peaks at ≈ 44° and ≈ 51° can be attributed to the presence of a not negligible curvature in the polished CoCrMo substrates, causing the actual value of ω to be higher than the nominal value in part of the irradiated surface of the sample. In order to confirm this assignment, an additional measure was made around the position of the peak with improved statistics, using a scan step size of 0.1° and a time per step of 400 s. The result, shown as an inset in Fig. 7, confirms the presence of a wide an asymmetric peak that can be deconvoluted into two Gaussian components centered at 38.0 ± 0.2° (G1) and 38.3 ± 0.03° (G2) and widths of 2.0 and 0.45°, respectively. Using the well-known Scherrer formula, the associated crystallite sizes can be estimated from the widths of the components, which turn out to be 4 nm for G1 and 17 nm for G2. Thus, G1 can be attributed to the presence of Ag nanocrystals as observed in HRTEM, while the origin of G2 is unclear.

Regarding the mechanical properties of the coatings, Fig. 8 shows the variation of the hardness as a function of the concentration of silver. As it can be seen, the hardness shows a slight tendency to decrease as the silver content increases, ranging from about 24 GPa for undoped coatings to 21 GPa for coatings with a silver concentration of 2.4 at. %. By comparison, the hardness of the N-DLC coating obtained by magnetron sputtering is 13.8 ± 0.5 GPa.

In addition to the high hardness, one of the most interesting results achieved in this work is the excellent adhesion of the Ag-DLC coatings on CoCrMo substrates. Fig. 9 displays an optical microscopy image corresponding to residual imprint generated by the Rockwell-C adhesion test, where no delamination or cracks have been generated. Similar results have been obtained for coatings up to 1.5 microns thick. This is a relevant result for those biomedical applications where a good tribomechanical behavior of the coating is required.

The analysis of the polarization curves shows that N-DLC coating appears to have a similar corrosion behavior to the CoCrMo substrate (Fig. 10). However, the coating displays a higher corrosion potential and a similar corrosion current density, but a reduced passivation area compared to the substrate. Moreover, when curves are compared at 0 Volts, it can be observed that N-DLC coating has a current density about a decade higher than that of the substrate. This indicates that the coating might introduce some local pinholes and imperfections that can accelerate corrosion, thus reducing the passive area as it can be observed in the Figure 10.

On the other hand, Ag-DLC coatings present a higher corrosion potential compared to N-DLC coatings and CoCrMo substrate. Additionally, Ag-DLC shows a reduced passive region and a passive current density lower than that of N-DLC and CoCrMo. The corresponding corrosion rates determined from the Tafel slopes agree with this assessment. Ag-DLC coatings exhibit an order of magnitude lower corrosion rate (Table 4). All of these features point out that Ag-DLC coatings are not detrimental in terms of corrosion resistance in comparison to CoCrMo substrate.

Finally, in view of possible biomedical applications, the cytotoxicity and the in vitro antibacterial properties of the coatings were studied. Concerning the cytotoxicity, the percentage of viable cells incubated with different dilutions of medium extracted from samples is shown in Fig. 11. Although a slightly decrease in cell viability is observed in non-diluted extracts, the percentage is above the recommended by the ISO standard to consider a biomedical sample as non-cytotoxic (>70%). This decrease in cell viability should not be attributed to the presence of Ag since no difference with the CoCrMo substrate and the N-DLC coating is observed. Hence, silver doped DLC coating with ~2% Ag could be considered as suitable for its use in biomedical applications.

To demonstrate the antibacterial properties of the Ag-DLC coating, a bacterial viability assay was performed. Noteworthy, as shown in Fig. 12 (a), for S. aureus strain a large amount of viable cells (green dots) is observed on N-DLC coating compared to the number of damaged cells (red dots). In contrast, when bacteria are cultured on Ag-DLC coating, the number of damaged cells considerably increases, observing very few viable cells (Fig. 12(b)). However, when bacteria cultured on the three surfaces are detached and plated on agar, the cell viability on Ag-DLC coating is approximately 80% in S. aureus and 60% in P. aeruginosa compared to non-coated surfaces and N-DLC coating (Fig. 12 (c)). Then, membranes are damaged when bacteria contact with Ag-DLC coating, but they are able to repair their membrane and restart their capability of proliferation when removed from the surface, a phenomenon observed by other authors [59-61]. Noteworthy, when bacterial cells are incubated in contact with Ag-DLC coating for a longer period, i.e. 24 h, their numbers significantly decreases compared to non-coated surfaces and N-DLC coating (Fig.12 (c)). This demonstrates that, although the bacterial cells can survive after short incubation periods, longer incubation periods may reduce biofilm formation due to a contact effect.

At 24 h, as observed after 4 h, the number of P. aeruginosa cells is lower than the number of S. aureus (Fig. 12 (c)). These differences should be attributed to the different resistances of bacterial strains to Ag, as observed previously by other authors [62-63]. In the case of N-DLC coating, cell viability at 4 and 24h (Fig. 12 (c)) corroborates the results obtained in Live/Dead (Fig. 12 (a)), therefore it does not exhibit bactericidal properties in both P. aeruginosa and S. aureus. Noteworthy, the effect of Ag-DLC coating on P. aeruginosa is remarkable, reducing their numbers to 30% after 24 h of incubation. This result is especially interesting considering that P. aeruginosa is a pathogenic bacteria that can rapidly develop resistance to different types of broad-spectrum antibiotics.

4. Conclusions

DLC coatings doped with variable concentrations of Ag were successfully prepared on CoCrMo substrates by means of pulsed filtered cathodic arc co-deposition using pure graphite and silver targets. The concentration of silver embedded in the coating was shown to be linearly controlled by adjusting the average arc current of the silver cathode. Aimed to obtain balanced mechanical, chemical, biocompatibility and bactericidal properties, Ag-DLC coatings with a silver concentration of about 2 at. % were prepared.

It has been shown that the application of the Ag-DLC coating decreases the surface free energy and increases the hydrophobicity, which potentially favors the antibacterial behavior. XPS measurements confirms that silver is in metallic state without creating chemical bonds with carbon. HRTEM and GIXRD analyses reveal the existence of Ag nanoparticles in the range of 2-4 nm size in an amorphous carbon matrix, which shows some fullerene-like structures or carbon nano-onions. No carbon or silver microparticles have been observed either at the substrate interface or within the coating. The carbon microparticles that have escaped the action of the magnetic filter have been segregated towards the surface during the growth of the coating. The Raman spectra of Ag-DLC coatings can be properly fitted using four Gaussians that could be attributed to different diamond and graphite structures.

The coatings have excellent adhesion, with hardness ranging from 24 to 21 GPa for undoped and silver-doped (2.4 at. %) samples, respectively. On the other hand, electrochemical corrosion studies indicate that the application of Ag-DLC coatings are not detrimental in terms of corrosion resistance in comparison to CoCrMo substrate. More importantly, the cytotoxicity tests performed with SaOS-2 cells demonstrate a good cytocompatibility of Ag-DLC coatings, which, in turn, proved to exhibit bactericidal activity against S. aureus and P. aeruginosa. In comparison, despite having similar roughness and surface free energy values, N-DLC coatings obtained by RF magnetron sputtering do not exhibit antibacterial properties. Further research will be carried out to assess the mechanisms and long-term bactericidal effect of silver-doped coatings to optimize their use in biomedical applications.

To conclude, the deposition methodology implemented in this work, consisting in the co-evaporation of independent silver and graphite cathodes, can be easily implemented in industrial cathodic arc PVD reactors, where rotating planetary sample holders and multiple cathodes are commonly used in the preparation of multicomponent and doped coatings.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 1

Experimental parameters of the different deposition stages used for the preparation of Ag-DLC coatings.

Stage	Substrate position	T (ºC)	P (Pa)	Ar flow (sccm)	V_{bias} (V)	Duration (min)	Thickness (nm)
Cr ion etching	Fixed	150	0.22	25.0	-600 (dc)	2.5	—
DLC layer	Fixed	60-70	0.10	10.6	-400 (pulsed)	45	535
Ag-DLC layer	Rotating	65-75	0.10	10.6	-400 (pulsed)	30	75

Table 2

Roughness parameters, contact angle and surface free energy of CoCrMo bare substrate, Ag-DLC coating and N-DLC coating.

Sample	Roughness (nm)		Contact angle (º)		Surface free energy (SFE) (mJ/m^2)		
	S_a	S_q	MQ Water	DMF	SFE	Polar energy	Dispersive energy
CoCrMo	6.2 ± 0.9	8.0 ± 1.0	50.0 ± 3.6	20.9 ± 3.0	54.6 ± 1.3	21.4 ± 4.0	33.3 ± 3.6
Ag-DLC	15.0 ± 2.0	20.4 ± 2.7	75.0 ± 3.3	52.6 ± 3.8	38.6 ± 2.7	8.2 ± 2.9	30.5 ± 5.1
N-DLC	11.5 ± 1.3	29.5 ± 4.2	76.4 ± 2.2	53.2 ± 4.1	38.7 ± 4.2	7.3 ± 3.1	31.3 ± 7.2

Table 3

Fitting parameters of the Raman spectrum with four Gaussians for Ag-DLC and N-DLC coatings in the spectral region 800-2000 cm^{-1}.

Sample	Peak position (cm^{-1})				Peak width (cm^{-1})				Peak area (relative)			
	D2	D1	G2	G1	D2	D1	G2	G1	A_{D2}/A	A_{D1}/A	A_{G2}/A	A_{G1}/A
Ag-DLC	1160	1417	1499	1569	215	268	57	135	0.106	0.652	0.008	0.233
N-DLC	1223	1398	1496	1573	244	205	59	146	0.261	0.430	0.009	0.300

Table 4

Intensity, potential and rate of corrosion of CoCrMo bare substrate, Ag-DLC coating and N-DLC coating.

Sample	I_{corr} (nA)	E_{corr} (mV)	V_{corr} (mm/year)
CoCrMo substrate	162	-809	4×10^{-4}
Ag-DLC coating	8	-44	1.9×10^{-5}
N-DLC coating	324	-336	1.8×10^{-4}

List of figure captions

Fig. 1.- Schematic of the filtered cathodic vacuum arc PVD system.

Fig. 2.- Variation of the concentration of silver in the coating as a function of the arc current of the silver cathode.

Fig. 3.- (a) SEM and (b) STEM image corresponding to the surface and the cross-section of a 2 % Ag-doped DLC coating, respectively.

Fig. 4.- (a) C1s XPS spectra and (b) XPS depth profile showing the atomic percentages of carbon and silver in a 2 % Ag-doped DLC coating.

Fig. 5.- HRTEM images of: (a) undoped DLC layer; (b) Ag-DLC layer with a 2 % Ag. The circles circumscribe some observed fullerene-like structures or carbon nano-onions.

Fig. 6.- Raman spectrum of 2 % Ag-DLC and N-DLC coatings, fitted with four Gaussians in the spectral region 800-2000 cm^{-1}.

Fig. 7.- GIXRD diffractograms at three different incident angles (ω) of 0.1°, 0.3° and 2.0° corresponding to a 2 % Ag-doped DLC coating. Inset: peak attributed to Ag (111) reflection obtained at $\omega = 0.1°$.

Fig. 8.- Hardness variation of the Ag-DLC coating as a function of silver concentration.

Fig. 9.- Daimler-Benz Rockwell-C adhesion test performed on a 2% Ag-doped DLC coating on a CoCrMo substrate.

Fig. 10.- Potentio-dynamic polarization curves obtained in Hank's solution of CoCrMo bare substrate, 2% Ag-doped DLC coating and N-DLC coating.

Fig. 11.- Viability of SaOS-2 cells, measured by enzymatic activity of lactate dehydrogenase (LDH), after incubating cells for 24 h with extract dilutions (1:0, 1:1, 1:10, 1:100 and 1:1000). Cell viability values are not statistically significant different for all dilutions ($p<0.05$).

Fig. 12.- Live/Dead S. aureus viability on N-DLC coating (a) and 2% Ag-doped DLC coating (b), showing viable cells in green dots and dead cells in red dots. Bacterial adhesion at 4 h and 24 h on Ag-DLC compared to non-coated substrate and N-DLC coating (c).

Credit Author Statement

No Credit Author Statement is included in this paper

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Highlights

- Ag-doped DLC coatings were prepared by pulsed filtered cathodic arc co-deposition.
- The coatings decrease the surface free energy and increases the hydrophobicity.
- Non-cytotoxic, hard, well-adhered and corrosion resistant coatings were obtained.
- The coatings exhibit bactericidal activity against S. aureus and P. aeruginosa.