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Preparation and antibacterial properties of Ag-containing diamond-like carbon films prepared by a combination of magnetron sputtering and plasma source ion implantation

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ABSTRACT

Ag-containing diamond-like carbon (DLC) films were prepared on austenitic type stainless steel SUS316L and silicon wafer substrates by a process combining reactive magnetron sputtering with plasma source ion implantation (PSII). An Ag disc was used as a target for the sputter source with an RF power of 100 W. A mixture of the gases Ar and C_2H_2 was introduced into the discharge chamber while a negative high voltage pulse was applied to the substrate holder. By changing the gas flow ratios the resulting Ag content of the films could be varied. The prepared films were composed of amorphous carbon with crystalline Ag, as observed by X-ray diffractometry and TEM. Additional sample characterizations were performed by X-ray photoelectron spectroscopy, secondary ion mass spectrometry and Raman spectroscopy. The surface morphology was observed by scanning electron microscopy. The antibacterial activity was determined using *Staphylococcus aureus* bacteria. All Ag-containing diamond-like carbon films exhibited an antibacterial activity with only small variations depending on the Ag content.

1. Introduction

A simple but effective ion implantation and deposition method is plasma source ion implantation (PSII), which has been used for many applications that are based on the surface modification of materials [1–3]. In the PSII process, a negative high voltage pulse is applied directly to a target and, thus, a plasma surrounding the substrate is generated. The ions from plasma forming hydrocarbon gases, such as methane or acetylene, lead to the implantation of carbon ions into the substrate surface. But this technique can also be applied to deposit diamond-like carbon (DLC) films. These films possess special properties such as chemical inertness, high hardness, low friction, high wear resistance and corrosion resistance. There have been attempts to improve the antibacterial properties of DLC films by the addition of certain elements, namely Ag. The incorporation of silver into DLC films (Ag-DLC) offers the possibility to provide films that are hemo-compatible and antibacterial [4–7]. Various methods have been used to prepare Ag-DLC films, e.g. pulsed laser deposition [8], ion beam deposition [9], plasma enhanced chemical vapor deposition [10] and filtered cathodic vacuum arc [11].

A simple, conventional source to provide metal during the coating process is a sputter source. When this is combined with PSII, it is not only possible to incorporate metal into a film, but also to implant metal ions into the substrate [12]. Hereby, a mixed zone at the interface is formed, thus improving the adhesion of the film. In our previous works we have reported on the plasma source implantation of sputtered Ag ions into materials and the effects of metal incorporation into DLC films on the composition, structure and phase formation [12–15]. In this study, we expanded the investigations to the influence of the silver content in DLC films on the structure, surface roughness and antibacterial properties of the films.

2. Experimental

Disks of commercial-grade austenitic stainless steel 316L that were polished to a mirror-like finish surface and silicon wafers (100) were used as substrates. The substrates were placed in the PSII apparatus and subjected to a PSII method combined with magnetron sputtering. The apparatus used is described in detail elsewhere [13].

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A mixture of argon and acetylene (C_2H_2) gases was fed into the vacuum chamber by controlling their flow rates through mass flow controllers. The flow rate of the acetylene gas was between 1.8 and 4.2 sccm, while the flow rate of argon was fixed at 20 sccm. While an increased flow of acetylene leads to a higher deposition rate of the DLC film because there is more source material available, it also decreases the amount of sputtered Ag due to deposition of hydrocarbons onto the sputtering target. Thus, the film composition can be changed via the relative gas flows. The gas pressure during implantation was set to around 1 Pa. An Ag disc with four inches (10 cm) in diameter was used as the sputtering target. The plasma was created by an RF source with 100 W at 3.56 MHz. A pulse voltage of −10 kV superposed to a DC voltage of −0.5 kV, with a repetition rate of 100 Hz and a pulse duration of 100 μs, was applied to the substrate holder during implantation. The process time was 1 h.

The thickness of the films was monitored by cross-sectional scanning electron microscopy (SEM). The surface morphology of the films was also observed by SEM and atomic force microscopy (AFM). The structure of the films was examined by glancing angle X-ray diffraction (XRD) with an incident angle of 0.5°. The chemical composition and bonding structure of the films were investigated using X-ray photoelectron spectroscopy (XPS) under Mg Kα X-ray irradiation, high resolution transmission electron microscopy (HRTEM) and Raman spectroscopy with an excitation wavelength of 514 nm of an argon ion laser. Depth profiles were obtained by secondary ion mass spectrometry using 3.5 keV O_2^+ primary ions detecting positive secondary ions. Distilled water contact angle measurements were made on the surface using a contact angle meter.

The antibacterial growth activity of the Ag-DLC coated disks was determined using *Staphylococcus aureus* (ATCC 29213). The disks were sterilized by being immersed in 70% ethanol, air-dried and exposed under UV light for 24 h before the experiment. Each disk was placed in individual wells of sterile 6-well culture plates with 3 ml of sterile Bacto Tryptic Soy Broth (BD biosciences) and 5 μL of bacterial suspension (1×10^4/ml). 3 ml of cell suspension without disk were used as positive control. The samples were incubated with constant agitation (200 rpm/min) at 37 °C and the absorbance at 570 nm was measured at certain time intervals. The antibacterial activity of the samples was calculated using the following relationship:

$$R(\%) = (B - C)/B \times 100 \qquad (1)$$

where R is the antibacterial effect (%), B is the mean number of bacteria in the samples without any disk, and C is the mean number of bacteria on the samples. The experiments were carried out in quintuplicate (i.e., five samples for each test) and repeated at least three times.

Antibacterial survival tests were carried out as follows. *S. aureus* cells were suspended in sterilized distilled water to a concentration of 1×10^5 cells/ml and 300 μl of the suspension was dispensed onto each sterilized disk. After incubation of indicated times at 37 °C, 50 μl of suspension on each disk were retrieved and diluted with 950 μl of Phosphate buffered saline. After stirring, 200 μl of the diluted suspension was placed onto Mannitol salt agar plates with egg-yolk. The bacteria were incubated for 16 h and the colonies formed were observed by optical microscope.

3. Results and discussion

The thicknesses of the films were in the range of 0.6–1.2 μm, which were measured by cross-sectional SEM. The deposition rates were 1.2 μm h^{-1} at a C_2H_2 flow rate of 2.45 sccm, 0.7 μm h^{-1} at 3.50 sccm, and 0.9 μm h^{-1} at 5.04 sccm.

Measurements of the water contact angle demonstrate an initial sharp rise of the contact angle with silver content up to angles between 90 and 100°, see Table 1. Consequently, silver enhances the hydrophobicity of the sample, since a hydrophobic surface has a contact angle higher than 70° [5].

The silver content in the films was determined by XPS. For the lowest flow rate of C_2H_2 a silver content of 27.0 at.% was found, with decreasing Ag content for increasing C_2H_2 flow rate, e.g. 2.3 at.% Ag at 5.0 sccm. The concentration of the silver in the DLC films could be controlled by changing the fraction of C_2H_2 in the gases, see Table 1 for the concentrations. These results are consistent with our previous reports for metal containing DLC [12–14].

The Ag phase was investigated with XRD. The silver concentration of 3.8 at.% shows only a weak broad Ag (111) peak, indicating that crystalline Ag has been formed. With an increase in the concentration to 5.9 at.% several higher reflexes for the Ag crystalline phase (200) and (311) were found. The intensity of the reflexes increased with the silver content in the films. The results of X-ray examination are confirmed by HRTEM. Electron diffraction gives more sensitive information on the phases present in a sample than X-ray diffraction. Therefore, selected-area electron diffraction patterns have been taken from the samples. Fig. 1 shows the results obtained by HRTEM imaging and the selected-area electron diffraction pattern of the 3.8 at.% Ag-containing DLC film. There are several areas that can be interpreted as small crystals with an extent of several nanometers, e.g. next to the lower right corner of the diffraction pattern inset in Fig. 1. The pattern itself is composed out of polycrystalline rings with small diffraction spots. The observed reflections have been indexed and allocated to the lattice distances of Ag. One can conclude that the Ag in the films is agglomerated in nanoscale silver crystals smaller than 10 nm.

The surface morphology was observed by SEM and AFM. A very smooth structure was observed for the lower content of silver. Previous observations have shown that for higher content Ag-containing films an increasing grain size with a corresponding higher surface roughness was found [15].

Fig. 2 shows an example of an AFM image of a 3.8 at.% silver DLC film deposited onto a silicon substrate. The average roughness *Ra* is 2.7 nm. This value is about ten times larger than the surface roughness for DLC films which was measured to be 0.23 nm. The influence of the silver content on the surface roughness and topography is evident. Since silver is not capable of forming a carbide, the small Ag clusters within the carbon matrix, as seen in the HRTEM image, might explain the higher surface roughness.

Fig. 3 shows the variations in Raman spectra of Ag-DLC films with increasing amount of silver concentration in the films. The Raman spectra show two prominent features, the D (disorder) and G (graphite) peaks (around 1350 cm^{-1} and around

Table 1
Raman spectroscopy and contact angle data of Ag-DLC films with various amounts of silver.

Ag concentration (at.%)	G band		I_D/I_G	Contact angle (degree)
	Peak (cm^{-1})	FWHM (cm^{-1})		
27.0	1589.1	112.9	2.11	93.3
14.0	1579.4	116.8	2.28	101.0
12.3	1582.5	116.6	2.13	89.0
6.9	1580.3	118.1	2.36	100.5
5.9	1572.9	125.4	2.24	88.2
3.8	1560.5	147.6	1.78	74.0
0	1553.6	153.6	1.59	65.6