

Biocompatible Silver-containing a-C:H and a-C coatings: A comparative Study

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ABSTRACT

Hydrogenated diamond-like-carbon (a-C:H) and hydrogen-free amorphous carbon (a-C) coatings are known to be biocompatible and have good chemical inertness. For this reason, both of these materials are strong candidates to be used as a matrix that embeds metallic elements with antimicrobial effect. In this comparative study, we have incorporated silver into diamond-like carbon (DLC) coatings by plasma based ion implantation and deposition (PBII&D) using methane (CH₄) plasma and simultaneously depositing Ag from a pulsed cathodic arc source. In addition, we have grown amorphous carbon – silver composite coatings using a dual-cathode pulsed filtered cathodic-arc (FCA) source. The silver atomic content of the deposited samples was analyzed using glow discharge optical spectroscopy (GDOES). In both cases, the arc pulse frequency of the silver cathode was adjusted in order to obtain samples with approximately 5 at.% of Ag. Surface hardness of the deposited films was analyzed using the nanoindentation technique. Cell viability for both a-C:H/Ag and a-C:/Ag samples deposited on 24-well tissue culture plates has been evaluated.

INTRODUCTION

In order to manufacture novel biocompatible coatings with tunable bio-reactions, a promising approach is to start from an existing biocompatible coating such as DLC and alloying it with bioactive elements. Diamond-like-carbons are well known to prevent inflammatory and allergic reactions but, by themselves, cannot produce any antibacterial bioactivity. In a recent article, R. Hauert (Swiss Federal Laboratories, EMPA) described the biocidal function of DLC films doped with certain toxic elements such as silver, copper and vanadium [1]. In general, the underlying idea is that by varying the alloy element concentration, it is possible to tailor the biological reactions of a composite metal-DLC film to a desired point where the biocidal effect of the metal is maximized without jeopardizing the biocompatibility of the material. In the present study, we attempt to compare both the viability of mouse MC3T3 osteoblastic cells and the mechanical properties of nanocomposite hydrogenated and hydrogen-free DLC-Ag coatings prepared using two different deposition techniques: i) plasma based ion implantation and deposition (PBII&D)[2] and by ii) metal plasma immersion ion implantation and deposition (MePIIID) [3].

EXPERIMENTAL DETAILS

The four doped and non-doped diamond-like carbon films discussed in this study have been deposited using the dual-cathode arc plasma source deposition system shown in figure 1. This system is equipped with a computer-controlled bias amplifier such as to synchronize substrate bias with the pulsed production of plasma. The hydrogen-free films were deposited in

a vacuum of about $0.67 \cdot 10^{-3}$ Pa. Alternate pulses from graphite and silver cathodes were used to manufacture the film with the desired composition. No bias voltage was applied during the deposition of the metallic component in order to avoid excessive re-sputtering of carbon atoms. Hydrogenated films were deposited by PBII&D. In this case, the system was operated in a continuously pulsed bias mode and methane (CH_4) was bled into the chamber at a flow rate of 30 sccm. The working pressure used the deposition of amorphous hydrogenated carbon films was about 9.33 Pa. Similarly, the silver containing

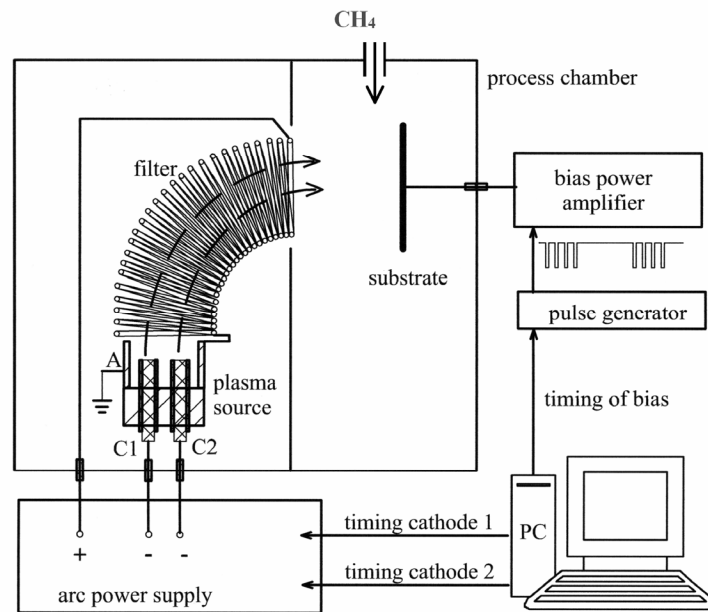


Figure 1. Schematic of plasma immersion implantation and deposition chamber.

amorphous hydrogenated film was deposited by PBII&D using methane and periodically pulsed arcing from a silver cathode.

Glow Discharge Optical Emission Spectroscopy (GDOES) depth profile analysis of the films was completed using a Jobin Yvon RF GD Profiler equipped with a 4 mm diameter anode and operating at a typical radio frequency discharge pressure of 650 Pa and power of 40 W.

Quantified profiles were obtained automatically using the standard Jobin Yvon QUANTUM Intelligent Quantification software. Nanoindentation experiments were performed using a surface force microscopy (SFM) apparatus equipped with a 67nm radius diamond tip. Nanoindentation experiments were performed with an atomic force microscope (Nanoscope II, Digital Instruments) equipped with a force transducer (Triboscope, Hysitron, Inc.). All nanoindentations were made with a Cube Corner diamond tip of nominal radius of curvature equal to ~ 67 nm, determined from calibration indentations produced on a standard fused quartz sample with various normal loads. A triangular loading function with loading and unloading time of 2 seconds was used in all nanoindentation experiments. In this study the hardness was defined as the ratio of the maximum load to the projected contact area of the diamond tip at the corresponding depth. The in-plane elastic modulus (E_r) of the samples was determined from

$$E_r = \frac{\sqrt{\pi}}{2} \frac{S}{\sqrt{A_c}}$$

where A_c is the projected contact area under maximum load and S is the slope of the initial portion of the unloading curve.

Hardness and reduced elastic modulus were determined from the unloading portion of the indentation curve of the force-displacement material response. The maximum Hertzian contact stress corresponding to the maximum loading force of 100 μ N was found to be around 1.5 GPa.

Cell survival and proliferation were determined using mouse MC3T3 osteoblastic cells that were seeded on coated glass cover slips at an initial density of 5 x 10⁴ cells per disc. The MTT assay was used to quantify the number of viable cells after 3 and 7 days of culture. Data from coated cover slips were normalized to those from uncoated glass control specimens.

DISCUSSION

Compositional Analysis

Table 1. Deposition and average silver content determined by glow discharge optical emission spectroscopy (GDOES).

| Sample | Composition | Deposition Technique | Silver Content (at.%) |
|--------|-------------|--------------------------------------|-----------------------|
| 1 | a-C:H | PBII&D (CH ₄) | 0 |
| 2 | a-C:H/Ag | PBII&D (CH ₄) + FCA (Ag) | 5.5 \pm 1 |
| 3 | a-C | MePIIID (C) | 0 |
| 4 | a-C/Ag | MePIIID (C) + FCA (Ag) | 5.6 \pm 1 |

The results of the quantitative analysis of silver for all the samples are shown in Table 1. In the case of sample #2, the repetition of the silver pulse was triggered at a rate of 8.4 times per minute, while for sample #4 the ratio of pulses of graphite to silver pulses employed was 12 to 1. Both of these two configurations resulted in films with about 5.5 at.% of Ag.

In Figure 2, the qualitative depth profile of the hydrogen and oxygen content of samples #1 (a-C:H) and #3 (a-C) is shown. Due to the lack of reference samples in the calibration, no quantitative data can be obtained from the measurements. Therefore, the intensity of the elementary signal is plotted (multiplied by a factor of 50) versus the experimental time. The first

interesting result is the high speed of the measurements. In only 5 seconds the H and O distribution throughout the coating are displayed. The interface between the carbon film and the substrate was located at the time when the substrate signal reached half of its value (not shown in the figure). Although no quantitative information was obtained, it is clear that the hydrogenated sample #1 has higher H and O contents than the a-C sample. However, the high H signal detected in the substrate of sample #1 could be related to a background increase due to changes in the emission lines because of the hydrogen present in the plasma [4]. After subtracting this background, the hydrogen signal remains significantly higher (~ 2 times) in the a-C:H sample. The presence of hydrogen in sample #3 (a-C) is likely caused by the presence of water vapor as a result of the deposition conditions (room temperature, $0.67 \cdot 10^{-3}$ Pa). Due to the importance of quantifying the hydrogen content in a wide range of samples, presently, we are working in collaboration with other groups in the implementation of hydrogen quantification protocols. This could lead to the use of GDOES as alternative to techniques such as ERDA, to monitor the depth distribution of H in a fast and accurate manner.

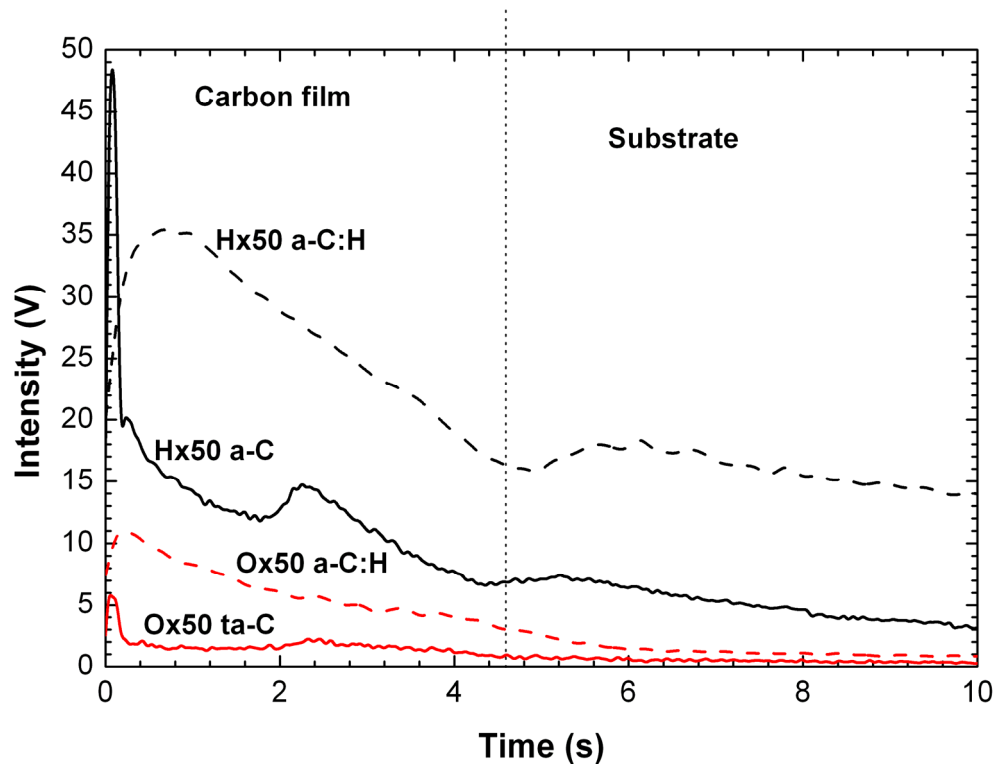


Figure 2. Intensity of hydrogen and oxygen by glow discharge optical emission spectroscopy (GDOES) for samples #1 (a-C:H) and #3 (a-C) with respect to time.

Nanoindentation

Fig. 3 shows the nano-indentation curves for all samples under $100 \mu\text{N}$ maximum normal load with a cubic corner diamond tip of $\sim 67 \text{ nm}$ radius. The additional of silver into a-C:H reduced the sample hardness and in-plane modulus, i.e. the maximum indentation depth was increased from 28 nm to 32.2 nm . The final residual displacements, which correspond to the permanent damage by the indentation, were the same for with and without silver. The energy dissipations during each cycle were 0.61 and 0.72 pJ . The addition of silver into a-C had reverse

effects on hardness and in-plane modulus. The a-C/Ag had smaller indentation depth than a-C, as well as smaller residual displacement. The energy dissipated of their cycles was 0.52 and 0.42 pJ, respectively. Smaller indentation depth, residual displacement and energy dissipation would give longer service life of the protective coating for the reason that material failure occurs by cyclic strain and accumulation of energy for cracking. These maximum indentation depths were more than 10% of the total film thicknesses, and therefore the sample hardness included a significant contribution from the silicon substrate; the actual hardness and modulus of the coating materials should be higher than the overall sample properties included in the figure.

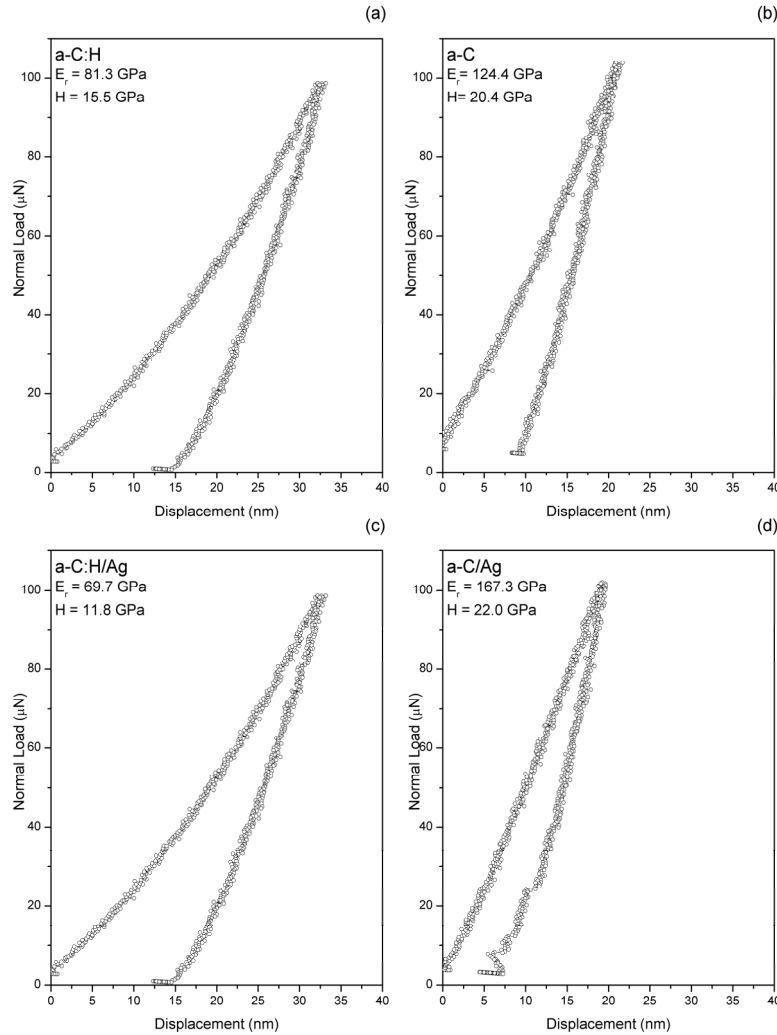


Figure 3. Load vs. displacement indentation curves at 100 μN maximum load. a) a-C:H. b) a-C. c) a-C:H/Ag d) a-C/Ag.

Cell Viability

The results for cell proliferation with respect to plastic control specimens for all the deposited samples are shown in figure 4. In comparison to hydrogenated carbon films, both doped and un-doped hydrogen-free films had higher cell viability. The results also show a slight increase in cell viability with time for sample #4 (a-C/Ag) whereas hydrogenated films (samples #1 and #2) had a drop of ten per cent of cells after 7 days of culture. The good cell attachment shown for silver doped films (about 98%) is a good indicator that this film is not toxic for cells. These results also underscore the importance of the use of the filtered cathodic arc technique for the deposition of biocompatible composite carbon films.

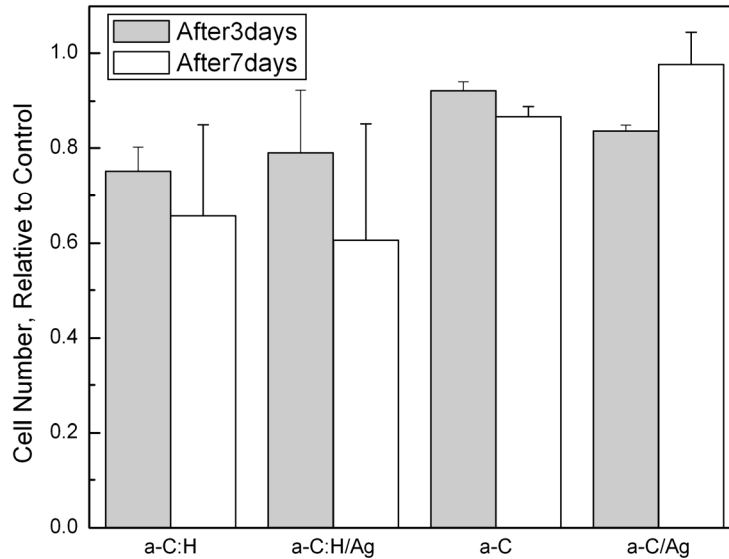


Figure 4. Quantitative adhesion of cells in comparison with the plastic positive control after three and seven days using standard MTT colorimetric assay.

CONCLUSIONS

Composite Ag/a-C:H and Ag/a-C coatings have been deposited by both PIII&D and MePIII&D techniques. Nanoindentation tests showed that hydrogen-free films had higher hardness and elastic modulus. Addition of about 5 at.% of silver resulted in a deterioration of the mechanical properties for hydrogenated films but not for the hydrogen-free ones. Cell proliferation tests showed higher cell viability of mouse MC3T3 osteoblastic cells on hydrogen-free DLC films. In future work, the authors plan to study the influence of different Ag concentrations in doped a-C films on the antibacterial efficacy against *Staphylococcus Aureus*.

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