Hemocompatibility and anti-bacterial properties of silver doped diamond-like carbon prepared by pulsed filtered cathodic vacuum arc deposition

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Abstract

Recent studies have shown that diamond-like carbon (DLC) films are suitable as surface coatings on biomedical devices. Doping of DLC with selective elements is an attractive method to enhance the biological and other properties of DLC. In this work, DLC films doped with silver (Ag) were deposited employing pulsed filtered cathodic vacuum arc (FCVA). Silver was chosen as the dopant because of its anti-bacterial properties. The structure and surface properties of the films were characterized by scanning electron microscopy (SEM), X-ray photoelectron spectroscopy (XPS), Raman spectroscopy and contact angle test whereas the biocompatibility of the Ag-doped DLC films was evaluated by platelet adhesion and anti-bacterial tests. Good platelet adhesion results were obtained from samples deposited using certain parameters and their biocompatibility was found to be better than that of the control sample made of low-temperature isotropic carbon (LTIC). Our results demonstrate that the Ag-doped DLC films are potentially useful biomaterials having both good blood compatibility and antimicrobial characteristics.

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1. Introduction

Diamond-like carbon (DLC) is known for its superior mechanical and chemical properties and thus has many applications in the magnetic [1], microelectronics [2], and optical [3] industries. DLC also has good biocompatibility and is an attractive coating material in biomedical devices including bone implants and cardiovascular devices [4–6]. In particular, doped DLC films have attracted much attention recently especially in the semiconductor field. By incorporating different elements such as nitrogen [7] or boron [8], the electrical properties of the materials can be altered. At the same time, doped carbon films are also potentially useful as biomaterials. Silver (Ag) is known to be a potent antibacterial agent that has been used in biomedical engineering with good effects [9,10]. It has also been reported that the tribological properties of DLC...
Films can be improved by doping with Ag [11] and Ag-doped DLC has been suggested to be potentially useful in biomedical applications [12,13]. In the work reported, we investigated both the hemocompatibility and antibacterial properties of Ag-doped DLC thin films synthesized by means of pulsed filtered cathodic vacuum arc (FCVA) deposition using a co-axial Ag–C target.

2. Experimental details

2.1. Film fabrication

A pulsed filtered cathodic vacuum arc (FCVA) equipped with a co-axial Ag–C target [14] depicted in Fig. 1 was utilized to deposit the Ag-doped DLC films on Si (100) using a sample bias voltage of 0, −400, or −600 V. The detailed experimental parameters are shown in Table 1. A pure Ag film was prepared as the control and a sample bias of −400 V was applied to improve the adhesion of the Ag film. Other materials such as tetrahedral carbon (ta-C), stainless steel (SS), and low-temperature isotropic pyrolytic carbon (LTIC) were also tested for comparison in our biomedical analyses.

2.2. In vitro platelet adhesion tests

In vitro tests were performed to evaluate the hemocompatibility of the Ag-doped DLC films. The technique used here assesses the surface thrombogenicity of the doped DLC and

### Table 1: Experimental parameters for fabrication of Ag-doped DLC films

<table>
<thead>
<tr>
<th>Sample</th>
<th>Bias (V)</th>
<th>Pulse length (μs)</th>
<th>Frequency (Hz)</th>
<th>Period</th>
</tr>
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<tbody>
<tr>
<td>Ag–C (a)</td>
<td>0</td>
<td>2000</td>
<td>5</td>
<td>3000</td>
</tr>
<tr>
<td>Ag–C (b)</td>
<td>−400</td>
<td>2000</td>
<td>5</td>
<td>3000</td>
</tr>
<tr>
<td>Ag–C (c)</td>
<td>−600</td>
<td>2000</td>
<td>5</td>
<td>3000</td>
</tr>
<tr>
<td>ta-C (control)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ag (control)</td>
<td>−400</td>
<td>2000</td>
<td>5</td>
<td>3000</td>
</tr>
<tr>
<td>Stainless steel (control)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LTIC (control)</td>
<td></td>
<td></td>
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</tbody>
</table>

### Table 2: Integrated area ratios of the D and G peaks (I_D/I_G), G peak position and full width at half maximum (FWHM)

<table>
<thead>
<tr>
<th>Sample</th>
<th>I_D/I_G ratio</th>
<th>G peak position</th>
<th>G peak FWHM</th>
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<tbody>
<tr>
<td>Ag–C (a)</td>
<td>1.14</td>
<td>1585.9</td>
<td>154.8</td>
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<td>Ag–C (b)</td>
<td>1.38</td>
<td>1583.3</td>
<td>144.2</td>
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<tr>
<td>Ag–C (c)</td>
<td>1.19</td>
<td>1580.0</td>
<td>138.5</td>
</tr>
<tr>
<td>ta-C (control)</td>
<td>–</td>
<td>1576.7</td>
<td>185.6</td>
</tr>
</tbody>
</table>

Fig. 2. Raman spectra of the Ag-doped DLC films: (a) Sample a, (b) Sample b, (c) Sample c, (d) ta-C.
examines the interaction between the materials and blood. Fresh blood plasma was collected from a healthy adult, and the yellow platelet rich plasma (PRP) was obtained by centrifuging for 15 min at 1000 revolutions per minute (rpm). The DLC and other samples, about 5×5 mm², were cleaned ultrasonically and then immersed and incubated at 37 °C in the PRP for 15 or 120 min. During incubation, some of the platelets interact with and adhere onto the surface. After rinsing, fixing, and critical point drying, the specimens were examined using optical microscopy (OM) and scanning electron microscopy (SEM). By employing the point counting method at a fixed magnification factor, the quantity and morphology of the adherent platelets were determined to assess the degree of platelet adhesion and activation. Ten fields on each sample were chosen randomly to obtain statistical averages.

2.3. In vitro anti-bacterial tests

In the in vitro anti-bacterial tests, the method of plate-counting [15,16] was employed to determine the antibacterial performance against *Escherichia coli* ATCC10536 (*E. coli*, gram negative). The samples were first washed with 70% ethanol to kill any bacteria on the surface. After drying, a 0.2 ml solution containing 2.0–5.0×10⁵ CFU (colony forming units)/ml of the bacteria was added onto the surface and then covered by a polyethylene film 4 cm×4 cm. The samples were incubated at a relative humidity (RH) of higher than 90% and temperature of 37 ±1 °C for 24 h. Afterwards, the samples were thoroughly washed with 20 ml of a 0.87% NaCl solution and...
containing Tween 80 at a pH of 7.0±2. For observation, 0.2 or 0.02 ml of the washing solution was added onto different dishes containing the nutrient agar. After 24 h of incubation under similar conditions, the active bacteria were counted using optical microscopy and the antibacterial effect was calculated using the following relationship:

\[ R(\%) = \left( \frac{(B-C)}{B} \right) \times 100\% \]

Fig. 5. (a) Ag 3d and (b) C 1s XPS spectra of: (i) Sample a, (ii) Sample b, (iii) Sample c, (iv) ta-C.
where $R$ is the antibacterial effect ($\%$), $B$ is the mean number of bacteria on the control samples (CFU/sample), and $C$ is the mean number of bacteria on the modified samples (CFU/sample).

### 3. Results and discussion

#### 3.1. Film characterization

The Raman spectra acquired from the Ag-doped DLC films are displayed in Fig. 2. The spectra closely resemble those of amorphous diamond-like carbon and can be resolved into the G (graphitic) and D (disordered) bands near 1550 cm$^{-1}$ and 1350 cm$^{-1}$, respectively. The ratio of the integrated areas under the D and G peaks $(I_D/I_G)$ as well as position and full width at half maximum (FWHM) of the G peaks are summarized in Table 2. The positions of the G and D peaks, FWHM of G peak and $I_D/I_G$ convey information about the $sp^3/sp^2$ bonding ratio, graphite cluster size, and disorder in these threefold coordinated islands. Salient G peaks are found in all samples. The single prominent peak in the ta-C (Fig. 2d) can be attributed to the high $sp^3$ content without doping. There is a slightly change in the $I_D/I_G$ ratio when the bias voltage is increased. The G peak tends to shift from 1585.9 cm$^{-1}$ to 1576.7 cm$^{-1}$ and the widths of the G peaks change from 154.8 cm$^{-1}$ to 138.5 cm$^{-1}$. The results indicate that there is more tetrahedral ($sp^3$) hybridization with the bias voltage.

The XPS depth profiles of the Ag-doped DLC films are displayed in Fig. 4. The XPS survey spectra of the doped DLC film (Ag–C (a)) and ta-C, indicating that Ag has been successfully incorporated into the carbon matrix. Fig. 5 displays the high resolution spectra of Ag 3d and C 1s. Both the Ag 3d$_{3/2}$ (374 eV) and Ag 3d$_{5/2}$ (368 eV) peaks are observed from the Ag-doped DLC. Comparing the results to those of the pure Ag control, the Ag ions can be inferred to exist in the metallic state, that is, without forming bonds with the matrix C atoms. Differences can be observed from the C 1s spectra acquired from the three Ag-doped DLC samples. The intensities of the C 1s peaks in samples a and b are lower than that in sample c. It is believed that the strong peak in sample c is caused by the high bias voltage on the substrate providing higher energetic C ions for film deposition and more effective formation of a tetrahedral-like structure with a high $sp^3$ content.

#### 3.2. Biomedical tests

Fig. 6 displays the statistical results of the platelet adhesion test whereas Figs. 7 and 8 show the SEM micrographs of adherent platelets test at ×1000 and ×3000 magnifications, respectively. Samples a and b show similar results but a larger number of adherent platelets is observed on sample c. The ta-C control exhibits the worst biocompatibility since both the total number and percentage of activation of adherent platelets are high. The poorer hemocompatibility of sample c and ta-C seems to be related to the tetrahedral-like structure which has a similar biological behavior as ta-C.

In addition to the platelet adhesion tests, anti-bacterial tests were conducted to investigate the other important property of biomaterials. The average number of bacteria and antibacterial effects of the doped samples are compared to those of Si and ta-C. As shown in Table 3, all the Ag-doped DLC films have excellent antibacterial effects (>98%). Even though sample c is the worst of the three samples, the average number of adhered bacteria is still very low (34) compared to ta-C (976) and Si (1938). Our results demonstrate unequivocally the excellent antimicrobial properties of Al-doped DLC.

#### 3.3. Wettability and surface energy

It is believed that the initial adsorption of protein from blood onto a material surface exerts a large influence on platelet adhesion and activation [17]. Therefore, it is important to investigate the interaction between the material surface and plasma proteins. The interfacial tension $(\gamma_{ij})$ between the condensed phase i and j can be evaluated by the following equation:

$$\gamma_{ij} = (\alpha_i - \alpha_j)^2 + (\beta_i - \beta_j)^2 + \Delta_{ij}. \quad (2)$$

Kaelble and Moacanin [18] define $\alpha_i$ and $\beta_i$ as respectively the dispersion $\sigma_\sigma = (\gamma_{ij}^\sigma)^{1/2}$ and polar $\beta_i = (\gamma_{ij}^p)^{1/2}$ components of the surface. The interface is dominated by Van der Waals interactions and the term $\Delta_{ij}$ describes the ion-covalent interaction that can be considered negligible. According to Sharma [19], the interfacial tension parameters for different proteins can be derived using the values $\alpha_i$ and $\beta_i$ for plasma proteins. Table 4 shows the calculated results of the Ag-doped DLC films.

It has been found that a low dispersion–highly polar surface favors weak adsorption of plasma proteins such as surface-treated stellite 21 [20] with $\alpha_i$ equal to 5.0 (mJ/m$^2$)$^{1/2}$ and $\beta_i \geq 5.0$ (mJ/m$^2$)$^{1/2}$. This kind of surface has excellent biocompatibility. As shown in Table 4, the interfacial tension of albumin is higher compared to those of fibrinogen and $\gamma$-
Globulin, suggesting that albumin preferentially adsorbs on the Ag-doped DLC and fibrinogen preferentially adsorbs on LTIC and ta-C. Preferential adsorption of albumin is known to passivate the surface of an implant and preferential adsorption of fibrinogen or globulin favors blood coagulation and platelet activation. Our results are consistent with the study of Hauert et al. [5] who have found that a high ratio of albumin to fibrinogen favors low surface platelets adhesion and reduces platelet activation and tendency of thrombus formation for the DLC. Furthermore, the interfacial tensions of three kinds of
plasma proteins on the Ag-doped DLC surface are significantly lower than those on the LTIC and Ta–C surfaces. As suggested by Ruckensten [21], a low blood-biomaterial interfacial tension is beneficial to blood compatibility. Since the conformation of plasma protein will change after adsorption onto a biomaterial surface, weak adsorption and less conformation of fibrinogen on the Ag-doped DLC film is beneficial.

A mechanically stable blood-biomaterial interface is considered as another surface energetic criterion of biocompatibility on a foreign surface. Since the cellular elements are compatible
with blood and their interface with the medium is also mechanically stable, a blood-biomaterial interfacial tension of about the same magnitude as the cell-medium interfacial tension ($\gamma_{SL} \approx 1 \text{–} 3 \text{ mJ/m}^2$) will render both long-term compatibility as well as a mechanically stable interface with blood. Table 5 shows the interfacial tension ($\gamma_{SW}$) between the materials and medium (water) and that all the Ag-doped DLC films have lower $\gamma_{SW}$ values than LTIC (mJ/m$^2$). This is another reason for the enhanced hemocompatibility of the doped DLC films.

4. Conclusion

The hemocompatibility and anti-bacterial characteristics of Ag-doped DLC films have been investigated. The sp$^3$ content of the doped films increases with the bias voltage applied to the samples during pulsed filtered cathodic vacuum arc deposition. The Ag atoms exist in the metallic form and do not bond with C in the matrix based on XPS analysis. Better platelet adhesion and activation are observed on the Ag-doped materials in comparison with undoped ta-C, Ag, stainless steel, and LTIC. Their surface anti-bacterial properties are also excellent thereby making the materials potentially useful in cardiovascular devices or implants.

Acknowledgments

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References


Table 3

<table>
<thead>
<tr>
<th>Samples</th>
<th>Average number of bacteria on samples</th>
<th>Antibacterial effects</th>
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<tbody>
<tr>
<td>Si control</td>
<td>1937.5</td>
<td>–</td>
</tr>
<tr>
<td>Ta–C control</td>
<td>976</td>
<td>–</td>
</tr>
<tr>
<td>Ag–C (a)</td>
<td>0</td>
<td>99.99%</td>
</tr>
<tr>
<td>Ag–C (b)</td>
<td>1</td>
<td>99.98%</td>
</tr>
<tr>
<td>Ag–C (c)</td>
<td>34</td>
<td>98.12%</td>
</tr>
</tbody>
</table>

Table 4

<table>
<thead>
<tr>
<th>Biological substance</th>
<th>(dyn/cm)$^{1/2}$</th>
<th>LTIC$^a$</th>
<th>Ta–C$^b$</th>
<th>Ag–C (a)$^c$</th>
<th>Ag–C (b)$^d$</th>
<th>Ag–C (c)$^e$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrinogen</td>
<td>4.972</td>
<td>16.8</td>
<td>12.2</td>
<td>21.2</td>
<td>8.7</td>
<td>12.5</td>
</tr>
<tr>
<td>γ-Globulin</td>
<td>5.428</td>
<td>13.1</td>
<td>28.1</td>
<td>76.8</td>
<td>9.7</td>
<td>67.2</td>
</tr>
<tr>
<td>Albumin</td>
<td>5.602</td>
<td>11.8</td>
<td>46.6</td>
<td>210.0</td>
<td>12.8</td>
<td>207.3</td>
</tr>
<tr>
<td>Whole blood</td>
<td>3.3</td>
<td>20.8</td>
<td>1.7</td>
<td>19.7</td>
<td>2.1</td>
<td>16.1</td>
</tr>
</tbody>
</table>

$^a$ For values of $\alpha_i=6.1$ (mJ/m$^2$)$^{1/2}$ and $\beta_i=2.4$ (mJ/m$^2$)$^{1/2}$.
$^b$ For values of $\alpha_i=5.8$ (mJ/m$^2$)$^{1/2}$ and $\beta_i=2.4$ (mJ/m$^2$)$^{1/2}$.
$^c$ For values of $\alpha_i=5.8$ (mJ/m$^2$)$^{1/2}$ and $\beta_i=2.8$ (mJ/m$^2$)$^{1/2}$.
$^d$ For values of $\alpha_i=5.5$ (mJ/m$^2$)$^{1/2}$ and $\beta_i=2.3$ (mJ/m$^2$)$^{1/2}$.
$^e$ For values of $\alpha_i=4.9$ (mJ/m$^2$)$^{1/2}$ and $\beta_i=3.6$ (mJ/m$^2$)$^{1/2}$.

Table 5

<table>
<thead>
<tr>
<th>Materials</th>
<th>Water contact angle</th>
<th>$\gamma_{SW}$ (mJ/m$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ag–C (a)</td>
<td>71.4</td>
<td>18.7</td>
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<tr>
<td>Ag–C (b)</td>
<td>79.6</td>
<td>22.7</td>
</tr>
<tr>
<td>Ag–C (c)</td>
<td>67.2</td>
<td>8.4</td>
</tr>
<tr>
<td>LTIC</td>
<td>74.9</td>
<td>24.1</td>
</tr>
</tbody>
</table>

$^*$ For values of $\gamma_{SW}=3$ mJ/m$^2$ will render both long-term compatibility as well as a mechanically stable interface with blood.