Characteristic of silver doped DLC films on surface properties and protein adsorption

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Abstract

Ag-incorporated diamond-like carbon (DLC) films were prepared on Si substrate using a hybrid deposition system composed of an end-Hall-type hydrocarbon ion gun and a silver DC magnetron sputter source. Ag was selected due to their potential values of biomaterial. The concentration of Ag in the films was varied from 0.1 to 9.7 at.% by controlling the fraction of Ar in the reaction gas mixture with benzene. In order to understand the influence of incorporated Ag on wettability, the surface energy and the protein adsorption as an indirect haemo-compatibility were measured. The surface energy of the Ag-incorporated DLC film decreased gradually with the increase of the Ag concentration. The haemo-compatibility was examined by the adsorption ratio of albumin/fibrinogen as an indirect method and improved with the increase of Ag concentration. The surface and biological behaviors of the films will be discussed in terms of the atomic bond characteristic and microstructure induced by Ag incorporation. Our results demonstrate that the Ag-incorporated DLC films are potentially useful as biomedical devices having good haemo-compatibility and hydrophobic characteristics.

Keywords: Diamond-like carbon (DLC); Protein adsorption; Surface energy; Wettability

1. Introduction

Diamond-like carbon films have been a good candidate for some application such as blood contacting devices \cite{1,2} and cells contacting materials \cite{3} due to their excellent mechanical properties such as low coefficient of friction \cite{4}, wear resistance properties \cite{5}, extreme good chemical inertness and biocompatibility \cite{6,7}. Therefore DLC films have been of interest as a protective coating in medical implants such as a replacement for hip \cite{8}, knee \cite{9}, coronary artery stents \cite{10} and mechanical heart valves \cite{11}. However, the biological behavior of the implant is strongly influenced by the chemical properties at the interface. For example, whenever a foreign surface of biomaterial is placed in contact with blood, the protein in blood first adsorbs to the foreign materials, resulting in the adhesion and activation of subsequent platelet. These induce the coagulation of blood when the activated platelets produce enzymatically activated substances. Consequently, the protein adsorption is the crucial event as a first process of haemo-compatibility between blood and the biomaterials because it influences the subsequent fabrication of thrombus \cite{12}. Therefore, it is very important to control the surface chemistry of an implant, particularly the composition, to produce a specific surface with a well-defined biological reaction. In this point of view, doped DLC films have attracted much attention recently. Several different research groups investigated the change of surface properties of DLC films by adding a third element such as Al \cite{13}, Ni, Fe and Si \cite{14}. It is well known that all of these properties can be changed within a certain range by the addition of other elements into the DLC. For example, H. Schulz et al.
reported that the addition of Al into DLC film decreases surface energy while contact angle increases from 70 to 98° [13] and J. S. Chen et al. showed an increase of the contact angle by the addition of Al and Fe into DLC film while a decrease of the contact angle by the addition of Ni [14]. At the same time, doping of Ag into DLC films is also potentially valuable as biomaterials. Ag has been reported as a powerful antibacterial agent that has been used in biomedical engineering with good effects [15–17]. Because Ag as a cytotoxic element, can be used in applications where no bacteria or cells should attach to a surface, such as temporary implants and surfaces of medical devices that inhibit cell proliferation and differentiation are required. However, doping of Ag into DLC film can make the change of wetting angle and surface energy and it can also affect the haemo-compatibility even though there are some contradictory results on the haemo-compatibility with wetting angle [18–21]. For example, F. Z. Cui et al. reported that the hydrophilic surfaces suppress the protein adsorption as well as platelet adhesion [18] and M. I. Jones et al. reported that the hydrophobic surface is better than the hydrophilic surface for coating on medical guide wire and protein absorption, platelet attachment and activation for cardiovascular applications [1]. It is unclear which property is more preferable. Thus, the haemo-compatibility of Ag-incorporated DLC films should be investigated with their composition, wetting angle, surface energy or atomic bonding structure together due to bio reaction to surface influenced by various factors, including surface energy, surface compositions and atomic bonding structure. In our work, we studied the characteristic of Ag doped DLC films on the surface properties and protein adsorption with detailed understanding of the composition, surface energy and structural effects.

2. Experimental

A schematic diagram of the hybrid deposition system used in our work is shown in Fig. 1. The deposition system composed of ion-beam deposition of benzene and magnetron sputtering of silver. The benzene was introduced into the end-Hall-type ion gun to obtain the hydrocarbon ions. Typical values of anode voltage and current are 90 V and 0.32 A, respectively. Argon sputtering gas was supplied to the sputter gun equipped with Ag target of a high purity (99.99%) with a diameter of 5 cm. Typical DC power supplied to the sputter gun was about 298 W (620 V, 0.48 A). The total gas flow used for Ag-incorporated DLC film was 18 sccm for all the samples and the fraction of Ar in the reaction gas was varied from 0.85 to 0.93 (15.3 to 17.7 sccm) to control the Ag concentration in the deposited films. The base pressure in the reactor was $2 \times 10^{-5}$ Pa, whereas the deposition pressure varied in the range of 0.08–0.15 Pa, depending on the fraction of Ar gas. Before deposition, the substrate was cleaned using the Ar ion beam with a pressure of 0.10 Pa and a bias voltage of $-400$ V for 30 min. And then, the buffer layer of DLC film was deposited for 8 min at a bias voltage of $-800$ V with an open shutter condition. The thickness of the buffer layer was $52 \pm 3$ nm. Subsequently, the Ag-incorporated DLC film with $150 \pm 20$ nm was deposited for 10 min at a bias voltage of $-200$ V. Pure DLC films were also prepared using only the hydrocarbon ion beam at a negative bias voltage of $-200$ V without operating the sputter gun. The deposition time was adjusted to obtain the thickness of $200 \pm 15$ nm for all the samples. A p-type Si (100) wafer with thickness of $500 \pm 10$ μm was used for the substrate, and this was placed on a rotating substrate holder placed about 22 cm from the ion gun. A thin Si (100) wafer of thickness $100 \pm 2$ μm was also used to calculate

Fig. 1. A schematic diagram of hybrid ion-beam deposition system.
the residual stress from the observed curvature of the film/substrate composite. The composition of the film was analyzed using Rutherford backscattering spectrometry (RBS) analysis employing a 2 MeV collimated $^{4}$He$^{+}$ ion beam. 2 MV Pelletron accelerator of National Electrostatic Corporation was used for the He ion acceleration. Home-made analysis chamber was used to collect the RBS spectra. Micro-Raman spectroscopy using Ar-ion laser having a wavelength of 514.5 nm was also used to characterize the atomic bond structure of the films.

The adsorption ratio of albumin and fibrinogen in plasma was determined to investigate the haemo-compatibility of the Ag-incorporated DLC films. It is just an indication that a good haemo-compatibility may be expected [1,2]. The albumin is known to reduce the adhesion of the platelets while fibrinogen is known to enhance the adhesion and activation of platelets. A total of 12 samples were prepared with a size of 1 cm × 1 cm and then placed into a plastic tube containing a 2 ml phosphate buffered saline (PBS) and shaken at 37 °C for 30 min and PBS was drained out. After that, they were divided into two groups and one was immediately transferred into a 2 ml of albumin solution and the other into a fibrinogen solution to avoid surface dehydration, respectively. The immersion into each plasma protein solution was divided into two groups again and then, one is maintained for 5 min to observe the initial adsorption effect and the other is maintained for 60 min to observe the long term adsorption effect, respectively. To completely remove the unadsorbed protein, all the samples were washed three times with 3 ml distilled water. Subsequently, a sodium dodecyl sulfate (SDS) solution of 2 ml was prepared by adding the samples into SDS then kept at 37 °C for overnight. Afterward, 100 μl of the plasma protein solution per each sample which came from the above explained SDS solution was placed into a 96 well plate and loaded also 120 μl micro Bicinchoninic acid kit for protein (BCA-1), respectively. Incubation at 60 °C for 60 min was followed and the absorbance of each wells was measured using enzyme linked immunosorbsant assay (ELISA) reader with a wavelength of 562 nm and evaluated each adsorbed plasma protein concentration from the calibration curve.

The surface energy composed of polar and dispersive components of the Ag-incorporated DLC film was evaluated by the measurement of contact angle using liquid drop goniometry. The interfacial tension between two condensed phases can be determined by Young’s equation [22], according to which

$$\cos \theta = \gamma_{LV} - \gamma_{SL}$$

where $\theta$ is the measured contact angle between liquid and solid, and $\gamma_{LV}$, $\gamma_{SV}$ and $\gamma_{SL}$ are the interfacial energies of the liquid/solid interface.
vapor, solid/vapor and solid/liquid interfaces, respectively. This equation can be rewritten as the Young–Duprê equation:

\[ W_a = \gamma_{LV} (1 + \cos \theta) = \gamma_{SL} + \gamma_{LV} - \gamma_{SL} \]  

(2)

where \( W_a \) is the adhesion energy per unit area of the solid and liquid surfaces. In the general form of Eqs. (1) and (2) then can be written:

\[ \gamma_{LV} (1 + \cos \theta) = 2 \sqrt{\gamma_L^P \gamma_S^P} + 2 \sqrt{\gamma_L^D \gamma_S^D} \]  

(3)

where \( \gamma_L^P \) and \( \gamma_S^P \) are the polar components of the surface energy of liquids phase and solid phase, respectively while \( \gamma_L^D \) and \( \gamma_S^D \) are the dispersive component of the surface energy of liquid and solid phase, respectively. Since \( \gamma_L^D \) and \( \gamma_S^D \) have been published for many liquids, it is possible to approximate \( \gamma_L^P \) and \( \gamma_S^P \) from a single measurement of \( \theta \) by the use of Eq. (3). Therefore, by measuring the contact angles of two different liquids with well known polar and dispersive components of surface energy (Table 1), Eq. (3) can be solved to determine the polar and dispersive components of the surface energy of the materials. The contact angle was measured using a liquid drop goniometry in atmospheric condition at room temperature at least five times for each sample.

3. Results and discussion

Fig. 2 shows the dependence of the Ag concentration in the DLC films on the Ar concentration in the gas mixture composed of Ar and benzene. When the Ar concentration was less than 89%, the incorporation of Ag in the DLC film was not detected probably due to a significant poisoning of the target caused by the high flux of carbon ions and the unstable operation of the magnetron sputter gun at the low sputtering yield conditions. However, as the Ar concentration increased from 89% to 93%, the Ag concentration in the film increased from 1.7 to 9.7 at.%. These results show that the Ag concentration in the film can be systematically controlled by changing the Ar concentration in the gas mixture. A similar tendency was reported for W incorporated DLC films using the similar hybrid ion-beam deposition system [23].

The contact angles formed with distilled water and formamide are shown in Fig. 3. As the concentration of Ag in DLC films increased, the contact angle also increased from 80° to 99° in case of distilled water and from 61° to 95° in case of formamide, respectively. Usually, a hydrophobic surface has a contact angle higher than 70°, while a hydrophilic surface has a contact angle lower than 70°. These results point that Ag-incorporated DLC film is more hydrophobic. The calculated surface energy (\( \gamma_S \)) of the films, its polar and dispersive components (\( \gamma_S^P \) and \( \gamma_S^D \)) determined from the linear fitting of Eq. (3) are shown in Fig. 4. As the concentration of Ag in DLC film increased from 0.1 to 9.7 at.%, the total surface energy decreased from 33 to 13 dyne/cm. The reductions in the total surface energy of Ag-incorporated DLC films are attributed to the reduction in both polar and dispersive component. The incorporation of Fe or Al into DLC films was also reported to reduce the total surface energy and its dispersive component presumably due to the increase in sp² content and resultant decrease in the atomic density [14,24]. Our results also showed the similar tendency with Raman spectroscopy observation on structure.

<table>
<thead>
<tr>
<th>Ag concentration (at.%)</th>
<th>I(D)/I(G)</th>
<th>FWHM of G peak (cm⁻¹)</th>
<th>Position of G peak (stressed) (cm⁻¹)</th>
<th>Position of G peak (unstressed) (cm⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.71</td>
<td>66.8</td>
<td>1562</td>
<td>1550</td>
</tr>
<tr>
<td>0.1</td>
<td>0.71</td>
<td>66.7</td>
<td>1562</td>
<td>1550</td>
</tr>
<tr>
<td>1.7</td>
<td>0.78</td>
<td>64.9</td>
<td>1562</td>
<td>1556</td>
</tr>
<tr>
<td>6.8</td>
<td>0.87</td>
<td>57.3</td>
<td>1568</td>
<td>1563</td>
</tr>
<tr>
<td>9.7</td>
<td>0.86</td>
<td>57.2</td>
<td>1567</td>
<td>1562</td>
</tr>
</tbody>
</table>

* Corrected position of the G peak from the position of G peak (stressed) by excluding the residual stress effect [25] using the stress data in Fig. 5.
The ratio $sp^2/sp^3$ determined by Raman spectra increased as Ag contents in the DLC film increased as shown in Fig. 5 and Table 2. The Raman spectra of amorphous carbon films can be resolved by two Gaussian peaks which are associated with D and G peaks of solid carbon materials, respectively. The D peak contributed to the disordered graphitic carbon and G peak contributed to the graphite carbon. When the Ag concentration increased from 1.7 to 9.7 at.%, the ratio of $I(D)/I(G)$ increased from 0.71 to 0.86, implying an increase of $sp^2$ bonds. Even though there were relatively large errors in determining the ratio of $I(D)/I(G)$, the position of G peak, and the full width at half maximum (FWHM) of each peak, the G-peak position is sufficiently small to allow structural changes of the films to be reflected. The error in the G-peak position, estimated to be ±2.0 cm$^{-1}$, is mainly due to uncertainty in the baseline correction so G-peak position of a film can be used to observe the structural variations in the film [25]. Fig. 5(b) showed the corrected G-peak positions where the residual stress effect was excluded. It is empirically known that the G-peak position of Raman spectra shifts to a higher wave number as the graphite component in the film increases [26]. The G-peak position in our films varied over a large range from 1550.2 to 1561.7 cm$^{-1}$ when the Ag concentration increased from 1.7 to 9.7 at.%. Therefore, the results of Raman including $I(D)/I(G)$ and G-peak position suggest that the atomic structure of carbon network was changed by the Ag incorporation except 0.1 at.% of Ag incorporation. There is no change of G-peak position at 0.1 at.% of Ag incorporation in which the atomic bond structure of the carbon network was not changed.

TEM structures of Ag-incorporated DLC film also support the Raman structure of film as shown in Fig. 6. When 0.1 at.% of Ag-incorporated into DLC film, Ag atoms are fully dissolved and this microstructure is the same to non-element incorporated DLC film where it showed the amorphous carbon matrix. Thus, we might suggest that there is no structure change. However, 1.7 at.% of Ag-incorporated DLC film showed amorphous carbon structure and partially segregated and formed crystalline Ag particles with a size of 2 nm. And further incorporating Ag into DLC film, Face Centered Cubic structure of Ag crystalline with a size range from 4 to 6 nm formed on the whole areas and their portion increased with the increase of Ag contents as shown in Fig. 6(c). It is well known that the structure of the film...
is sensitive to the haemo-compatibility [24,27,28]. Thus in order to study the fundamental change in haemo-compatibility of Ag-incorporated DLC films the protein adsorption is used to characterize the films. Adsorptions of albumin and fibrinogen are two primary factors which are related to haemo-compatibility. The adsorption ratio of albumin/fibrinogen obtained in this work, as determined by mean absorbance values, is shown in Fig. 7. The higher value corresponds to the lower number of adhering platelets and consequently the lower tendency of thrombus formation [1,2]. It clearly shows that the more Ag-incorporated DLC film exhibits a higher albumin/fibrinogen ratio compared with the DLC and less incorporated DLC film. The adsorption ratio of albumin/fibrinogen is 0.91 for 0.1 at.% of Ag-incorporated DLC film, 0.93 for 3.7 at.% of Ag-incorporated DLC film while 0.96 for 9.7 at.% of Ag-incorporated DLC film exhibits a higher albumin/fibrinogen ratio as an indication of the good haemo-compatibility with the increase of Ag contents in the film. The adsorption ratio of albumin/fibrinogen is 0.91 for 0.1 at.% of Ag-incorporated DLC film, 0.93 for 3.7 at.% of Ag-incorporated DLC film while 0.96 for 9.7 at.% of Ag-incorporated DLC film which immersed in PBS solution for 60 min, respectively. It is reported that the haemo-compatibility of Ag-incorporated DLC film increased with Ag contents in the film and affects the protein adsorption ratio compared with the DLC and less incorporated DLC film. Therefore, the structure, surface energy and protein adsorption analysis show that a lower surface energy is related to the increase of sp²/sp³ content in the film and affects the protein adsorption ratio as an indication of the good haemo-compatibility with the increase of Ag contents in the film.

4. Conclusions

Ag-incorporated DLC films have been deposited by a hybrid ion-beam deposition technique under the control of various fractions of Ar and benzene gas. By adding Ag into the DLC film, the bonding structure, surface energy, wetting angle and protein adsorption ratio of DLC films were modified except 0.1 at.% of Ag incorporation. The increase of Ag contents in DLC film resulted in the increase of sp² bonding in the amorphous carbon matrix revealed by Raman spectroscopy. The wetting angles and protein adsorption ratios of the Ag-incorporated DLC film also increased with Ag contents in the films. Thus, our results demonstrated that Ag incorporating into DLC film is one of the potential methods for synthesizing hydrophobic film and suggested a possibility of biomaterials.

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References

[22] T. Young, Philos. Trans. R. Soc. Lond. 9 (1805) 255.