Structure and properties of annealed amorphous hydrogenated carbon (a-C:H) films for biomedical applications

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Abstract

When a biomaterial comes in contact with a biological body, biological substance denaturation will be concomitant with the physical interaction between the biomaterial surface and biological substance, such as blood protein denaturation resulting from electrical charge transfer. In this study, we investigated the relationship between the physical properties of amorphous hydrogenated carbon (a-C:H) and its blood compatibility. The films were fabricated using plasma immersion ion implantation-deposition (PIII-D), followed by annealing in vacuum between 200 and 600 °C. A series of a-C:H films with different structures and chemical bonds were characterized by Raman, elastic recoil detection (ERD) and atomic force microscopy (AFM). The results indicate that hydrogen effusion and film graphitization are promoted at high annealing temperature. The physical properties and surface characteristics of the films including the carrier concentration and mobility, resistivity, band gap and surface wettability were also examined. Their changes with anneal temperature are due to the increase of sp² bonding cluster caused by graphitization. The thrombogenesis of the films was evaluated employing in vitro platelet adhesion tests. The adhesion, activation, and morphology of the platelets were investigated using scanning electron microscopy (SEM). The results were correlated with the biological data to elucidate the blood compatibility mechanism of a-C:H films. The platelet adhesion and activation of a-C:H is affected by annealing. It is believed that the possible factors affecting blood compatibility are the adhesion energy of blood plasma, band gap, carrier type and concentration. Improving the electronic structure of a-C:H films is critical to the abatement of platelet activation.

Keywords: Plasma immersion ion implantation-deposition; Hydrogenated amorphous carbon (a-C:H) film; Annealing; Blood compatibility

1. Introduction

The high hardness, low coefficient of friction and excellent surface finish of amorphous diamond-like carbon (DLC) make it attractive as a coating in biomedical implants such as joints, orthopedic pins and screws, dental prostheses, and medical guide-wires [1–4]. Recently, it has received much attention due to its good blood compatibility and has been suggested as a potential biomaterial in blood contacting-devices [5–13]. However, reports on the relationship between blood compatibility and film characteristics are quite limited [6–9], although some results have shown that cell adhesion onto DLC coatings is related to the bonding structure (i.e. fraction of sp³ bonds) [9], surface energy and hydrophobicity [7,8], and film thickness [12].

When a biomaterial comes in contact with a biological body, biological substance denaturation will be concomitant with the physical interaction between the biomaterial surface and biological substance [14–17]. For example, when a foreign surface is placed in contact with blood, the initial response of the living components of blood to the biomaterial must depend on its surface properties such as blood protein and coagulation factor denaturation resulting from electrical charge of protein transfer to the material surface [14,17]. If a biomaterial exhibits very strong foreign body reactions with blood, thrombus formation will ensue leading to implant failure. Therefore, the characteristics of the coatings play an important role on the blood compatibility [18]. The unique characteristic of amorphous carbon is mainly due
to that carbon atoms can exist in both sp$^3$-type tetrahedral bonds and sp$^2$-type trigonal bonds in a predominantly amorphous state coexisting with 20–40 at.% of hydrogen. The fraction of sp$^3$ bonds and hydrogen content in the films can be modulated by using different methods to cater to different materials needs [19,20]. However, the relationship between the physical characteristic of amorphous carbon films and blood compatibility is not well understood in spite of the potential benefits offered by it as a blood contacting material and this paper reports our recent findings in this important area.

2. Experimental

Hydrogenated amorphous carbon films were fabricated at room temperature using plasma immersion ion implantation-deposition (PIII-D) [21]. The a-C:H deposition was carried out in a mixture of acetylene (C$_2$H$_2$) and argon. The C$_2$H$_2$ influx and C$_2$H$_2$/Ar gas-flow ratio were kept constant at 10 sccm and 0.5 sccm, respectively, throughout the deposition process. The working pressure was approximately 1.22×10$^{-1}$ Pa. The plasma was sustained by radio frequency (RF) with a power of 500 W. The substrate DC bias voltage was −900 V and the PIII-D process time was 2 h. Prior to film deposition, the substrates underwent Ar$^+$ sputter cleaning for 15 min to remove surface contaminants and surface oxide using 500 W RF and a substrate DC bias voltage of −500 V. The base pressure was approximately 2×10$^{-3}$ Pa. Transparent quartz pieces, silicon wafers (100) and the wafers covered with thermally grown oxide were used as substrates. After PIII-D, annealing was carried out at 200–600 °C for 30 min at reduced pressure (<1×10$^{-3}$ Pa).

The structural information of the films was determined by Renishaw RM 3000 Micro-Raman system at room temperature using a 25 mW He–Ne laser (633 nm). Graphite was used as a control sample in the analyses. Hydrogen in the films was detected by means of elastic recoil detection. The surface morphology of the a-C:H films was characterized using an extended multimode nano-scope atomic force microscope (AFM). The optical transmittance was measured by UV–Visible spectroscopy. The electrical resistivity of the annealed a-C:H films was determined by four-point probe at room temperature. The carrier concentration and mobility at room temperature were measured using the van-der-Pauw–Hall effect technique. Wettability examinations were performed using the sessile drop method using a JY-82 contact angle goniometer. At least four drops of each liquid of each sample were used in this measurement in order to get good statistical averages. The three physiological liquids used in this test were double distilled water, normal saline, and human platelet poor plasma (PPP).

![Fig. 1. Intensity ratios of the D-band to G-band, width and position of G-band with annealing temperature derived from Raman spectra.](image)

Platelet adhesion experiments were conducted to evaluate the surface thrombogenicity of the films in vitro. Samples of a-C:H films were washed and then immersed into human platelet rich plasma (PRP) for 15 min at 37 °C. After rinsing, fixing and critical point drying, the specimens were examined using scanning electron microscopy (SEM) and optical microscopy to evaluate the quantity and morphology of the adherent platelets. Five fields of view were chosen at random to obtain good statistical results.

3. Results and discussion

3.1. Composition and bonding structure

Raman spectra (not shown here) were acquired from the as-deposited a-C:H films and the films annealed at 200–600 °C for 30 min. The spectrum of the as-deposited film shows a broad peak at approximately 1560 cm$^{-1}$ and an obvious shoulder at a lower frequency, that are commonly referred to as the G and D lines of graphite, respectively. It also indicates that the band shifts to two separate D- and G-bands when the annealing temperature is high. The G-band is associated with the optically allowed $E_{2g}$ zone center of crystalline graphite at approximately 1585 cm$^{-1}$ whereas the D-band at 1355 cm$^{-1}$ is the disorderallowed zone edge modes of the graphite that is only Raman active due to the lack of long-range order [19,20]. These two bands can be used to monitor the structural modification of the a-C:H films since they contain structural information of the films. The position and width of the G-peak and $I_D/I_G$ ratio as a function of the annealing temperature are shown in Fig. 1. The shift in the G-band indicates the increase in size and number of the sp$^2$ carbons, and the increase in the observed $I_D/I_G$ intensity ratio suggests that there is an increase in the number of ordered aromatic rings within the samples. The emergence of
the D-band as well as the increase of the \( \frac{I_D}{I_G} \) ratio show that the material has become nano-crystalline graphite [22]. The steep changes observed in the three curves at 300–400 °C indicate that graphitization is promoted at higher annealing temperature arising from a diffusive mechanism. Similar results have been reported by Ogwu [23].

The elastic recoil detection (ERD) spectra acquired from the films annealed at 400 and 600 °C are exhibited in the Fig. 2. They are quite similar to that of the as-deposited film, with a sharp peak at the surface originating from hydrogen atoms in the subsurface region [24]. It indicates that the hydrogen depth profile is less affected by thermal annealing under our experimental conditions. The decrease of the H intensity with increasing temperature suggests out-diffusion of hydrogen under thermal annealing [22,23]. Fig. 3 exhibits the three-dimensional AFM morphology of the as-deposited a-C:H film and the film annealed at 600 °C. In contrast to the as-deposited a-C:H film, the surface morphology of the annealed film shows some inhomogeneities although the mean roughness (RMS) only changes slightly from 1.4 to 1 nm. It appears to stem from the growth of sp\(^2\) clusters or the formation of microcrystalline graphite [22].

3.2. Physical properties and wettability

The optical band gap \( E_g \) of the a-C:H films was derived from the Tauc’s plot and the dependence of \( E_g \) on annealing temperature is shown in Fig. 4a. The optical band gap of the as-deposited a-C:H film is 2.2 eV, and it decreases gradually from 1.9 to 0.8 eV when the annealing temperature increases from 200 to 600 °C. As the band gap does depend on the configuration of the sp\(^2\) sites, the growth of the sp\(^2\) cluster with increasing annealing temperature is the main reason for the observed band gap narrowing [20].
The resistivity $\rho$ and carrier concentration $n$ at room temperature are plotted in Fig. 4b–c as functions of annealing temperature. Both the resistivity and Hall mobility decrease with increasing annealing temperature and the trends are consistent with the Raman shift of the G-line. The steep changes approximately 400 °C in the two curves also agree with the Raman results. Thus, it appears that the increase of the electrical conductivity in the annealed a-C:H film is related to an increase in the sp² bonding carbon and ordered sp² cluster caused by graphitization. The carrier concentration increases with annealing temperature and rises sharply approximately 500 °C. The type of majority carriers in the films can be determined by Hall effect measurements. The Hall voltage is negative for n-type and positive for p-type, and so a positive Hall voltage obtained from the samples annealed at high temperature 500–600 °C shows a p-type characteristic, which probably correlates to the increase of the darling bond defects caused by H effusion [25]. The resistivity of the as-deposited a-C:H film could not be measured using our equipment.

The contact angles of three physiological liquids on the films are shown in Table 1. The results show that all the surfaces have a hydrophobic nature with much higher contact angles at approximately 80°. In contrast with the results of the as-deposited film, the contact angles of water and normal saline change less, while the contact angles of blood plasma increases from 67.4 to approximately 77.4. As the annealing temperature increases, the contact angles of water, normal saline and blood plasma vary randomly in a narrow range of 4.0°, 4.7° and 1.6°, respectively.

### 3.3. Platelet adhesion and activation

Fig. 5 exhibits the statistical results of the platelets adhered on the a-C:H films of different anneal temperature from PRP. In contrast with the results acquired from the as-deposited film, the number of adherent platelets on the annealed films decreases, fluctuates slightly from 200 to 500 °C, and increases slightly at 600 °C. This platelet adhesion tendency is contrary to that of the contact angle of blood plasma. The adhesion work ($W_a$) of blood plasma can be calculated by the Young equation, $W_a = \gamma(1 + \cos \theta)$. Apparently, the annealed films possess a lower $W_a$ than the as-deposited film, and so adhesion of blood plasma on the film surface becomes more difficult after annealing and the number of platelets adhered on the annealed films is much less. Fig. 5 also indicates that the percentage of un-activated platelets varies in another way that depends on the annealing temperature. The un-activated platelet percentages of the annealed films are close to that of as-deposited one when the annealing temperature is lower than 400 °C, and the percentages decrease when the anneal temperature is higher than 500 °C. The curve changes steeply at 400–500 °C like the curves in Fig. 4. It thus appears that annealing at a relatively low temperature cannot cause retrogression of blood-compatibility of our a-C:H films, and the activation of platelets adhered on the a-C:H film surfaces is related to the change of the physical properties of the films.

The interaction between blood and artificial materials is very complicated and the detailed mechanism is still not clear. In a simplistic view, when blood comes in contact with an artificial surface, the first event is protein adsorption. If the adsorbed protein such as fibrinogen is denatured, the coagulation factors or platelets will be activated causing a series of cascade reaction of blood coagulation and finally thrombosis. It has been shown that denaturing of fibrinogen and coagulation factor FV.

<table>
<thead>
<tr>
<th>Anneal temperature (°C)</th>
<th>Double distilled water</th>
<th>Normal saline</th>
<th>Human plasma (PPP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>300</td>
<td>79.0 ± 0.91</td>
<td>73.7 ± 1.01</td>
<td>76.5 ± 0.39</td>
</tr>
<tr>
<td>400</td>
<td>79.1 ± 0.87</td>
<td>74.5 ± 0.28</td>
<td>77.2 ± 0.21</td>
</tr>
<tr>
<td>500</td>
<td>82.3 ± 0.40</td>
<td>78.4 ± 0.59</td>
<td>77.9 ± 0.34</td>
</tr>
<tr>
<td>600</td>
<td>78.4 ± 0.52</td>
<td>75.5 ± 0.54</td>
<td>78.1 ± 0.55</td>
</tr>
<tr>
<td>As-deposited</td>
<td>80.1 ± 0.45</td>
<td>73.5 ± 0.74</td>
<td>67.4 ± 0.98</td>
</tr>
</tbody>
</table>
FVIII, FIX depends on transfer of its charges to the material, and this process is related to the electronic structure and properties of the material [14,17]. Fibrinogen has an electronic structure similar to that of a semiconductor. Its band gap is 1.8 eV [26], and the Fermi level lies at midgap. Huang et al. [17,27] suggested an energy band model to explain the relationship between fibrinogen denaturation and band structure. Fibrinogen electron transfer from their occupied valence band into the free states of the material surface causes the decomposition of the protein. Based on this model, factors that can affect the fibrinogen denaturation include the band gap, local states in the band gap, carrier concentration and so on. When a material possesses a wider band gap than fibrinogen, there are less local states in the band gap, lower carrier concentration and n-type structure, and consequently, fibrinogen denaturation will be inhibited. Thus, it is reasonable that the as-deposited a-C:H film with a larger band gap and lower carrier concentration possesses lower surface activation of adherent platelets. It is probably the reason for the blood-compatibility retrogression at temperature higher than 400 °C and the significant change in the electronic characteristics such as narrowing of the band gap (narrower than band gap of fibrinogen), order-of-magnitude increase of the carrier concentration and of p-type conductivity.

4. Conclusion

Hydrogenated amorphous carbon thin films were fabricated using plasma immersion ion implantation-deposition (PIII-D) followed by annealing in vacuum between 200 and 600 °C. The Raman and ERD results indicate that hydrogen effusion and film graphitization are promoted when the annealing temperature is high. Changes of band gap, resistivity, carrier concentration and mobility with annealing temperature can be attributed to the increase of the sp² bonding cluster caused by graphitization. The platelet adhesion and activation of a-C:H is affected by annealing. Annealing at a relatively low temperature cannot cause retrogression of blood-compatibility of our a-C:H film, and the activation of platelets adhered on the a-C:H film surfaces is enhanced at temperature higher than 400 °C and related to the change of the physical properties of the film such as narrowing of the band gap (narrower than the band gap of fibrinogen), increasing in the carrier concentration and p-type conductivity. Hence, the blood compatibility of a-C:H films is affected by the electronic structure and improving the electronic structure is important for the abatement of platelet activation.

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References