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Biocompatibility of calcium and phosphorus doped diamond-like carbon thin films synthesized by plasma immersion ion implantation and deposition

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Abstract

Diamond-like carbon has superior mechanical and chemical properties and has been widely studied as coatings in the optics, magnetic media, semiconductor and biomedical industry. In order to enhance the properties and performance of the materials, elemental doping and multi-layer deposition have been proposed. In cardiovascular biomedical applications such as artificial heart valves, low-temperature isotropic pyrolytic carbon (LTIC) is the most widely used material, but its blood compatibility is still not adequate. Diamond-like carbon (DLC) is a potential substitute due to its good biocompatibility and mechanical properties. In addition, the blood compatibility may be further enhanced by introducing some biologically friendly elements. In this work, calcium and phosphorus doped DLC films were fabricated by plasma immersion ion implantation and deposition (PIII and D). The structure and biological properties were assessed using X-ray photoelectron spectroscopy (XPS), scanning electron microscopy (SEM), contact angle measurement, and platelet adhesion test. Reduced platelet adhesion was obtained from the calcium or phosphorus doped DLC compared to LTIC, suggesting that doping DLC with calcium or phosphorus enhances its surface blood compatibility.

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Keywords: Diamond-like carbon; Doping; Blood compatibility; Plasma immersion ion implantation and deposition

1. Introduction

Diamond-like carbon (DLC) films have high wear resistance and a low coefficient of friction, making them useful coating materials in mechanical components such as drills and cutting tools. There are many other applications in which DLC has good potential, for example the microelectronics [1], magnetic [2] and optical [3] industries. DLC films also have good biocompatibility and are attractive coating materials for artificial heart valves, stents or bone implants [4–6]. Our previous studies have shown that nitrogen [7] and phosphorus [8] doped DLC films in contact with blood show a smaller number of adhered or activated platelets than either traditional DLC films or low-temperature isotropic pyrolytic carbon (LTIC). The doped materials can thus minimize complications arising from thrombosis and have high potential in artificial cardiovascular implants.

In this work, we investigate calcium and phosphorus doped DLC films as biomaterials. Calcium is an essential bone constituent and one of the vital elements. Although other dopants such as Ti [9–11], W [12], and B [13,14] have been studied, Ca doped DLC has not yet been studied extensively [15]. Here, Ca doped DLC, Ca and P doped DLC and P doped DLC were fabricated by plasma immersion ion implantation and deposition (PIII and D). In vitro platelet adhesion tests were conducted to evaluate the hemocompatibility of the various materials. Since surface wettability is a crucial factor influencing platelet behavior and consequently the blood compatibility of the materials, contact angle measurements were conducted to determine the relationship between hemocompatibility and surface energy.

2. Experimental details

Ca, Ca and P, and P doped DLC films were synthesized by plasma immersion ion implantation and deposition (PIII

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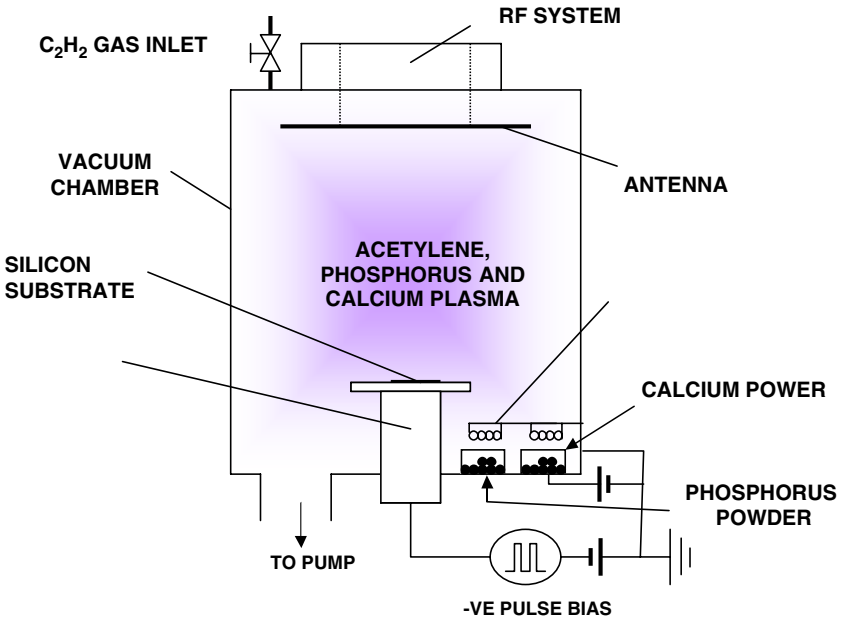

Fig. 1. Schematic diagram of the PIII-D system.

and D) [16]. Two small containers with 99.9% pure red phosphorus and 98.9% pure calcium powders were placed individually inside the plasma chamber as shown in Fig. 1. Phosphorus and calcium were evaporated by heated filaments while a positive bias voltage was applied to the calcium container. Acetylene (C2H2) and Ar gases were introduced into the chamber, and the mixed gas plasma was ignited by a 500 W radio frequency (RF) source for film deposition. Si (100) substrates were placed on the sample platen connected to a high negative pulsed voltage. Before deposition, the wafers were cleaned by argon sputtering for 15 min and the experimental details are shown in Table 1.

The surface morphology of the films was characterized using scanning electron microscopy (SEM). The chemical structure and elemental depth profiles were determined by X-ray photoelectron spectroscopy (XPS). The contact angle measurement was conducted using the sessile drop technique performed on a contact angle goniometer at room temperature. Five fluids, water, glycol, tritolyl phosphate, formamide, and diiodomethane, were used in our tests. Each test was conducted six times on different locations to obtain statistical averages. The biocompatibility of the films was estimated utilizing in vitro platelet adhesion and the detailed procedures can be found elsewhere [17].

Table 1
Experimental parameters for the synthesis of Ca, CaP and P doped DLC films

Sample	Ar/C2H2 (sccm)	Bias voltage (kV)	Bias frequency (Hz)/ pulse length (μs)	Deposition time (min)
Ca-DLC	0/60	15	100/100	150
CaP-DLC	5/10	20	100/100	60
P-DLC	10/10	20	100/100	60

3. Results

The surface biocompatibility was assessed using in vitro platelet adhesion tests. Fig. 2 displays the morphology and the quantity of the adhered platelets on the samples. The statistical results are presented in Table 2 and Fig. 3. Both the P-DLC and Ca-DLC films show smaller numbers of adhered and unactivated platelets than LTIC. Our results show that either Ca or P doping alone can enhance the hemocompatibility but not when acting together.

Figs. 4–6 display the XPS depth profiles acquired from the Ca, P, and Ca and P doped DLC films, respectively. The Ca-DLC profile shows a sharp film interface at about 25 nm but the interfaces in the P and Ca and P doped samples are not as well defined. The sharper interface in the Ca-DLC film is the result of a lower bias voltage (15 kV) and longer deposition time (150 min). Interface mixing is promoted by higher impact energy resulting in collision cascades that carry atoms across the interfacial boundary.

4. Discussion

Surface wettability can influence the surface blood compatibility of the films by promoting the adsorption and unfolding of proteins. Albumin and fibrinogen first adsorb on a foreign surface when blood comes in contact with it. The degree of conformational changes of these adsorbed proteins influences the behavior of platelets in the blood plasma. Since albumin and fibrinogen are well known platelet adhesion inhibiting [18] and activating proteins [19], respectively, the reaction between the surface and the proteins that compromises their function will play an important role [20]. Yang et al. [21] have reported that the albumin can reduce the adhesion and activation of platelets

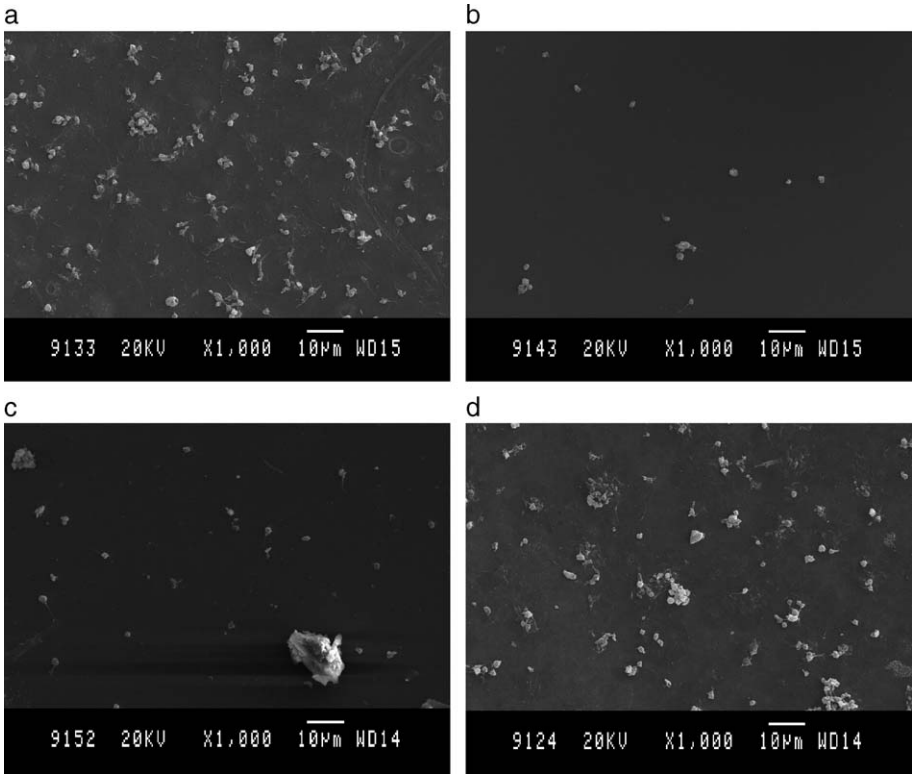

Fig. 2. SEM photos showing adhered platelets on (a) CaP-DLC, (b) P-DLC, (c) Ca-DLC, and (d) LTIC control.

on the materials. Liu et al. [22] conducted a series of blood contact tests on polyacrylonitrile (PAN) samples that were treated with immobilized human serum albumin, collagen, or heparin. Their study demonstrated that a sample covered with absorbed albumin shows improved hemocompatibility. Here, we study the relationship between the surface energy and wettability of the doped DLC films and platelet adhesion and activation.

In our experiments, five fluids, water, glycol, tritolyl phosphate, formamide, and diiodomethane, were used. The interfacial tension between two condensed phases can be determined by Young equation (Eq. (1)) [23] and van Oss equation (Eq. (2)) [24]:

$$W_a = \gamma_l(1+\cos\theta); \tag{1}$$

$$W_a = 2\sqrt{\gamma_l^p \gamma_s^p} + \sqrt{\gamma_l^d \gamma_s^d}; \tag{2}$$

where W_a is the work of adhesion, θ is the contact angle, γ_l^p and γ_l^d are the polar and dispersive components of the liquid phase, respectively, and γ_s^d and γ_s^p are the polar and dispersive components of the solid phase. We can obtain the following equation from Eqs. (1) and (2):

$$\gamma_l(1+\cos\theta) = 2\sqrt{\gamma_l^p \gamma_s^p} + 2\sqrt{\gamma_l^d \gamma_s^d}. \tag{3}$$

By measuring the contact angles of two different fluids with known polar and dispersive components (Table 3), Eq. (3) can be solved to determine the polar and dispersive components of the interfacial energy of the materials.

The interfacial energies (γ_{sp}) between plasma proteins and samples are shown in Table 4. Ca-DLC (0.11) and P-DLC (0.08) have lower value of $\gamma_{sp}^p/\gamma_{sp}^d$ for albumin (γ_{sp}^p and γ_{sp}^d represent the polar and dispersive components of the interfacial energy between proteins and materials) than CaP-DLC and LTIC,

Table 2
Statistical results derived from platelet adhesion tests

Sample	Number of adhered platelets	Number of non-activated platelets
CaP-DLC	141	5
P-DLC	16	14
Ca-DLC	28	13
LTIC	99	23

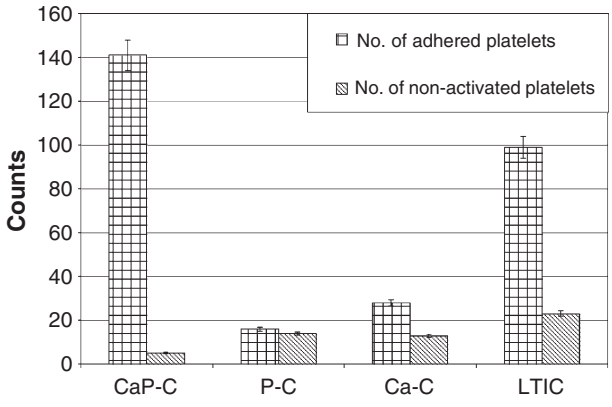

Fig. 3. Quantity of platelets adhered on CaP-C, P-C, Ca-C and LTIC.

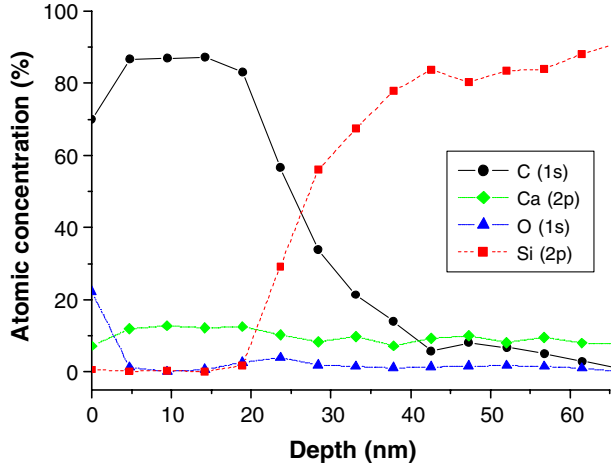

Fig. 4. Elemental depth profiles acquired from the Ca-DLC film.

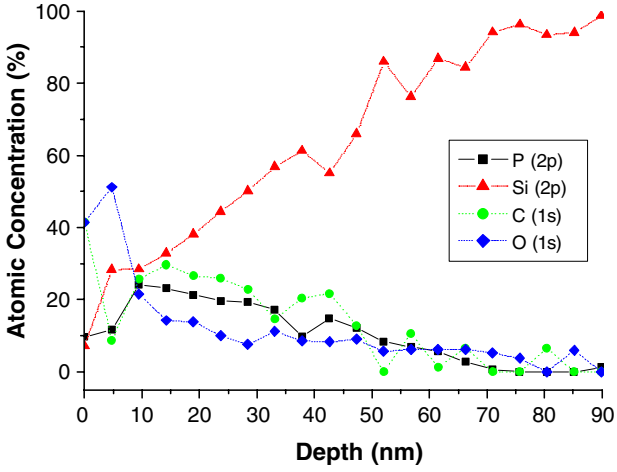

Fig. 5. Elemental depth profiles acquired from the P-DLC film.

suggesting stronger adhesion of albumin. The $\gamma^{p}_{sp}/\gamma^{d}_{sp}$ ratio for fibrinogen is also small, but the total interfacial energy, γ_{sp}, is less than that of albumin, which means that less conformational changes should occur. Our results suggest that these surface energies are the primary factors for the good compatibility

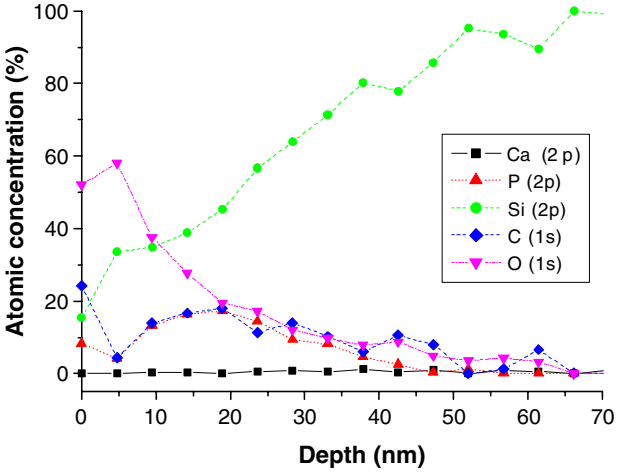

Fig. 6. Elemental depth profiles obtained from the CaP-DLC film.

Table 3
Surface tension and surface tension components (dispersive and polar) of different testing liquids used in the contact angle tests

Liquid	γ (nJ/cm^2)	γ_l^p (nJ/cm^2)	γ_l^d (nJ/cm^2)
Water	72.8	21.8	51.0
Glycol	48.3	29.3	19
Tritolyl phosphate	40.9	39.2	1.7
Formamide	58.2	39.5	18.7
Diiodomethane	50.8	48.5	2.3

Table 4
Interfacial energies between materials and plasma proteins

Biological substance	Ca-DLC		CaP-DLC		P-DLC		LTIC	
	γ_{sp}	$\gamma^{p}_{sp}/\gamma^{d}_{sp}$	γ_{sp}	$\gamma^{p}_{sp}/\gamma^{d}_{sp}$	γ_{sp}	$\gamma^{p}_{sp}/\gamma^{d}_{sp}$	γ_{sp}	$\gamma^{p}_{sp}/\gamma^{d}_{sp}$
Fibrinogen	6.7	0.04	45.4	4.62	6.5	0.02	16.8	12.2
Albumin	11.2	0.11	35.8	6.31	10.7	0.08	11.8	46.6

Table 5
Contact angle (θ_w) and interfacial energy (γ_{sw}) between different materials (samples) and water

Materials	θ_w (°)	γ_{sw} (nJ/cm^2)
Ca-DLC	87.2	6.2
CaP-DLC	51	57.6
P-DLC	49	5.1
LTIC	74.9	24.2

observed on Ca-DLC and P-DLC in our platelet adhesion test. Table 5 shows the results of the contact angles and calculated interfacial energies (γ_{sw}) between water and the films. Ca-DLC and P-DLC have the lowest value of interfacial energy (γ_{sw}=6.2 and 5.1, respectively) with water (medium), indicating that both films have closer interracial tension (1–3 nJ/cm^2) with the cell-medium than CaP-DLC and LTIC. Hence, our platelet results are consistent with the calculated surface energy.

5. Conclusion

Ca, Ca and P, and P doped DLC films were synthesized by plasma immersion ion implantation and deposition (PIII and D) and their blood compatibility was evaluated using platelet adhesion tests. Contact angle tests were conducted to determine the surface energy and wettability. Our results show strong adhesion of albumin with low conformational change of fibrinogen on both the Ca-DLC and P-DLC films. Both materials also exhibit less adhesion and activation of platelets. However, DLC films doped with both Ca and P show inferior blood compatibility which is explained by its high values of $\gamma^{p}_{sp}/\gamma^{d}_{sp}$ and interfacial energy (γ_{sp}) of fibrinogen. These high energy values inhibit albumin adhesion and cause serious conformational change in fibrinogen, respectively. These effects could provoke the activation of adhered platelets and cause serious thrombus.

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