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Platelet adhesion on phosphorus-incorporated tetrahedral amorphous carbon films

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ABSTRACT

The haemocompatibility of phosphorus-incorporated tetrahedral amorphous carbon (ta-C:P) films, synthesized by filtered cathodic vacuum arc technique with PH_3 as the dopant source, was assessed by in vitro platelet adhesion tests. Results based on scanning electron microscopy and contact angle measurements reveal that phosphorus incorporation improves the wettability and blood compatibility of ta-C film. Our studies may provide a novel approach for the design and synthesis of doped ta-C films to repel platelet adhesion and reduce thrombosis risk.

1. Introduction

Amorphous carbon (a-C) and tetrahedral amorphous carbon (ta-C) are considered as potential biomedical materials because of their prominent chemical inertness, high wear resistance, excellent corrosion resistance and good biocompatibility [1–3]. Furthermore, doped or alloyed a-C films with silicon, fluorine, nitrogen or phosphorus are suggested to be more compatible when contacting with blood [4–8]. For example, Saito et al. found that the addition of fluorine into diamond like carbon (DLC) films significantly improved antithrombogenicity of DLC films [4]. Kwok et al. prepared phosphorus-doped DLC films by plasma immersion ion implantation deposition and confirmed the decreased protein adsorption and platelet attachment on the films [8].

Filtered cathodic vacuum arc (FCVA) technology is an excellent method to deposit biocompatible ta-C films because of the high deposition rate over large areas [9,10]. In the present paper, phosphorus-incorporated ta-C (ta-C:P) films are synthesized by FCVA technique. PH_3 widely used in the semiconductor industry is adopted as the dopant source. Contact angle, in vitro platelet adhesion and activation and scanning electron microscopy (SEM) measurements are performed to determine the physicochemical properties and platelet adhesion behaviors of ta-C and ta-C:P films. The relation between the surface state and haemocompatibility is analyzed by the interfacial tension theory.

2. Experiment

80–100 nm ta-C and ta-C:P films were synthesized on silicon wafers by FCVA system with 10 sccm PH_3 (purity 99.9999%) as the dopant source [11]. The substrate bias voltage was −80 V for the preparation of ta-C films and −20, −80 and −200 V for three ta-C:P films, respectively. These four specimens were labeled as ta-C and ta-C:P-x (x = 20, 80 and 200, respectively) correspondingly.

Surface wettability was examined by the sessile drop technique using a CAM 101 contact angle goniometer (Finland) with water and glycol as wetting agents. Each test was conducted six times on different locations of specimens to obtain statistical averages. The interfacial tension between two condensed phases could be determined by the Young and Van Oss equations [12,13]. The polar component (γ_S^p) and dispersive component (γ_S^d) of the surface energy (γ_S) for the films were obtained by measuring the contact angles of water and glycol on the films. In vitro platelet adhesion experiments were performed via human whole blood taken from a healthy donor. All films were cleaned and incubated in human platelet-rich plasma for 60 min at 37 °C. After rinsing,

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Table 1
Parameters of contact angle, surface energy and interfacial energy with water for different materials

Material	Contact angle (°)		Surface energy (dyn/cm)			γ_S^p/γ_S^d	Interfacial energy (dyn/cm)
	Water	Glycol	γ_S	γ_S^p	γ_S^d		
ta-C	78.45	45.02	38.06	5.75	32.31	0.18	23.54
ta-C:P-20	59.10	35.44	41.94	26.07	15.87	1.64	4.62
ta-C:P-80	56.07	34.39	44.20	30.61	13.59	2.25	3.56
ta-C:P-200	58.22	37.22	42.41	28.77	13.64	2.11	4.12

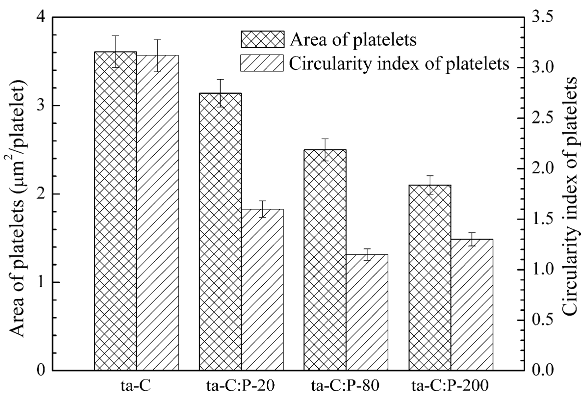

Fig. 1. Area and circularity index of platelets adhered to different surfaces.

fixing, and critical point drying, the quantity and morphology of the platelets adhered to the specimens were examined using SEM (HITACHI S-4700, Japan). Six fields were chosen at random to obtain statistical averages of the adherent platelets counts at unit area. Two parameters were selected to estimate the spreading and morphology of platelets on the materials. The degree of platelet spreading was assessed by the area of platelets (A) which was defined as the ratio of the total areas of adherent platelets (A_p) in a randomly sampled area to the number of adherent platelets (N) in the same area: $A = A_p/N$. The platelet morphology was evaluated by circularity index (C) proposed by Park et al. [14]: $C = P^2/4\pi A$, where P was the perimeter of platelets. Image analysis software was used to distinguish the labeled platelet cytoskeletons from the background and measure their perimeters and areas.

3. Results and discussion

3.1. Contact angle measurement and surface energy calculation

The parameters of contact angle, surface energy and interfacial energy with water for different films are displayed in Table 1. Data indicate that all ta-C:P films represent more hydrophilic surfaces, and the interfacial tensions between ta-C:P films and water are more closer to the cell–medium interface tension (1–3 mJ/m^2) as reported by Kwok et al. [8]. Phosphorus incorporation also increases the γ_S and the ratio of γ_S^p to γ_S^d, γ_S^p/γ_S^d. It is usually believed that the material with low interfacial tension and high polar-dispersive ratio can represent good haemocompatibility. So ta-C:P films may be potential biocompatible materials.

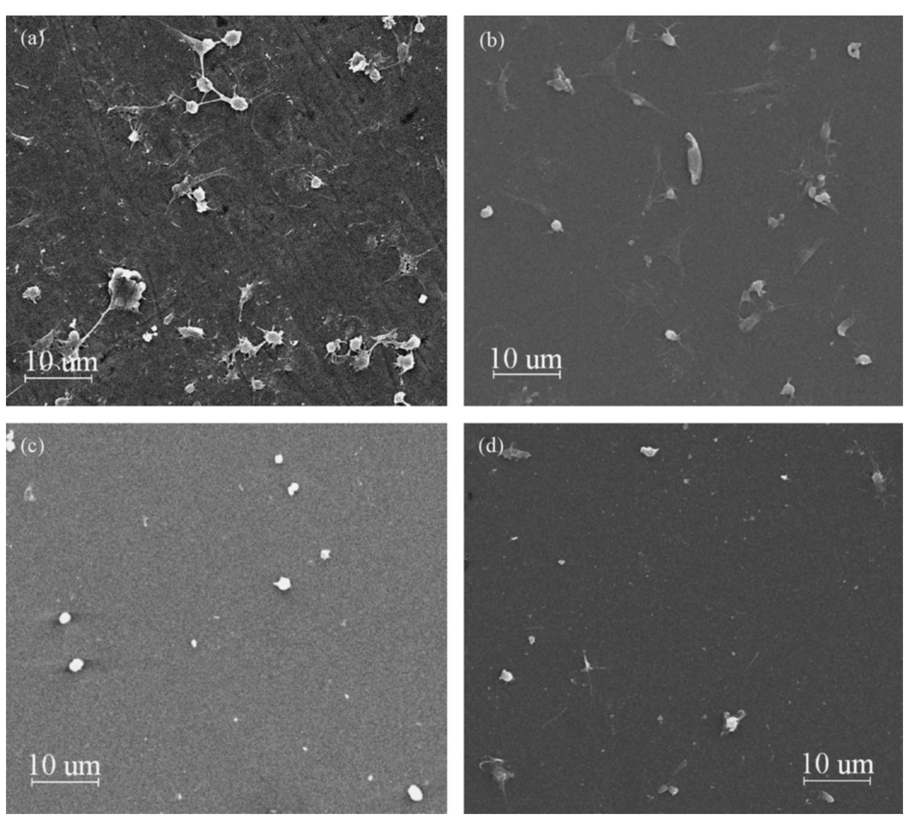

Fig. 2. Morphology of adherent platelets on (a) ta-C, (b) ta-C:P-20, (c) ta-C:P-80, and (d) ta-C:P-200 film surface.

3.2. Platelet adhesion and activation

Platelet adhesion tests exhibit significant difference in the behaviors of platelets adhered to different surfaces. The statistically average count of adherent platelets on ta-C surface is about 4.9×10^3 in the area of per mm^2 at 60-min incubation time. The mean numbers of platelets attached to ta-C:P films are significantly lower than that attached to ta-C surface and less than 1.7×10^3 at the same area. According to the viewpoint of Goodman et al. [15], platelet activation can be assessed by the spreading degree of platelets or *A* of platelets. Unactivated platelets are 2 μm in diameter [6]. With the increasing activation, the platelets can have a size up to ~5 μm at a high state of activation. For ta-C film, a wide coverage of platelets implies a high degree of platelet spreading and a high state of activation (Fig. 1). Compared to ta-C film, these three ta-C:P films show the less areas of platelets and therefore the lower spreading and activation.

Additionally, the morphological change of the adherent platelets is a common qualitative criterion to evaluate the activation of adherent platelets on the material surfaces. Based on the model proposed by Park et al. [14], *C* of the platelet on a material is correlated with the haemocompatibility of blood-contacting surface. When the measured platelet is a perfect circle, $C = 1$. An observed increase in *C* indicates the decreased haemocompatibility of the surface and activated state of the platelets. ta-C film with a high *C* exhibits a significant activity toward platelets and bad haemocompatibility, and may be more thrombogenic. Comparatively, the *C* of ta-C:P films is more closed to 1, indicating unactivated or low-activated states of platelets on ta-C:P surfaces.

The high-magnification morphologies of platelets adhered to the film surfaces shown in Fig. 2 also confirm the conclusions mentioned above. The platelets on ta-C film are much flatter with spreading pseudopodia and more wider spreading than those on ta-C:P films. Comparatively, most of the adherent platelets on ta-C:P films remain isolated and disc shaped without or slight pseudopodium occurrence. It is inferred from above results that the numbers, the degrees of pseudopodia and aggregations of the adhered platelets on ta-C:P surfaces are markedly lower than those on ta-C film. Therefore, phosphorus doping does improve the blood compatibility of ta-C film [8]. This can be explained by the suggestion that phosphorus incorporation increases the polar component γ_S^p of surface energy for ta-C film (Table 1). ta-C:P-80 with highest phosphorus content [11] has the highest γ_S^p/γ_S^d and lowest interfacial tension with water, and represents the best blood compatibility. This is consistent with the results of Yang et al. [3]. Additionally, the sp^2/sp^3 ratio of ta-C:P films may effect the haemocompatibility of the films. Although phosphorus concentration of ta-C:P-200 is slightly lower than that of ta-C:P-20, the quantity of sp^2 bonds in ta-C:P-200 and sp^2/sp^3 ratio ($I_{(D)}/I_{(G)}$ ratio) are higher due to the action of higher-energetic plasma (200 V substrate bias) [11]. Therefore, phosphorus impurity and substrate bias contribute to the better compatibility with blood of ta-C:P-200 compared with that of ta-C:P-20.

4. Conclusions

Phosphorus-incorporated tetrahedral amorphous carbon (ta-C:P) films are fabricated by filtered cathodic vacuum arc technique under different substrate biases. All ta-C:P films exhibit more hydrophilic surfaces than undoped ta-C film. Platelet adhesion tests show that ta-C:P films with less spreading area and circularity index of platelets represent lower adhesion and activation of platelets in terms of the higher polar-dispersive ratio and similar cell–medium interfacial energy with water. The presented ta-C:P appears to be a promising film material for blood contacting devices, such as rotary blood pumps, artificial organs and heart valves. However, more basic studies and long-term implantation are needed.

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