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Wettability and biocompatibility of nitrogen-doped hydrogenated amorphous carbon films: Effect of nitrogen

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Abstract

Amorphous carbon films have been applied in biomedical fields as potential biocompatible materials with wettability that can be adjusted by doping with other elements, including F, Si, Ti, O and N. In this study, nitrogen-doped hydrogenated amorphous carbon (a-C:H:N) films were deposited by PIII-D using $C_2H_2 + N_2$ gas mixtures. The biocompatibility and anti-thrombotic properties of the films were assessed in vitro. The surface morphology and surface wettability of the films were characterized using atomic force microscopy (AFM) and a contact angle method. The results show no cytotoxicity for all films, and films with appropriate nitrogen doping possess much better endothelial cell growth and anti-thrombotic properties.

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Keywords: Plasma immersion ion implantation–deposition (PIII–D); Nitrogen-doped hydrogenated amorphous carbon (a-C:H:N); Wettability; Biocompatibility

1. Introduction

Some inorganic films, such as diamond-like carbon (DLC) and carbon nitride (CN), have been applied in the biomedical field as potential biocompatible coating materials because of their chemically inert behavior and their biological properties [1–4]. However, there have been few studies addressing the antecedent causes of their good biocompatibility, and clinical use requires a more thorough understanding of the various mechanisms. Undoubtedly, the wettability of biomaterials can strongly influence their biocompatibility. In recent studies, it has been suggested that the wettability of amorphous carbon films can be adjusted by doping with other elements, for example F, Si, Ti, O and N [5–7]. In the work described here, we compare the wettability and biocompatibility of nitrogen-doped hydrogenated amorphous carbon (a-C:H:N) films with the wettability and biocompatibility of nitrogen-free a-C:H films as potential blood-contacting biomedical materials, and we suggest a possible origin of their biocompatibility.

2. Experimental

The films were fabricated on silicon wafers (1 0 0) using plasma immersion ion implantation–deposition (PIII–D), a technique that is well suited for the treatment of specimens with complex geometry such as artificial joints and mechanical heart valves. The process parameters are listed in Table 1. This fabrication method has been described in

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Table 1
Synthesis parameters of the films

Sample indication	Gas mixture	C_2H_2 flow rate (%)	Pressure (10^{-4} Torr)	RF power (W)	Negative bias (V)	Time (min)
I: a-C:H	$C_2H_2 + Ar$	33	9.1	500	150	90
II: a-C:H:N	$C_2H_2 + N_2$	33	5.0	500	150	70

detail elsewhere [8]. Film number I, made with Ar gas contains less N than film number II, made with N_2 gas. Therefore, film I will be identified as C:H and film II as C:N:H. Film composition was measured by X-ray photoelectron spectroscopy (XPS). Surface morphology of the a-C:H and a-C:H:N films was characterized using an extended multimode nanoscope atomic force microscope (AFM). Wettability examinations were performed using the sessile drop method.

The biocompatibility and anti-thrombotic properties of the films were assessed in vitro. Human micro-vascular endothelial cell line (HMEC) was seeded onto sample surfaces to a density of $1 \times 10\ ml^{-1}$. After 6 h incubation at 37.8 °C in a humidified atmosphere containing 95% air and 5% CO_2, endothelial cells (ECs) grown on the surfaces were fixed and simultaneously stained for vinculin, actin and nuclei using corresponding antibodies and fluorescence stain dyes [9]. The morphology of the cells was observed by a phase contrast microscope equipped with a fluorescence light source. The MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay is a widely used method in assessment of cytotoxicity and cell viability [10]. MTT activity, as a marker for cell metabolism, was also assayed after three days incubation using an ELISA microplate reader at 570 nm. After incubation in human platelet-rich plasma (PRP) for 15 min at 37 °C, samples of the a-C:H and a-C:H:N films were examined using optical microscopy to evaluate the quantity and morphology of the adherent platelets.

3. Results and discussion

XPS results (the spectra are not shown here) indicate that more nitrogen was incorporated in the a-C:H:N films (C/N = 0.127) than in the a-C:H films (C/N = 0.026). Fig. 1 exhibits the three-dimensional AFM morphology of the a-C:H and a-C:H:N films. In contrast to the a-C:H films, the surface morphology of the a-C:H:N films show much greater roughness, which appears to stem from the effect of N incorporation on the amorphous carbon bond structure and the growth of sp^2 clusters (the Raman spectra are not shown here). The results of measurements of contact angle with distilled water show that the a-C:H surface has a hydrophobic nature with a contact angles of 79°. In contrast to the a-C:H films, the a-C:H:N surfaces were more hydrophilic with a lower contact angle of 63°. This appears to be affected mainly by incorporation of nitrogen in the films.

Fig. 2 shows photomicrographs of ECs stained for actin stress fibers (red), vinculin (green) and nucleus (blue)

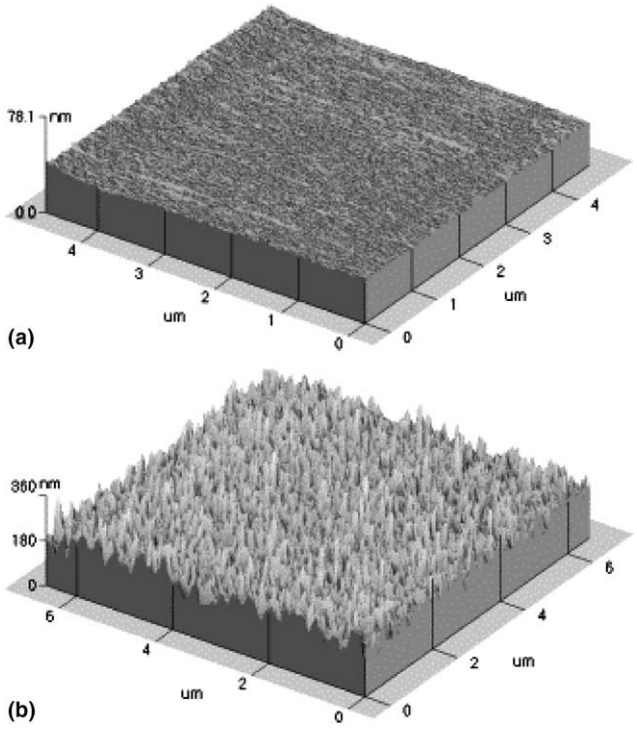

Fig. 1. AFM images of: (a) a-C:H film and (b) a-C:N:H film.

grown on the sample surfaces. ECs on the a-C:H and a-C:H:N films formed well-polymerized actin cytoskeleton and vinculin adhesion complexes with a distribution pattern of green sharp spikes at the terminating points of red fibers. No significant morphological differences were found between cells grown on the various films, while there was a greater cell density with spreading cells on a-C:H:N films than that on a-C:H films. No obviously increased cell death or immediate toxicity could be found. The results of MTT assay for cell growth on the a-C:H and a-C:H:N films are shown (Fig. 3) with a control sample of titanium (Ti), a notable non-cytotoxic material. Both samples I and II show relative higher metabolic activity than Ti. Interestingly, there is a slight trend to a higher metabolic activity on the a-C:H:N film compared to the a-C:H film. Since the pattern of stress fibers appeared to correlate with cell growth [9] and MTT assay can reflect the level of cell metabolism [10], the well-defined distribution pattern of actin fibers, adhesion protein and relative high MTT activity indicate a regular, good cell growth on the a-C:H, but somewhat limited compared to the a-C:H:N films with greater hydrophilicity and roughness.

Fig. 4 shows the statistical results of platelets adhered on a-C:H and a-C:H:N films. The stainless steel, a common

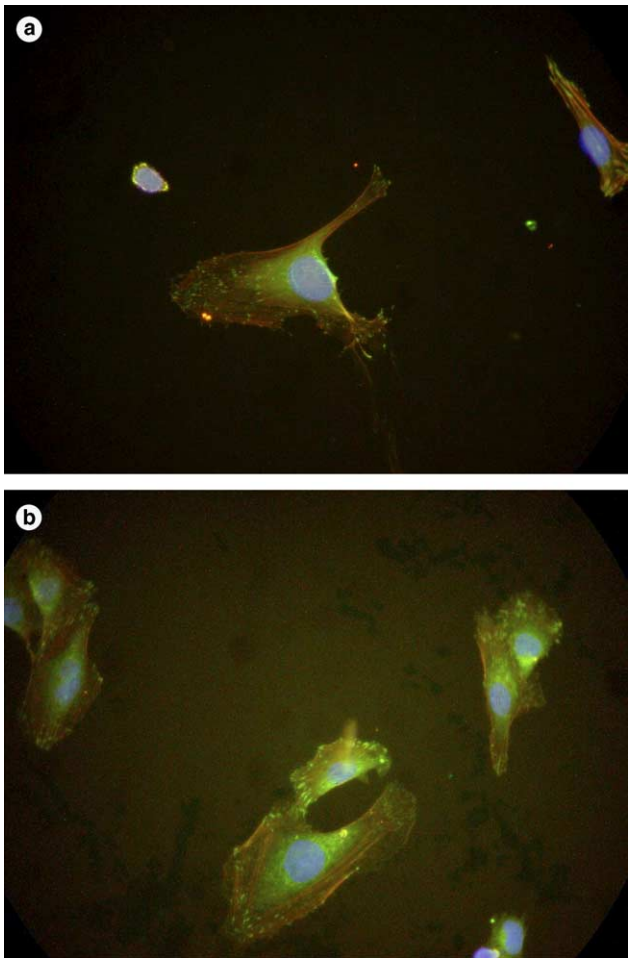

Fig. 2. Photomicrographs of human microvascular endothelial cells fluorescently stained for actin stress fibers (red), vinculin (green) and nucleus (blue) on: (a) a-C:H film and (b) a-C:N:H film. (For interpretation of the references to the colour in this figure legend, the reader is referred to the web version of this article.)

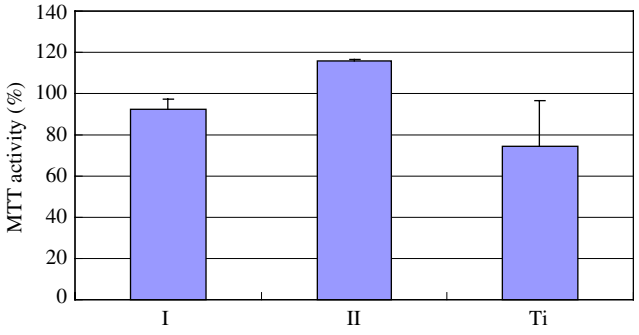

Fig. 3. Metabolic activity of EC grown on a-C:H film, a-C:N:H film and Ti, tested by MTT assay.

material used in blood contacting devices such as blood vessel stent, is used as a control material. The highest adherent number and the lowest inactive percentage of platelets are obtained at the stainless steel surface. In contrast to the results for the a-C:H films, the number of adherent platelets on a-C:H:N films decreases and the percentage of unactivated platelets increases. The results thus indicate that the platelets can barely attach to the surface of a-C:H:N film and can thus hardly be activated by it. It appears that the platelet adhesion and activation are related to the variation of wettability and roughness caused by N incorporation.

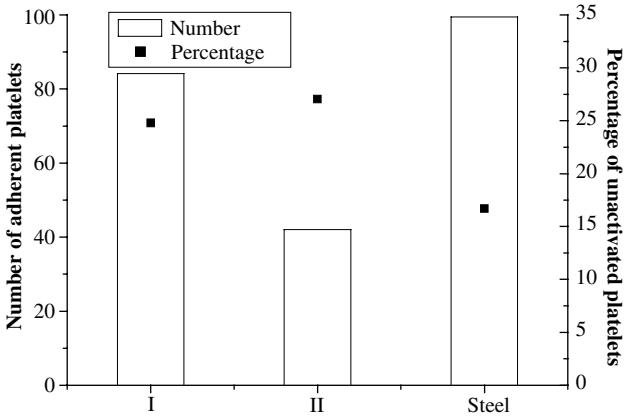

Fig. 4. Statistical results of platelets adhered on the surface of a-C:H and a-C:N:H films (15 min incubation in PRP).

4. Conclusion

a-C:H:N films were fabricated by PIII-D using $C_2H_2 + N_2$ gas mixtures. Incorporation of nitrogen in the amorphous carbon can increase the film hydrophilicity and roughness. No obviously increased cell death or immediate toxicity was found in all films, while a-C:H:N films possesses better endothelial cell growth and anti-thrombotic properties than a-C:H films. This suggests that nitrogen incorporation can improve the biocompatibility of our a-C:H films, and is related to the variation of composition, wettability and roughness of the films.

Acknowledgements

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