Hemocompatibility of nitrogen-doped, hydrogen-free diamond-like carbon prepared by nitrogen plasma immersion ion implantation–deposition

Sunny C.H. Kwok,[1] Ping Yang,[1,2] Jin Wang,[1,2] Xuanyong Liu,[1] Paul K. Chu[1]
[1]*Department of Physics and Material Science, City University of Hong Kong, Tat Chee Avenue, Kowloon, Hong Kong*
[2]*School of Materials Science and Engineering, Southwest Jiaotong University, Chengdu, 610031, People's Republic of China*

Received 26 June 2003; revised 2 March 2004; accepted 15 March 2004
Published online 18 May 2004 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jbm.a.30070

Abstract: Amorphous hydrogenated carbon (a-C:H) has been shown to be a potential material in biomedical devices such as artificial heart valves, bone implants, and so on because of its chemical inertness, low coefficient of friction, high wear resistance, and good biocompatibility. However, the biomedical characteristics such as blood compatibility of doped hydrogen-free diamond-like carbon (DLC) have not been investigated in details. We recently began to investigate the potential use of nitrogen-doped, hydrogen-free DLC in artificial heart valves. In our experiments, a series of hydrogen-free DLC films doped with nitrogen were synthesized by plasma immersion ion implantation–deposition (PIII-D) utilizing a pulsed vacuum arc plasma source and different N to Ar (F_N/F_{Ar}) gas mixtures in the plasma chamber. The structures and properties of the film were evaluated by Raman spectroscopy, Rutherford backscattering spectrometry (RBS), and X-ray photoelectron spectroscopy (XPS). To assess the blood compatibility of the films and the impact on the blood compatibility by the presence of nitr gen, platelet adhesion tests were conducted. Our resul indicate that the blood compatibility of both hydrogen-fr carbon films (a-C) and amorphous carbon nitride films a better than that of low-temperature isotropic pyrolytic ca bon (LTIC). The experimental results are consistent with th relative theory of interfacial energy and surface tension i cluding both dispersion and polar components. Our resul also indicate that an optimal fraction of sp^2 bonding desirable, but an excessively high nitrogen concentratic degrades the properties to an extent that the biocompatib ity can be worse than that of LTIC. (PACS codes: 81.05U 87.68.+z,52.77.Dq,68.55.Nq.) © 2004 Wiley Periodicals, Ir J Biomed Mater Res 70A: 107–114, 2004

Key words: hydrogen-free diamond-like carbon; nitrog implantation; plasma immersion ion implantation–depo tion (PIII-D); blood compatibility; carbon nitride films

INTRODUCTION

When natural heart valves undergo degenerative processes such as calcification of leaflets due to infection, aging, or dietary problems, artificial heart valve replacement is a solution. Low-temperature isotropic pyrolytic carbon (LTIC) is the most common material for artificial heart valves. Although LTIC is the most widely used materials in commercial products, i blood compatibility is still not adequate, and as result, patients must continue to take blood anticoa ulation medicine.[1] Therefore, other materials are beir investigated as potential replacements. Amorpho carbon or diamond-like carbon (DLC) films are pote tial biomedical materials due to their chemical ine ness, low coefficient of friction, high wear resistanc and biocompatibility.[2–4] In fact, previous studies ha shown that amorphous hydrogenated carbon (a-C:I and DLC thin films with the proper sp^3/sp^2 rat exhibit good blood compatibility.[5–9]

Hydrogen-free DLC or tetrahedral amorphous ca bon (ta-C)[10–12] films have attracted considerable inte est due to their favorable properties such as superi mechanical properties and chemical resistance cor pared to a-C:H films. Moreover, ta-C films doped wi nitrogen are expected to be excellent candidates f use as biocompatibility coatings on biomedical ir plants due to not only their excellent properties b

Correspondence to: P.K. Chu; e-mail: paul.chu@cityu.edu.hk

Contract grant sponsor: Hong Kong CityU Strategic Research; contract grant numbers: 7001389, 7001447
Contract grant sponsor: NSF; contract grant number: NSFC39870199
Contract grant sponsor: State Key Basic Research; contract grant number: G1999064706
Contract grant sponsor: High Technology Project (of the PRC); contract grant number: 00-863-102-09-01

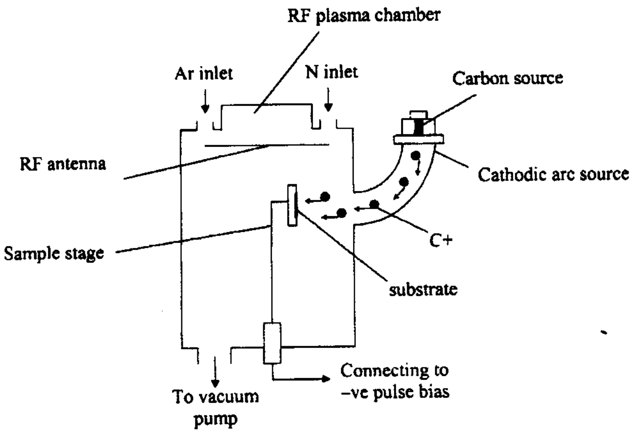


Figure 1. Schematic of PIII-D equipment used for the fabrication of a-C:N films.

also their chemical composition containing only carbon and nitrogen, which are biologically compatible. However, biocompatibility studies such as blood compatibility have been quite limited.[13]

In this work, the blood compatibility of C—N films was investigated. Nitrogen-doped DLC films were synthesized by plasma immersion ion implantation–deposition (PIII-D)[14] by operating a carbon-filtered cathodic arc source in concert with a nitrogen/argon plasma. Different mixtures of nitrogen to argon gases were introduced into the plasma to synthesize a series of thin films. The structure and composition of the thin films were determined, and *in vitro* platelets adhesive test was conducted to assess the impact of nitrogen on the haemocompatibility.

EXPERIMENTAL

The plasma immersion ion implantation–deposition (PIII-D) machine equipped with a carbon cathodic arc plasma source is depicted schematically in Figure 1. Details of the instrument can be found elsewhere.[15] Silicon substrates were precleaned ultrasonically in acetone, ethanol, and deionized water before inserting into the vacuum chamber. The chamber was pumped down to a base pressure of 1×10^{-3} Pa, and the samples were then sputtered cleaned by argon ions for 10 min prior to film deposition. Carbon ions were subsequently introduced into the chamber using the cathodic arc via a curved magnetic duct to eliminate macroparticles. Argon and nitrogen gases were simultaneously bled into the chamber using separate flow meters to control their individual flow rates. The experimental conditions are shown in Table I.

The chemical composition of the amorphous carbon nitride films was determined by Rutherford backscattering spectrometry (RBS), and X-ray photoelectron spectroscopy (XPS) was used to determine the N content and C—N bonds. Raman spectroscopy was employed to characterize the microstructure of the films.

Contact angle measurements were performed on the solid surface using doubly distilled water and diiodomethane. The contact angles of the test liquids on the sample surface were measured by the sessile drop technique using a contact angle geniometer (JY-82, China). The accuracy of this technique is typically ±2 degrees. Results are the mean of five measurements taken on different regions of the sample surface. To avoid crosscontamination of liquids, a dedicated microsyringe was used for each liquid.

The blood compatibility behavior of the films was evaluated utilizing *in vitro* platelet adhesion tests.[16] To investigate the plateletsinteraction with the film, the platelet rich plasma (PRP) was employed. It was extracted from human blood by centrifugmg for 15 min at 1000 revolution/min. The lighter substances including platelets were separated from the plasma during centrifuging. The samples were immersed into this solution for 20 and 120 min. After rinsing, fixing, and critical point drying, the adherent platelets on sample surface were examined using optical microscopy (OM) and scanning electron microscopy (SEM). Counting was performed over five fields of view (1.9 × 0.4 mm) chosen at random and the results were averaged. As shown in Figure 2 to be discussed in more details in the next section, surface thrombogenicity is indicated by the morphology change or activation of the adhesive platelets, for instance, adhesion quantity and morphology (shape), especially its aggregation and extent of pseudopodium. The details pertaining to this issue will be discussed in the next section.

RESULTS AND DISCUSSION

The Raman spectra acquired from the a-C and a-C:N films are displayed in Figure 3. The spectra that

TABLE I
Experimental Conditions for the Synthesis of the a-C:N Films

Sample	a-C	a-C:N-1	a-C:N-2	a-C:N-3
Base pressure ($\times 10^{-3}$ Pa)	2.1	2.0	1.9	2.0
Working pressure ($\times 10^{-2}$ Pa)	6.5	6.8	7.6	9.2
N gas flow (sccm)	0	10	20	30
Ar gas flow (sccm)	10	10	10	10
Flow ratio (F_N/F_{Ar})	0	1	2	3
Sample voltage (kV)			−10	
Pulse width (μs)			100	
Pulsing frequency (Hz)			50	
Time (min)			30	

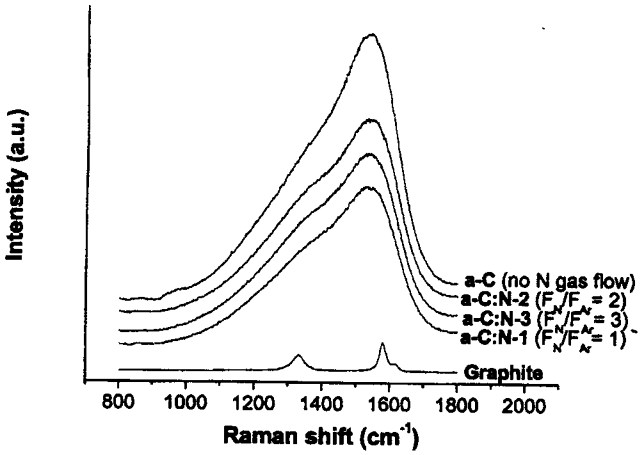


Figure 3. Raman spectra of a-C:N films produced with different F_N/F_{Ar} ratios.

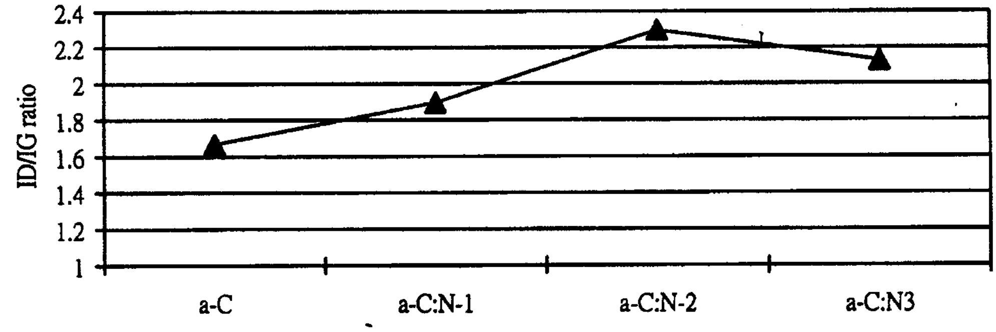
closely resemble those of amorphous diamond-like carbon can be deconvoluted into the G (graphitic) and D (disordered) bands near 1550 and 1350 cm^{-1}, respectively.[17–19] The spectra can be fitted using two Gaussian components. The ratio of the integrated areas under the D and G peaks (I_D/I_G) as well as position and full width at half maximum (FWHM) of the G peaks are summarized in Figure 4. The positions of the G and D peaks, FWHM of G peak and I_D/I_G can be correlated with the sp^3/sp^2 bonding ratio, graphite cluster size, and disorder in these threefold coordinated islands. Salient G peaks are found in all the a-C:N samples especially a-C films. The prominent peak in a-C can be attributed to the use of the cathodic arc source without nitrogen plasma. Most C 1s atoms possess sp^3 characteristics. As shown in Figure 4(a), the I_D/I_G ratio does not increase continuously with the F_N/F_{Ar} gas flow ratio increasing from 1 to 3. It attains the highest value at F_N/F_{Ar} = 2. At the same time, the G peak tends to shift from 1543.8 to 1552.2 cm^{-1} when the width of the G peaks changes from 144.8 to 139.1 cm^{-1} as shown in Figure 4(b). These results indicate that the amount of sp^2 bonding increases or graphitization has taken place with nitrogen doping and that the extent of graphitization is related to the F_N/F_{Ar} gas flow ratio.[20]

The C1s and N1s XPS spectra acquired from the a-C:N films are fitted using three to four Gaussian subpeaks.[21–23] The different peaks represent different chemical bonds such as C—C, sp^2 CN, sp^3 CN, N—O, and C—O[24] in the films. The bonds with the corresponding binding energies are shown in Table II. The XPS peak area fractions of the different constituents of the C1s and N1s peaks are calculated and shown in Figure 5. It can be observed that the sp^3 C—N bond decreases and sp^2 C—N bond increases when the flow ratio rises from 1 to 3. These results are consistent with the Raman spectra that indicate promotion of graphitization when the F_N/F_{Ar} flow ratio increases.

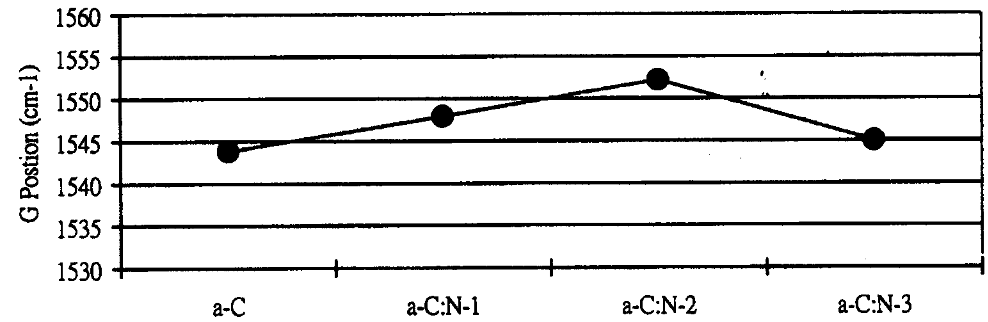
The RBS spectra of a-C:N films with different F_N/F_{Ar} flow ratio are displayed in Figure 6. No nitrogen peak appears in a-C, but the nitrogen peak intensity increases with the F_N/F_{Ar} flow ratio. It is consistent with previous results that a higher nitrogen flow rate increases the number nitrogen atoms incorporated into the films due to the enhancement in the interaction probability between N_2 molecules and C^+ ions.[25] Our results also indicate that N expedites film graphitization.

Platelet adhesion test is one of the simple and preliminary approaches to evaluate the blood compatibility of materials. Good surface thrombogenicity of the films is indicated by a small quantity and less activation or morphological change of the adherent platelets. Similar to the methodology of other researchers, we categorize the activated adherent platelets in terms of the shape and extent of pseudopodium.[26] In Figure 2(a), most of adhesive platelets on the amorphous carbon films are isolated and round, and very little destruction can be observed. On the other hand, as shown in Figure 2(d), most of the platelets on the LTIC exhibit pseudopodium indicative of some extent of activation. Figure 7 displays the statistical results based on the platelet test on the a-C and a-C:N samples. It can be observed that the nonhydrogenated carbon film (a-C) as well as amorphous carbon nitride (a-C:N) films (except for a-C:N-3) exhibit better biocompatibility than LTIC,. The number and percentage of activated platelets are relatively small on a-C:N-1 and a-C:N-2. However, a-C:N-3 deposited with the highest N to Ar gas ratio exhibits substantial platelet activation. This observation is consistent with the trend observed in the Raman spectra. A steep decrease in the I_D/I_G ratio and shift of the G peak are observed in a-C:H-3. When the F_N/F_{Ar} ratio is too high, the probability of graphitization goes up and the amount of sp^2 bonding in the film will consequently increase.

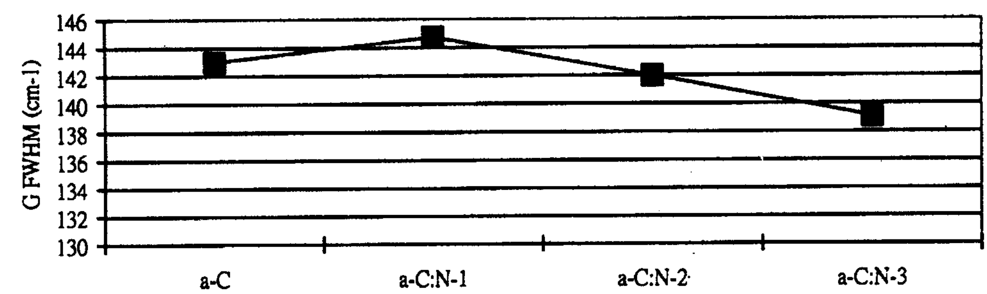
To investigate the enhancement mechanism, Figures 8 and 9 reveal the calculated results of the surface tension (between samples and water) as well as interfacial energy of protein (fibrinogen and albumin) of the a-C and a-C:N films. These parameters provide an objective explanation on the wettability properties of the films relative to the adhesive behavior of platelets. Referring to Figure 8, the contact angle of water and surface tension of a-C and a-C:N-2 are relatively low, indicating that the films become more hydrophilic. The trend in the surface tension is similar to the results of the interfacial energy between the samples and protein shown in Figure 9. According to the study of Yang et al.,[27,28] the adhesion of protein is strong when both the dispersive and polar components of the surface tension have similar contributions to the interfacial energy. On the other hand, weak adhesion of protein occurs if only one of the components is dominant in the interfacial energy. Thus, in Figure 9, low



(a) I_D/I_G ratio



(b) Position of G peaks



(c) FWHM of G peaks

Figure 4. (a) I_D/I_G ratio, (b) position, and (c) FWHM of G peaks in the Raman spectra of acquired from the a-C:N film

values of $\gamma^{P}_{sp}/\gamma^{d}_{sp}$ indicate preference of protein adhesion. It prohibits multilayer absorption of protein inducing the activation and adhesion of platelets, and thrombogenesis results.

All in all, our results show that nitrogen-doped hydrogen-free amorphous carbon films with the proper nitrogen concentration, possess better biocompatibility than LTIC. However, an excessively high nitrogen content actually degrades the blood compatibility of the film.

TABLE II
Bonding Energy with Corresponding Bonding Types

	C 1s	N 1s
Bonding	Energy (eV)	Energy (e
C—C	284.5	—
C—N (sp2)	285.7	400.5
C—N (sp3)	287.7	398.4
C—O	288.5	—
N—O	—	402.3

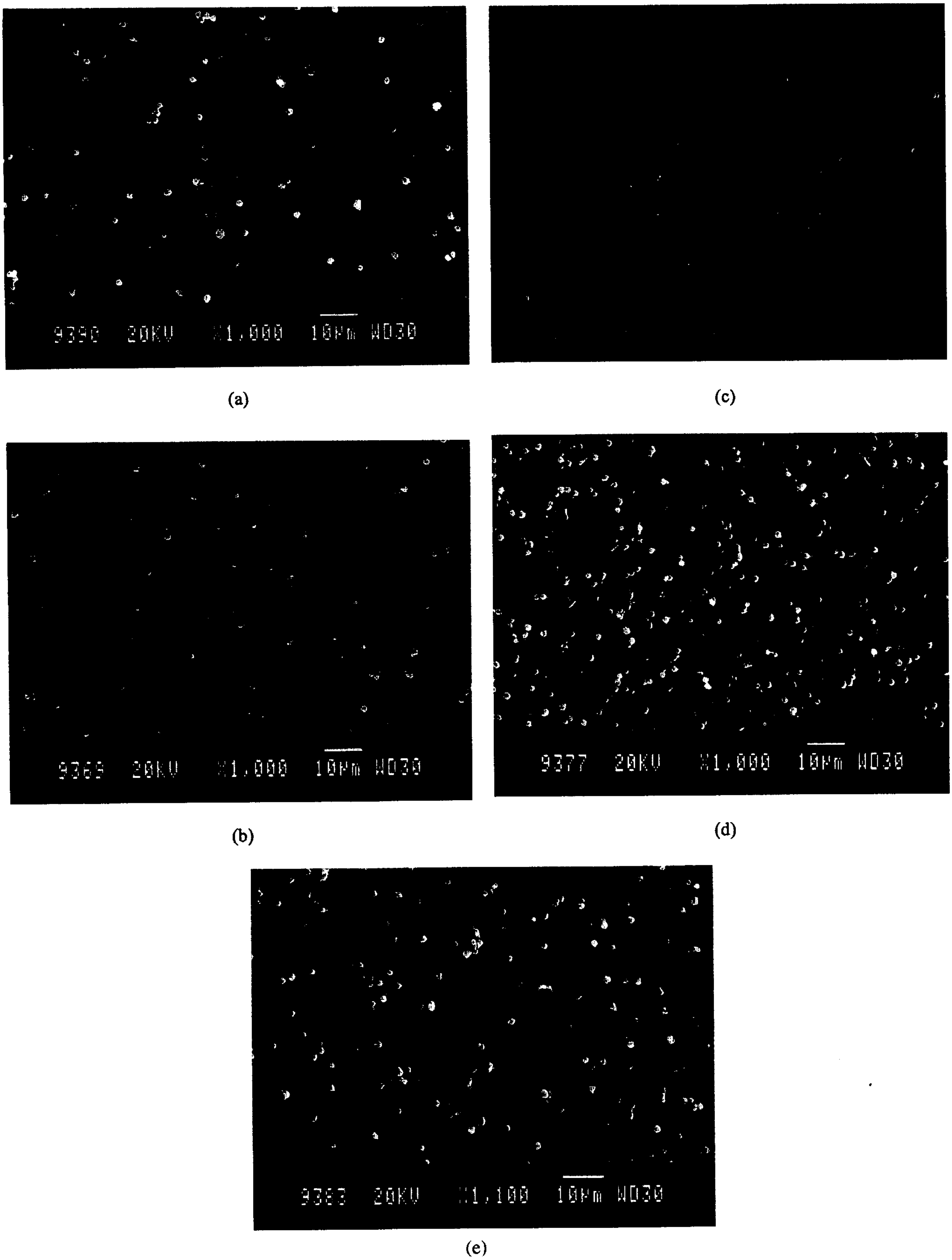


Figure 2. Representative SEM micrographs showing the morphology and quantity adherent platelets on: (a) a-C, (b) a-C -1 (c) a-C:N – 2, (d) a-C:N – 3, and (e) LTIC.

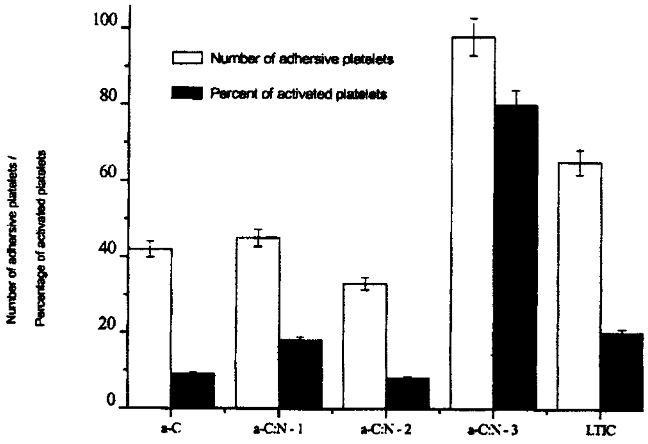


Figure 7. Number of adherent platelets and percentage of activated platelets on the a-C:N films deposited with different F_N/F_{Ar} flow ratios.

CONCLUSION

Nonhydrogenated carbon (a-C) films and hydrogen-free amorphous carbon nitride films with different nitrogen contents were produced using dual metal and gas source PIII-D. The amount of nitrogen is critical to the haemocompatibility of the materials. Our results indicate that the blood compatibility of hydrogen-free carbon films is better than that of LTIC and it can be further improved by the addition of nitrogen. Our data suggest that an optimal fraction of sp^2 bonding is desirable, and an excessively high nitrogen concentration degrades the blood compatibility to the extent that it may be worse than that of LTIC. Our results indicate that graphitization induced degradation of the wettability properties appears to be the culprit. In summary, our work shows that nitrogen doping of hydrogen-free DLC can improve the blood compatibility of the materials, but care must exercised to select the proper nitrogen concentration.

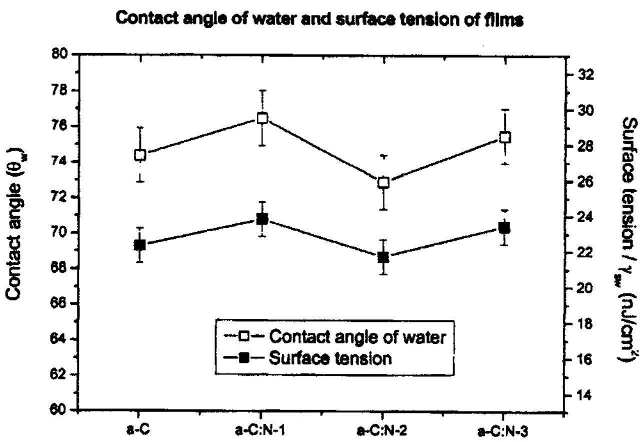
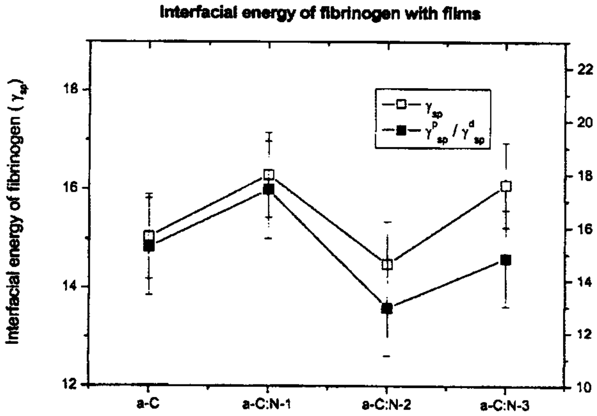
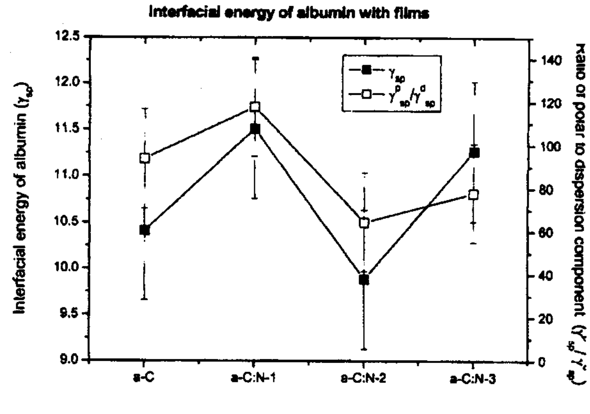


Figure 8. Contact angle of water and surface tension of films.



(a)



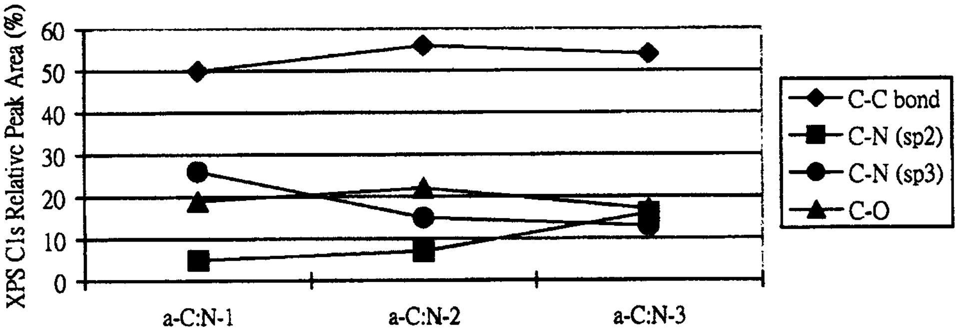
(b)

Figure 9. Interfacial energy of protein (a) fibrinogen albumin of films.

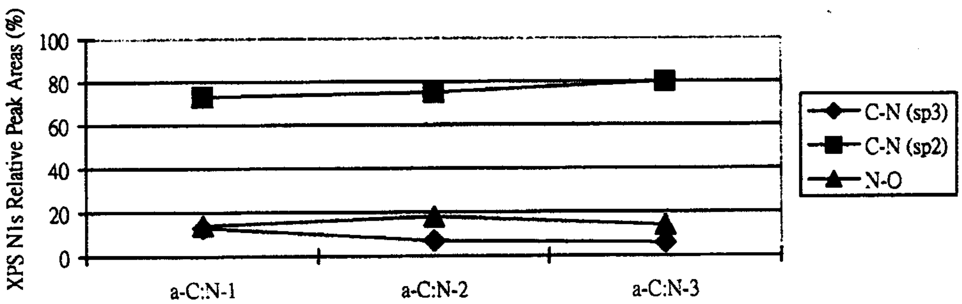
We thank W.Y. Cheung of the Chinese University of H Kong for performing the RBS analysis.

References

1. Wang XH, Zhang F, Li CR, Zheng ZH, Wang X, Liu XH, C AQ, Jiang ZB. Improvement of blood compatibility of artif heart valves via titanium oxide film coated on LTIC. Sur Coatings Technol 2000:128–129;36–42.
2. Cui FZ, Li DJ. A review of investigations on biocompatibili diamond-like carbon and carbon nitride films. Surface C ings Technol 2000;131:481–487.
3. Grill A. Diamond-like carbon coatings as biocompatible m rials—An overview. Diamond Related Mater 2003;12:166–
4. Rodil SE, Olivares R, Arzate H, Muhl S. Properties of car films and their biocompatibility using in-vitro tests. Diam Related Mater 2003;12:931–937.
5. Chen JY, Wang LP, Fu KY, Huang N, Leng Y, Leng YX, Y P, Wang J, Wan GJ, Sun H, Tian XB, Chu PK. Blood comp bility and sp^3/sp^2 contents of DLC synthesized by PII Surface Coatings Technol 2002;156:289–294.
6. Yang P, Kwok SCH, Chu PK, Leng YX, Chen JY, Wan Huang N. Haemocompatibiltiy of hydrogenated amorph



(a) C1s



(b) N1s

Figure 5. Results derived from the XPS spectra: (a) C1s, (b) N 1s.

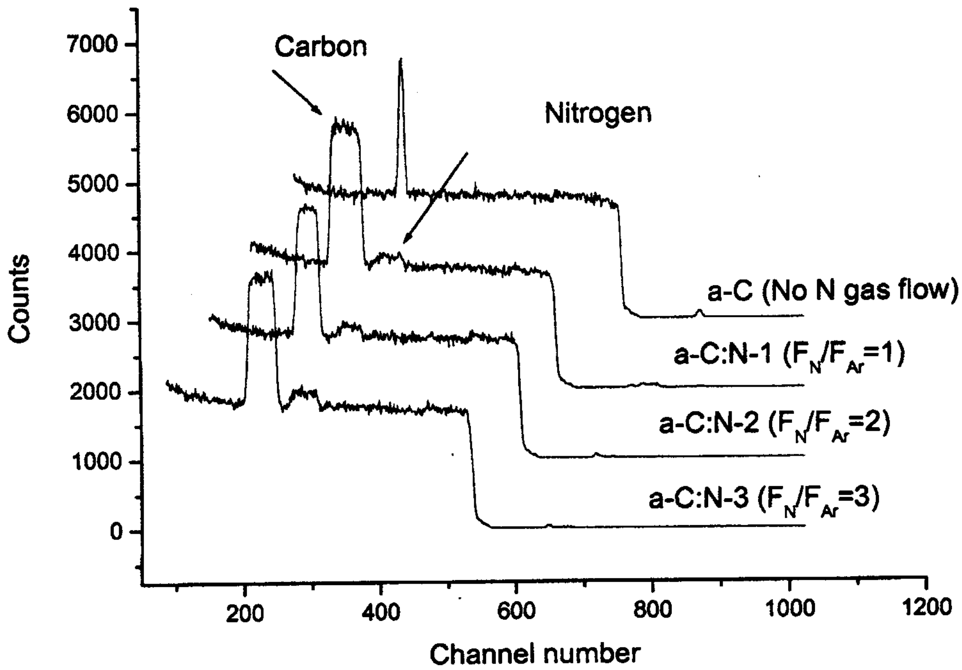


Figure 6. RBS spectra acquired from the a-C:N films deposited with different F_N/F_{Ar} flow ratios.

carbon (a-C:H) films synthesized by PIII-D. Nuclear Instrum Methods Phys Res B 2003;206:721–725.

7. Leng YX, Chen JY, Yang P, Sun H, Wan GJ, Huang N. Mechanical properties and platelet adhesion behavior of diamond-like films synthesized by pulsed vacuum arc plasma deposition. Surface Sci 2003;531:177–184.
8. Sheeja D, Tay BK, Nung LN. Feasibility of diamond-like carbon coatings for orthopaedic applications. Diamond Related Mater 2004;13:184–190.
9. Hauert R. A review of modified DLC coatings for biological applications. Diamond Related Mater 2003;12;583–589.
10. Inaba H, Fujimaki S, Sasaki S, Hirano S, Todoroki S, Furusawa K, Yamasaka M, Shi X. Properties of diamond-like carbon films fabricated by the filtered cathodic vacuum arc method. Jpn J Appl Phys 2002;41:5730–5733.
11. Chang YY, Wang DY, Wu WT. Characterization of nitrogen-doped a-C:H films deposited by cathodic-arc activated deposition process.Diamond Related Mater 2003;12;2077–2082.
12. Zhang XW, Ke N, Cheung WY, Wong SP. Synthesis and structure of nitrogenated tetrahedral amorphous carbon thin films prepared by a pulsed filtered vacuum arc deposition. Diamond Related Mater 2003:12;1–77.
13. Tessier PY, Pichon L, Villechaise P, Linez P, Angleraud B, Mubumbila N, Fouquet V, Straboni A, Milhet X, Hildebrand HF. Carbon nitride thin films as protective coatings for biomaterials: Synthesis, mechanical and biocompatibility characterizations. Diamond Related Mater 2003;12:1066–1069.
14. Chu PK, Chen JY, Wang LP, Huang N. Plasma-surface modification of biomaterials. Mater Sci Eng Rep 2002;36:143–206.
15. Chu PK, Tang BY, Wang LP, Wang XF, Wang SY, Huang N. Third-generation plasma immersion ion implanter for biomedical materials and research. Rev Sci Instrum 2001;72:1660–1665.
16. Chen JY, Leng YX, Tian XB, Wang LP, Huang N, Chu PK, Yang P. Antithrombogenic investigation of surface energy and optical bandgap and hemocompatility mechanism of $Ti(Ta^{+5})O2$ thin films. Biomaterials 2002;23:2545–2552.
17. Lifshitz Y. Diamond-like carbon—Present status. Diamond Relative Mater 1999;8:1659–1676.
18. Cheng YH, Tay BK, Lau SP, Shi X, Qiao XL, Sun ZH, Chen JG, Wu YP, Xie CS. Influence of nitrogen ion energy on the Raman spectroscopy of carbon nitride films. Diamond Relative Mater 2001:10:2107–2278.
19. Ferrari AC, Rodil S, Robertson J. Resonant Raman spectra of amorphous carbon nitrides: The G peak dispersion. Diamond Relative Mater 2003;12:905–910.
20. Yang P, Huang N, Leng YX, Chen JY, Fu RKY, Kwok SCH, Leng Y, Chu PK. Activation of platelets adhered on amorphous hydrogenated carbon (a-C:H) films synthesized by plasma immersion ion implantation-deposition (PIII-D). Biomaterials 2003;24:2821–2829.
21. Zhou ZM, Xia LF. The investigation of carbon nitride films prepared at various arc currents by vacuum cathode arc method. J Phys D Appl Phys 2002;35:1991–1996.
22. Dementjev AP, Graaf AD,van de Sanden MCM, Maslakov KI, Naumkin AV, Serov AA. X-Ray photoelectron spectroscopy reference data for identification of the C_3N_4 phase in carbon-nitrogen films. Diamond Relative Mater 2000;9:1904–1907.
23. Petrov P, Dimitrov DB, Krastev V, Georgiev C, Popov C. X-ray photoelectron spectroscopy characterization of CN_x thin films deposited by electron beam evaporation and nitrogen ion bombardment. Diamond Relative Mater 2000;9:562–565.
24. Merel P, Tabbal M, Chaker M, Moisa S, Margot J. Direct evaluation of the sp^3 content in DLC films by XPS. Appl Surface Sci 1998;136:105–110.
25. Zhou ZM, Xia LF. The investigation of carbon nitride films prepared at various arc currents by vacuum cathode arc method. J Phys D Appl Phys 2002;35:1991–1996.
26. Goodman SL. Sheep, pig, and human platelet-material interactions with model cardiovascular biomaterials. J Biomed Mater Res 1999;45:240–250.
27. Zhang XW, Ke N, Cheung WY, Wong SP. Synthesis and structure of nitrogenated tetrahedral amorphous carbon thin films prepared by a pulsed filtered vacuum arc deposition. Diamond Relative Mater 2003;12:1–7.
28. Yang P, Huang N, Leng YX, Chen JY. Comparative study of blood compatibility between TiO2-x film and LTI-carbon. Chin J Biomed Eng 2002;21:90–93.