



Wettability and protein adsorption on ultrananocrystalline diamond/amorphous carbon composite films

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ABSTRACT

Ultrananocrystalline diamond/amorphous carbon (UNCD/a-C) composite films have been prepared by microwave plasma chemical vapour deposition (MWCVD) from 17% CH₄/N₂ mixtures and modified with O₂ and CHF₃ plasmas, which changed the surface termination from hydrogen to oxygen and fluorine, respectively. X-ray photoelectron spectroscopy (XPS) showed that successful oxidation and fluorination of the UNCD surface has been achieved with surface O or F concentrations of ca. 12 at.%. None of the plasma modification processes led to a change of the film topography as studied by atomic force microscopy (AFM); for all samples the rms roughness was in the range of 10–12 nm. The UNCD/a-C films with different terminations were characterized by contact angle measurements with water, formamide and benzyl alcohol; from the results obtained the surface energy was calculated. The adsorption of albumin and fibrinogen to the different UNCD/a-C samples was assessed by an inverted enzyme-linked immunosorbent assay (ELISA). The determined albumin/fibrinogen ratios, which could be used to evaluate the tendency of thrombus formation, are correlated with the surface properties of as-deposited and modified UNCD/a-C films.

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1. Introduction

The chemical inertness and biocompatibility of diamond, together with its other exceptional properties like extreme hardness, low friction, high wear resistance, etc., have directed the efforts of the scientists in recent years towards the application of diamond thin films in biomedicine and biotechnology [1–6]. However, the very high roughness of polycrystalline diamond (PCD) films prevents their application in many bio-related fields, where smooth surfaces are required. Ultrananocrystalline diamond (UNCD) films, in pure form or as composites of diamond nanocrystallites embedded in an amorphous matrix, offer a solution of this problem, as they are very smooth and retain to a great extent the outstanding properties of PCD coatings. As a result, the possible application of UNCD films in biosensors [7], as coatings for implants [8] and templates for immobilization of biomolecules [9–12] have already been investigated. In almost all of these cases a surface modification followed by functionalization is required. The first step, achieved by plasma, photo-, electro- or thermochemistry, is rather fundamental since it tailors the surface termination and as a consequence, the hydrophilicity/hydrophobicity of the surface. This is of particular importance for

biointerfaces because tuning the surface hydrophobicity could promote specific biomolecule adsorption and/or suppress biofouling.

In this paper we report on the results of plasma modification of the surface of UNCD/a-C composite films and their characterization with respect to the surface composition, topography, wettability and protein adsorption.

2. Experimental

2.1. Deposition and surface modification

Ultrananocrystalline diamond/amorphous carbon (UNCD/a-C) composite films were prepared by MWCVD from 17% CH₄/N₂ mixtures in a deposition set-up described in details elsewhere [13]. The experiments were performed at a substrate temperature of 600 °C, a working pressure of 22 mbar, and a MW plasma input power of 800 W; the duration of the deposition process was 390 min. The films were grown onto monocrystalline (100) silicon wafers, which were etched in NH₄F/HF and then pre-treated ultrasonically in a suspension of nanocrystalline diamond powder (250 nm grain size, 50 mg) and ultradisperse diamond powder (3–5 nm grain size, 80 mg) in n-pentane to enhance the nucleation density.

The surface of the as-deposited films is hydrogen terminated; nuclear reaction analysis (NRA) revealed an H surface concentration of ca. 14 at.% [14]. These samples are referred as UNCD_H in the following.

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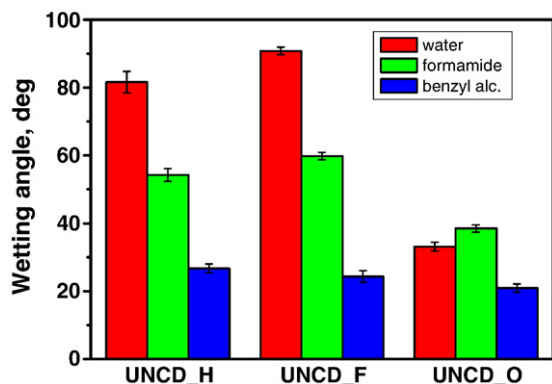


Fig. 1. Wettability of UNCD/a-C films with different surface terminations with water, formamide and benzyl alcohol.

In order to modify the surface of the UNCD/a-C films, they were subjected to plasma treatments in an O_2 MW plasma (Technics Plasma E 100) at room temperature for 10 min (UNCD_O) and in a CHF_3 RF plasma at 66 Pa and room temperature for 10 min (UNCD_F). It can be supposed that the temperature, induced during these treatments is rather low to cause any structural changes in the films.

2.2. Surface analyses

The surface composition of UNCD/a-C films with different terminations was investigated by X-ray photoelectron spectroscopy (XPS) and time of flight secondary ion mass spectroscopy (TOF-SIMS); details of the set-ups and measurement procedures have been reported earlier [15]. The films were also characterized by atomic force microscopy (AFM) in tapping mode to probe the influence of the plasma treatment on their topography.

Wettability studies were performed in dynamic sessile drop mode against water, formamide and benzyl alcohol with a DataPhysics contact angle goniometer. The contact angle measurements were carried out with five drops of each liquid on each sample in order to get reasonable statistical averages. The surface energy of the samples, divided into a dispersive and a polar component, was calculated according to the method of Owens and Wendt [16].

The protein adsorption on UNCD surfaces with different termination was studied by inverted enzyme-linked immunosorbent assay (ELISA) with albumin and fibrinogen. Standardized human plasma (dilution 1:10 in phosphate buffer solution) was incubated for 30 min on the coatings. After rinsing with phosphate buffer (pH = 7.4), the primary antibody, either anti-human albumin (anti-alb) or anti-human fibrinogen (anti-fib), depending on the protein to be detected, was added and incubated for 10 min. The dilution of the primary antibodies was 1:400 in phosphate buffer. Following another rinsing, a peroxidase-linked secondary antibody, immunoglobulin G (IgG), was added (dilution 1:500 in phosphate buffer) and incubated for another 10 min. With a final rinsing the unbound antibody-enzyme conjugates were removed. The following step was the addition of a stain which is converted by the enzyme into a color signal to be detected. This conversion process was stopped after 6 min by addition of sodium dodecyl sulfate (SDS) solution. The remaining bound stain was proportional to the protein quantity adsorbed to the UNCD surface. The adsorption was analyzed with a spectrometer SpectraMax M2 (Molecular Devices) at an absorbance wavelength of 405 nm.

3. Results and discussion

3.1. Basic bulk and surface properties

The UNCD/a-C films deposited under the conditions described above have been comprehensively characterized with respect to their crystal-

linity, composition and bonding structure [17,18]. They are composed of diamond nanocrystallites with sizes of 3–5 nm as determined by X-ray diffraction, which are embedded in an amorphous carbon matrix. The ratio of the volume fractions of the two phases is close to unity. Investigations of the films with Raman spectroscopy, XPS and Auger electron spectroscopy showed the presence of sp^2 -bonded carbon atoms (up to 15 at.%) [18]. Although no H_2 was added to the precursor gas mixture, the UNCD/a-C films contain about 8–9 at.% H in the bulk, as revealed by NRA, originating from the CH_4 molecules and bonded predominantly in sp^3 - CH_x groups [14].

The surface composition of UNCD/a-C films with different terminations has been investigated by XPS [19]. It was found that the oxygen content of the O_2 plasma treated surface is about 12 at.%, in contrast to 2 at.% for the H-terminated as-deposited sample, indicating an oxidation of the surface. The plasma treatment with CHF_3 leads to a surface fluorine concentration of almost the same value (12 ± 2 at.%) indicating that a fluorination process has taken place on the surface. Having in mind that the hydrogen surface concentration of the as-grown samples is about 14 at.% [14], the XPS results indicate a change of the surface termination by the plasma processes.

Closer analyses of the XPS peaks combined with the results from TOF-SIMS revealed that in the case of the oxygen plasma treatment the terminating hydrogen atoms are replaced by O and OH groups rather than by carboxylic acid groups [19]. CHF_3 plasma treatment has led to a substitution of the C–H bonds by C–F rather than to the deposition of a $C_xH_yF_z$ polymer, in agreement with the XPS observation that even slight argon sputtering (3 keV for 1 min) is sufficient to remove the fluorine completely from the surface [19].

AFM studies showed for all samples under investigation the characteristic topography of UNCD/a-C films, composed of structures with diameters of several hundreds nanometers, which themselves possess a substructure. The rms surface roughness values lie in the narrow range of 10–12 nm which are typical for our films [20]. These results indicate that the plasma treatments, under the experimental conditions described above, do not lead to changes of the surface structure; especially, grain boundary etching by any of them can be excluded within the limits of the parameters used in this study.

3.2. Wettability and surface energy

Dynamic contact angle measurements were used to probe the wettability of UNCD/a-C films with different surface terminations. The water contact angle increases from $\theta = 82 \pm 3^\circ$ for UNCD_H to $\theta = 91 \pm 1^\circ$ for UNCD_F, but decreases to $\theta = 33 \pm 1^\circ$ for UNCD_O (Fig. 1). These values slightly differ (by few degrees) from those obtained in static mode [19], most probably due to the different method and/or the sample storage duration. The treatment in CHF_3 plasma strongly impairs the water wetting, while the replacement of the surface H atoms by O

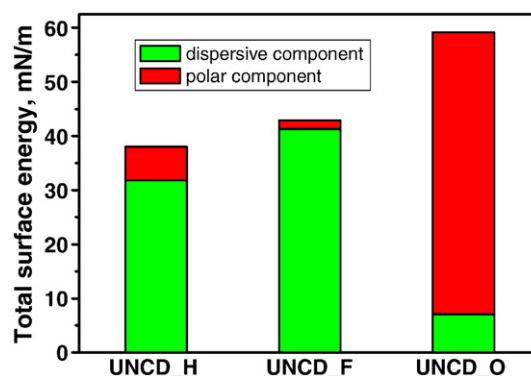


Fig. 2. Total surface energy of UNCD/a-C films with different surface terminations shown with its dispersive γ_s^d and polar γ_s^p components.

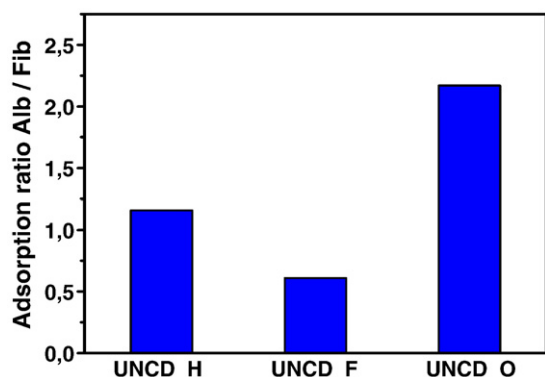


Fig. 3. Adsorption ratio albumin/fibrinogen on UNCD/a-C films with different terminations.

and OH in the oxygen plasma makes the surface more hydrophilic. A similar trend is observed for θ of the other polar test liquid, formamide, while the influence of the surface termination on the benzyl alcohol contact angles is weaker, due to the polarity which is lower in comparison with the other two test liquids.

Based on these wettability data, the surface energy of the UNCD/a-C films with different terminations was determined. The total surface energy γ_s can be described as a sum of a dispersive γ_s^d and a polar γ_s^p component. The first is related to van der Waals and induced dipole forces, while the second one comprises dipole–dipole interactions, hydrogen bridge bonds, acceptor–donor and acid–base interactions [21]. Both plasma processes increase the total surface energy of the UNCD/a-C films (Fig. 2). However, the contribution of the two components is different: an increase of the dispersive component for UNCD_F and a large contribution of the polar one for UNCD_O. Since the polar component is responsible for donor–acceptor interactions at the solid–liquid interface, better wettability with the polar test liquids (water and formamide) was observed in the case of O-terminated UNCD/a-C films (Fig. 1). The values of γ_s obtained for the UNCD/a-C films (between 38.0 and 59.2 mN/m) are in the same range as those of as-deposited (36.3–47.6 mN/m) [22] and surface modified (47.0–65.8 mN/m) [23] PCD films, as well as of NCD films (50.0–70.4 mN/m) [24]. The surface energy difference between the H- and O-terminated UNCD/a-C films of 21.2 mN/m is very close to those for PCD films with the same termination ($\Delta\gamma_s = 18.8$ mN/m).

3.3. Protein adsorption

The first event that occurs when a foreign material comes into contact with blood is plasma protein adsorption, followed by platelets interaction with the adsorbed protein layer [25,26]. In order to evaluate the haemocompatibility of a surface, in the sense of decreased possibility of thrombus formation, an important parameter to be initially studied is the adsorption of two plasma proteins, albumin and fibrinogen. HSA (Human Serum Albumin) is the most abundant protein in the human blood plasma. It has been found that HSA adsorption on a surface inhibits thrombus formation. Fibrinogen takes part in blood coagulation, facilitates the adhesion and aggregation of platelets, and plays an important role in the processes of haemostasis and thrombosis [27,28]. Therefore the enhancement of HSA adhesion against that of fibrinogen is highly desirable for a successful functionalization of haemocompatible coatings. Dion et al. proposed that the higher the ratio albumin to fibrinogen, the lower the number of adhering platelets and the lower the tendency of thrombus formation [29,30].

The effect of the surface termination of the UNCD/a-C coatings on the adsorption of albumin and fibrinogen was studied by inverted ELISA. The ratio of albumin to fibrinogen adsorption was calculated from the individual levels of both proteins adsorbed on the surfaces (Fig. 3). The oxygen terminated layers exhibit a higher albumin to

fibrinogen ratio as compared with the fluorine and hydrogen terminated films. It has been pointed out that the variation of the albumin and fibrinogen adsorption ratio is strongly related to the associated surface energies since fibrinogen, being itself hydrophobic, preferentially adsorbs on hydrophobic surfaces, but albumin (with a hydrophilic nature) on hydrophilic surfaces during competitive binding [31]. The O-terminated UNCD/a-C layers have a hydrophilic surface (contact angle of water 33°) with a greater polar component of the surface energy, leading to a higher albumin/fibrinogen ratio. The F-terminated films show the lowest protein ratio, which is related to the hydrophobic nature of these surfaces (contact angle of water 91°) and the predominant dispersive component of the surface energy. Therefore the albumin adsorption is much greater on the oxygen terminated surfaces and vice versa – the fibrinogen is preferentially adsorbed on the hydrophobic fluorine and hydrogen terminated surfaces. These results are in accordance with the literature data where the albumin/fibrinogen adsorption ratio increases with the surface hydrophilicity, e.g. for hydrophilic DLC (Alb/Fib > 2) [32], and for hydrophobic a-C:H (Alb/Fib = 1.0–1.2) [33] and a-C (Alb/Fib = 1.0–1.1) [21]. They also indicate that UNCD/a-C coatings with improved blood compatibility can be prepared applying different surface modifications. Further experiments including platelets adhesion on the coatings and ELISA with greater number of samples for improved statistics are in progress, and the results will be reported later.

4. Conclusions

UNCD/a-C composite films were deposited by MWCVD and subjected to O₂ and CHF₃ plasmas for surface modification, which resulted in a replacement of the terminating C–H bonds by C–O or C–OH and C–F_x groups, respectively, without changing the surface roughness. The as-deposited H-terminated films are hydrophobic, as revealed by contact angle measurements; the hydrophobicity is increased for the F-terminated coatings, while the O-terminated ones are hydrophilic. The plasma treatments increase the surface energy of the UNCD films with different contributions of the dispersive and polar components. The surface termination affects the protein adsorption; the albumin/fibrinogen adsorption ratio increases with the hydrophilicity of the UNCD surface suggesting the possibility of preparation of such coatings with good haemocompatibility.

References

- [1] C.E. Troupe, I.C. Drummond, C. Graham, J. Grice, P. John, J.L.B. Wilson, M.G. Jubber, N.A. Morrison, *Diamond Relat. Mater.* 7 (1998) 575.
- [2] R. Müller, M. Adamschik, D. Steidl, E. Kohn, S. Thamasett, S. Stiller, H. Hanke, V. Hombach, *Diamond Relat. Mater.* 13 (2004) 1080.
- [3] X. Xiao, J. Wang, C. Liu, J.A. Carlisle, M. Mech, R. Greenberg, D. Guven, R. Freda, M.S. Humayun, J. Weiland, O. Auciello, *J. Biomed. Mater. Res. B* 77 (2006) 273.
- [4] R.J. Hamers, J.E. Butler, T. Lasseter, B.M. Nichols, J.N. Russell Jr., K.Y. Tse, W. Yang, *Diamond Relat. Mater.* 14 (2005) 661.
- [5] T. Strother, T. Knickerbocker, J.N. Russell Jr., J.E. Butler, L.M. Smith, R.J. Hamers, *Langmuir* 18 (2002) 1968.
- [6] T. Knickerbocker, T. Strother, M.P. Schwartz, J.N. Russell Jr., J.E. Butler, L.M. Smith, R. J. Hamers, *Langmuir* 19 (2003) 1938.
- [7] J.A. Carlisle, O. Auciello, *Electrochem. Soc. Interface* 12 (2003) 28.
- [8] M.J. Papo, S.A. Catledge, Y.K. Vohra, C. Machado, *J. Mater. Sci. Mater. Med.* 15 (2004) 773.
- [9] W. Yang, O. Auciello, J.E. Butler, W. Cai, J.A. Carlisle, J.E. Gerbi, D.M. Gruen, T. Knickerbocker, T.L. Lasseter, J.N. Russell Jr., L.M. Smith, R.J. Hamers, *Nature Mater.* 1 (2002) 294.
- [10] W. Yang, O. Auciello, J.E. Butler, W. Cai, J.A. Carlisle, J.E. Gerbi, D.M. Gruen, T. Knickerbocker, T.L. Lasseter, J.N. Russell Jr., L.M. Smith, R.J. Hamers, *Mat. Res. Soc. Symp. Proc.* 737 (2003) F4.1.1.
- [11] J. Wang, M.A. Firestone, O. Auciello, J.A. Carlisle, *Langmuir* 20 (2004) 11450.
- [12] A. Härtl, E. Schmich, J.A. Garrido, J. Hernando, S.C.R. Catharino, S. Walter, P. Feulner, A. Kromka, D. Steinmüller, M. Stutzmann, *Nature Mater.* 3 (2004) 736.
- [13] C. Popov, M. Novotny, M. Jelinek, S. Boycheva, V. Vorlicek, M. Trchova, W. Kulisch, *Thin Solid Films* 506–507 (2006) 297.
- [14] W. Kulisch, T. Sasaki, F. Rossi, C. Popov, C. Sippel, D. Grambole, *Phys. Stat. Sol. (RRL)* 2 (2008) 77.
- [15] W. Kulisch, C. Popov, S. Bliznakov, G. Ceccone, D. Gilliland, L. Sirghi, F. Rossi, *Thin Solid Films* 515 (2007) 8407.

- [16] D.K. Owens, R.C. Wendt, *J. Appl. Polym. Sci.* 13 (1969) 1741.
- [17] C. Popov, S. Bliznakov, W. Kulisch, *Diamond Relat. Mater.* 16 (2007) 740.
- [18] C. Popov, W. Kulisch, S. Bliznakov, B. Mednikarov, G. Spasov, J. Pirov, M. Jelinek, T. Kocourek, J. Zemek, *Appl. Phys. A* 89 (2007) 209.
- [19] C. Popov, W. Kulisch, S. Bliznakov, G. Ceccone, D. Gilliland, L. Sirghi, F. Rossi, *Diamond Relat. Mater.* 17 (2008) 1229.
- [20] W. Kulisch, C. Popov, H. Rauscher, L. Sirghi, T. Sasaki, S. Bliznakov, F. Rossi, *Diamond Relat. Mater.* 17 (2008) 1116.
- [21] M. Stüber, L. Niederberger, F. Danneil, H. Leiste, S. Ulrich, A. Welle, M. Marin, H. Fischer, *Adv. Eng. Mater.* 9 (2007) 1114.
- [22] F. Pinzari, P. Ascarelli, E. Cappelli, G. Mattei, R. Giorni, *Diamond Relat. Mater.* 10 (2001) 781.
- [23] L. Ostrovskaya, V. Perevertailo, V. Ralchenko, A. Dementjev, O. Loginova, *Diamond Relat. Mater.* 11 (2002) 845.
- [24] L. Ostrovskaya, V. Perevertailo, V. Ralchenko, A. Saveliev, V. Zhuravlev, *Diamond Relat. Mater.* 16 (2007) 2109.
- [25] J.D. Andrade, in: J.D. Andrade (Ed.), *Surface and Interfacial Aspects of Biomedical Polymers*, Plenum Press, New York, 1985.
- [26] K.K. Chittur, *Biomaterials* 19 (1998) 301.
- [27] E. Lee, S. Kimm, *ASAIO J.* 3 (1980) 355.
- [28] S. Slack, T. Horbet, *J. Biomater. Sci. Polym.* 2 (1991) 227.
- [29] I. Dion, C. Baquey, *Int. J. Artif. Organs* 15 (1992) 617.
- [30] I. Dion, X. Roques, *Biomed. Mater. Eng.* 3 (1993) 51.
- [31] A.K.H.A. Rich, *J. Cell Sci.* 50 (1981) 1.
- [32] W.J. Ma, A.J. Ruys, R.S. Mason, P.J. Martin, A. Bendavid, Z. Liu, M. Ionescu, H. Zreiqat, *Biomaterials* 28 (2007) 1620.
- [33] P. Yang, N. Huang, Y.X. Leng, J.Y. Chen, R.K.Y. Fu, S.C.H. Kwok, Y. Leng, P.K. Chu, *Biomaterials* 24 (2003) 2821.