COMPARISON OF SURFACE PROPERTIES OF DLC AND ULTRANANOCRYSTALLINE DIAMOND FILMS WITH RESPECT TO THEIR BIO-APPLICATIONS

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Introduction

DLC layers are entirely amorphous or contain micro- or nanocrystalline diamond or graphite and possess a disordered structure with a mixture of carbon bonding configurations. Furthermore, DLC can be hydrogen free (a-C) or containing hydrogen (hydrogenated amorphous carbon (a-C:H)). DLC films exhibit excellent physical and chemical properties, as well as high level of biocompatibility [1]. The films are dense, mechanically hard, smooth, abrasion resistant, IR transparent, chemically inert, resistant to attack by both acids and bases, have a low coefficient of friction, low wear rate, and are biocompatible and thromboresistant [2-3]. DLC coatings can be adherent on various biomaterials; neither toxicity toward certain living cells nor inflammatory response or loss of cell integrity were reported [4]. DLC shows an excellent hemocompatibility, a decreased tendency of thrombus formation and coated heart valves and stents are already commercially available [5]. The properties of the DLC coatings depend strongly on the hydrogen content and sp^3/sp^2 ratio which, in turn, depend on the deposition process and its parameters. The range of the properties of the DLC produced by different methods and under different process parameters is considerable.

Diamond is a material with quite a number of excellent properties [6], like extreme hardness, high elastic modulus, high wear resistance, optical transparency in a broad spectral range, resistivity controllable by the level of dopants, etc. which make it a promising candidate for diverse applications. Due to its outstanding electrochemical properties, superior chemical inertness and biocompatibility, artificially grown diamond has been recognised as an extremely attractive material for both (bio-)chemical sensing and as an interface to biological systems. This holds for all forms of diamond: monocrystalline (natural or artificial) and poly- (PCD), nano- (NCD) and ultrananocrystalline (UNCD) films.

In the current work the surface and antibacterial properties of DLC and UNCD films including the nature of the surface bonding and termination, wettability, surface energy and tests with Gram-positive and Gram-negative bacteria were studied and discussed.

Experimental

Deposition: The DLC layers were prepared by pulsed laser deposition (PLD) with a KrF excimer laser with energy densities from 4 $J \cdot cm^{-2}$ to 14 $J \cdot cm^{-2}$ and PLD combined with simultaneous bombardment of the growing film with argon ions of various energies. The number of the laser pulses on the rotating graphite target (0.5 Hz) was adjusted to reach approximately the same layer thickness (80 nm for layers on Ti-6Al-4V substrates (ISO 5832-3) and 100 nm on Si (111) substrates) and were between 2000 and 4000. Before deposition the substrates were RF cleaned (13.56 MHz) in 5 Pa argon for two minutes [7, 8]. The DLC films were deposited at room temperature in argon ambient (0.01-0.15 Pa) .In case of bombardment with Ar^+, an ion gun eH200 (Kaufman and Robinson, Inc.) was applied with parameters adjusted to reach a maximum value of sp^3 bonds [9], i.e. working argon pressure of 0.01 - 0.1 Pa, cathode current of 0.5 A

and 0.15 A, and ion energy of 40 eV. The ion gun regulated the gas flow needed to maintain constant cathode current and ion energy.

For the deposition of the UNCD films microwave plasma enhance chemical vapour (MWCVD) deposition was used. The process gas mixture contained 17% methane in nitrogen with a total flow of 300 sccm, leading to a pressure of 2.3 kPa. The substrate temperature was kept at 600°C and the input MW power at 800 W. The deposition time was 360 min in order to achieve UNCD films with thicknesses of ca. 1 µm. They were deposited on monocrystalline (100) Si wafers, ultrasonically pretreated in a suspension containing 80 mg ultradisperse diamond powder (mean grain size 3-5 nm) and 50 mg nanocrystalline diamond powder (mean grain size 250 nm) in 75 ml n-pentane. The pretreatment provided a nucleation density of about 10^{10} cm^{-2} allowing the deposition of closed and uniform films.

The schemes of the deposition systems are shown in Figure 1; the deposition parameters are summarized in Table 1.

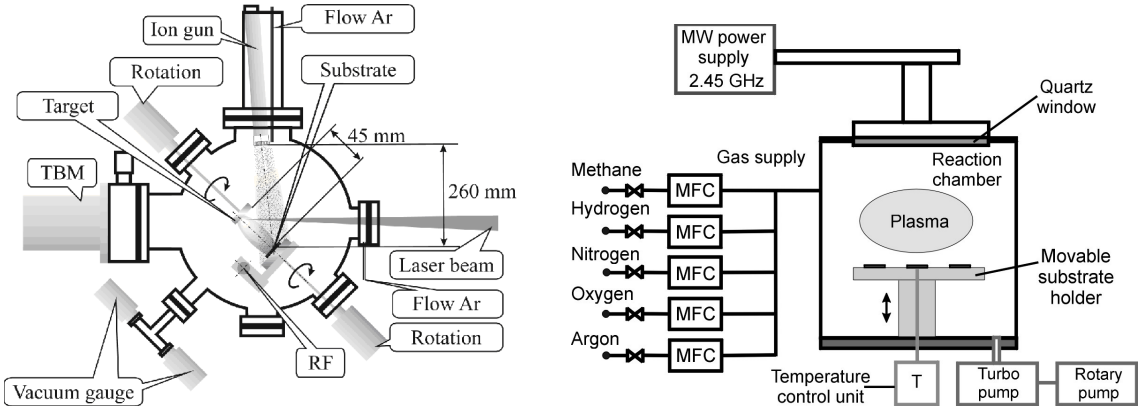

Figure 1: Scheme of PLD deposition system with ion bombardment (left) and MWCVD (right)

Table 1: Deposition conditions, composition, bonding nature and rms roughness of DLC and UNCD films

Sample	Laser energy density [J cm-2]	Ion energy [eV], ion current [A]	Composition			Bonds [%]		Roughness rms [nm]
			C [at%]	O [at%]	N [at%]	sp^2	sp^3	
DLC4	4	-	-	-	-	42	58	0.3
DLC8	8	-	-	-	-	35	65	-
DLC10	10	-	-	-	-	32	68	0.3
DLC10 0.15 A	10	40 and 0.15	92.7	6.1	1.2	19	81	0.2
DLC10 0.5A	10	40 and 0.5	91.2	8.2	0.6	29	71	4.2
DLC10 1A	10	40 and 1.0	94.1	5.9	0.0	27	73	-
DLC14	14	-	-	-	-	-	-	0.6
UNCD	-	-	98,1	1,2	0,7	10	90	9-14

Characterization: The morphology and topography of the DLC and UNCD films were investigated by scanning electron microscopy (SEM, Hitachi S-4000) and atomic force microscopy (AFM, Nanoscope Dimension 3100).

The content of diamond (sp^3) and graphitic (sp^2) bonds were determined by X-ray photoelectron spectroscopy (XPS) applying an ADES-400 spectrometer (VG Scientific, U.K.) with Mg Kα radiation (1253.6 eV) and a hemispherical electron energy analyzer. The photoelectron spectra were recorded with a pass energy 100 eV or 20 eV for survey scan and for the narrow scans of C1s and O1s core spectra, respectively. The inelastic electron background was subtracted using the Shirley`s procedure [10].

Wettability studies were performed by static contact angle measurements using a contact angle meter (DSA100, Krüss Co.) with three test liquids: distilled water, diiodomethane and ethylene glycol. The measurement was performed at room temperature by the sessile drop method with a drop volume of approximately 1±0,2 µl. The surface free energy with its dispersive (γ^d) and polar (γ^p) components was

calculated using the Fowkes method. The component γ^d related to van der Waals and induced dipole forces, while γ^p describes short-range dipole-dipole interactions, hydrogen bridge bonds, acceptor-donor and acid-base interactions.

The antibacterial properties of DLC and UNCD films were investigated using two representative bacteria typically used in antimicrobial testing, i.e. strain *Bacillus subtilis* and *Escherichia coli*. *Bacillus subtilis* is a Gram-positive bacterium commonly found in soil, while *Escherichia coli* is a Gram-negative bacterium found in large intestine of mammals. Microbial cells *E. coli* were grown in nutrient media LB Broth (Luria/Miller), microbial cells *B. subtilis* in nutrient media MPB (pepton, beef extract, NaCl, pH 7,0). For preparation of the solid media, the nutrient media was supplemented with 2% bacteriological agar as solidifying agent. The tested thin films were placed into sterile chambers and covered with 1 ml of the overnight cultures *E. coli* or *B. subtilis* diluted to cell concentration of 10^6 CFU/ml. The sterile chambers were cultivated at 35°C and aliquots of 100 µl were taken and diluted up to 10^{-6} after 8 hours. 100µl of each diluted sample were spread over the agar plates, incubated for 48 hours and then the number of growing colonies was counted.

Results and Discussion

The morphology and topology of the films were studied by scanning electron microscopy (SEM) and atomic force microscopy (AFM), which revealed closed, uniform and homogeneous DLC and UNCD coatings. The DLC layers are extremely smooth with rms roughness between 0.2 and 0.6 nm; only DLC 10, 0.5 A shows higher rms roughness of 4.2 nm, probably due to the higher energy of the bombarding ions used during the deposition. The rms roughness of UNCD layers was on the order of 9 - 14 nm.

The XPS survey spectra reveled only carbon and oxygen peaks for the DLC films. The O concentration (6 - 9%) was the same for all samples under investigation. The percentage of sp^2 and sp^3 hybridized carbon atoms in them was estimated by peak-fitting of the high resolution C1s core spectra using Gaussian functions. It was found that depending on the laser energy density and the energy of the bombarding Ar^+ the amorphous films contained from 58% to more than 80% of sp^3 bonds (see Table 1). The ion bombardment increased in general the content of the sp^3 bonds due to increased mobility of the condensing atoms and implantation of atoms under the surface [9, 11]. The UNCD films were composed of diamond nanocrystallites (3-5 nm in diameter) embedded in an amorphous carbon matrix, containing up to 30% sp^2 bonds, also determined by deconvolution of the C1s core spectra. The ratio of the crystalline and amorphous fractions is closed to 1.

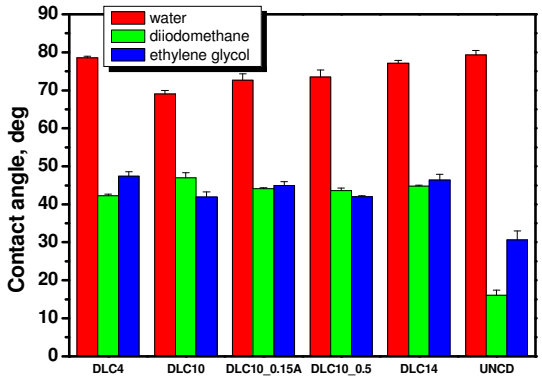

Figure 2: Wettability of DLC and UNCD films with water, diiodomethane and ethylene glycol

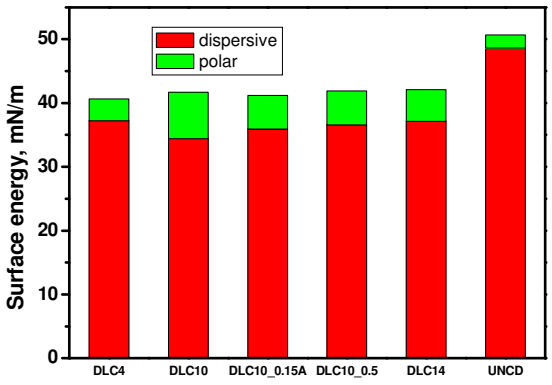

Figure 3: Total surface energy γ of DLC and UNCD films shown with dispersive γ^d and polar γ^p components

Contact angle measurements were used to probe the wettability of the DLC and UNCD films. The water contact angle for all DLC films was between 69° and 79°, for UNCD it was 79° (Fig. 2). In a similar way the contact angles for the other two test liquids, ethylene glycol (less polar than water) and diiodomethane (non-polar) do not differ substantially for all DLC films, while they are lower for the

UNCD films. In this case the surface is hydrogen terminated, as shown in previous studies, which determines the wettability. Based on these data, the surface energy of the DLC and UNCD was calculated. The total surface energy γ can be described as the sum of a dispersive γ^d and a polar γ^p component.There are not significant changes in the surface free energies for DLC layers deposited under different conditions. However, the surface energy of the UNCD layers is slightly higher, with higher disperse and lower polar component (Fig. 3)

The bactericidal efficacy of different diamond-like-carbon thin films on *E. coli* and *B. subtilis* strain was estimated according the formula:

$$\text{(bactericidal efficacy) (\%)} = \frac{\text{(alive number in reference group)} - \text{(alive number in experiment group)}}{\text{(alive number in reference group)}} \times 100\ \%$$

All DLC and UNCD films under investigation showed very good antibacterial efficiency as shown in Figure 4.

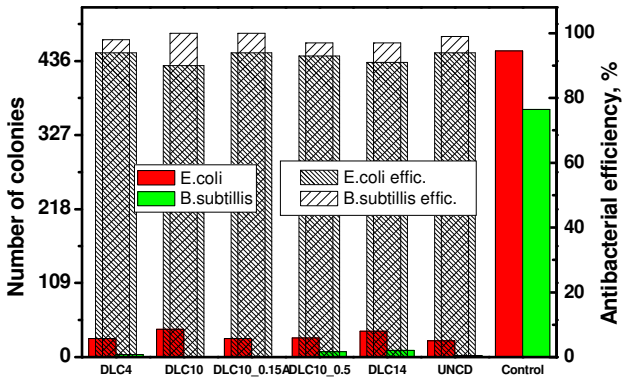

Figure 4: Number of *E. coli* and *B. subtillis* colonies on DLC and UNCD films and the respective antibacterial efficiency

Conclusions: DLC and UNCD films under investigation exhibit high antibacterial efficiency which could be related to their surface properties including high smoothness, hydrophobicity and high dispersive component of the surface energy.

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