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Cell adhesion and growth on ultrananocrystalline diamond and diamond-like carbon films after different surface modifications

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ABSTRACT

Diamond and diamond-like carbon (DLC) films possess a set of excellent physical and chemical properties which together with a high biocompatibility make them attractive candidates for a number of medical and biotechnological applications. In the current work thin ultrananocrystalline diamond (UNCD) and DLC films were comparatively investigated with respect to cell attachment and proliferation after different surface modifications. The UNCD films were prepared by microwave plasma enhanced chemical vapor deposition, the DLC films by pulsed laser deposition (PLD). The films were comprehensively characterized with respect to their basic properties, e.g. crystallinity, morphology, chemical bonding nature, etc. Afterwards the UNCD and DLC films were modified applying O_2 or NH_3/N_2 plasmas and UV/O_3 treatments to alter their surface termination. The surface composition of as-grown and modified samples was studied by X-ray photoelectron spectroscopy (XPS). Furthermore the films were characterized by contact angle measurements with water, formamide, 1-decanol and diiodomethane; from the results obtained the surface energy with its dispersive and polar components was calculated. The adhesion and proliferation of MG63 osteosarcoma cells on the different UNCD and DLC samples were assessed by measurement of the cell attachment efficiency and MTT assays. The determined cell densities were compared and correlated with the surface properties of as-deposited and modified UNCD and DLC films.

1. Introduction

Different carbon-based materials naturally attract the interest for diverse applications in medicine and biotechnology since carbon is the element of life. Two of the most promising materials for applications as coatings of implants, active parts of biosensors, platforms for neuronal growth, etc. are diamond-like carbon (DLC) and diamond. Their tremendous impact on a number of technological solutions (e.g. coatings of hard disk platters and heads, of razors, of cutting tools, etc. just to mention a few of the most popular ones) has stimulated also intensive research on possible applications in different biomedical and biotechnological fields. DLC films are amorphous, composed of a mixture of sp^2- and sp^3-bonded carbon atoms with variable ratios and may contain hydrogen, depending on the deposition technique and conditions. The properties of DLC vary in wide ranges with the sp^2/sp^3 ratio and the H concentration in the films. As a result of their optimization DLC layers with outstanding friction properties, high hardness and wear resistance, combined with good chemical inertness and biocompatibility [1–3] can be deposited. Nowadays DLC films have found various biomedical applications, especially in cases where low cell adhesion is required (e.g. as coatings on hip joints, artificial heart valves, coronary stents, etc.) [4,5].

Diamond exhibits supreme mechanical and tribological properties and biocompatibility which make it an ideal biomaterial [6]. This holds also for the different types of diamond films – mono-, poly-, nano- and ultrananocrystalline (according to their crystallinity) – making them suitable candidates for the coating of implants [7,8] or as platforms in different types of biosensors

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[9–13]. Ultrananocrystalline diamond (UNCD) films composed of diamond crystallites smaller than 10 nm have attracted a special interest since they combine the excellent diamond properties with a rather smooth surface.

For different bio-applications different bioactivities are usually required which can be tailored by the surface termination. The surface enters into direct contact with the bio-entities (DNA, RNA, proteins, cells, tissues); thus its properties, like wettability, surface conductivity and charge, etc., determine the further interaction. The surface modification of DLC and UNCD films with a number of plasma and photochemical processes has been successfully demonstrated [5,14–19]. In the present work we have comparatively investigated the surface modifications of DLC films prepared by pulsed laser deposition (PLD) and UNCD films applying an UV/O_3 treatment and O_2 or NH_3/N_2 plasmas with respect to the surface termination, wettability and surface energy achieved. Furthermore, these results were related to those concerning the attachment and proliferation of osteosarcoma cells on differently prepared DLC and UNCD surfaces.

2. Experimental

2.1. Deposition of DLC and UNCD films

The DLC layers were prepared by the pulsed laser deposition (PLD) technique with an excimer laser (KrF, λ = 248 nm, pulse duration τ = 20 ns, frequency 10 Hz). The number of laser pulses on the rotating target of high purity graphite was 2000 for a laser energy density of 12 J cm^{-2} and 4000 pulses for a laser energy density of 6 J cm^{-2}. Initially the chamber was evacuated down to a pressure of 5×10^{-5} Pa or lower, afterwards Ar was introduced with a gas flow of 10 sccm, leading to a working pressure of 0.25 Pa. Silicon (1 0 0) wafers were used as substrates after radiofrequency discharge cleaning (power 100 W) for five minutes at 5 Pa of argon. The substrates were not intentionally heated during the deposition. The target-substrate distance was 45 mm.

The UNCD films were prepared by microwave plasma enhanced chemical vapor deposition (MWCVD) from a gas mixture containing 17% methane in nitrogen with a total flow of 300 sccm. The substrate temperature was kept at 600 °C, the input MW power at 800 W and the working pressure at 2.3 kPa. The deposition time was 360 min in order to achieve UNCD films with thicknesses of ca. 1 μm. Monocrystalline (1 0 0) Si wafers, cleaned in NH_4F/HF and 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 were used as substrates. The pretreatment provided a nucleation density on the order of 10^{10} cm^{-2} allowing the deposition of closed and uniform films.

2.2. Surface modifications of DLC and UNCD films

The DLC and UNCD films under investigation were subjected to three modifications:

- UV/ozone treatment
  The modification of DLC and UNCD films was performed with light illumination in air without controlling the humidity, applying an UV low pressure mercury grid lamp (BHK Inc., Claremont, CA, area approx. 125 mm × 125 mm, input power 600 W) emitting a.o. an ozone-forming wavelength of 185 nm. The distance between the sample and the UV lamp was 4 cm. During the illumination (10 min) the chamber was closed; afterwards it was flushed with nitrogen for at least five minutes.
- Oxygen plasma treatment
  The oxygen plasma modification (2.45 GHz) was carried out in an oxygen asher (TePla 200-G) for 5 min at 250 W discharge power and 67 Pa working pressure.
- Ammonia plasma treatment
  The amination was performed in an inductively coupled plasma (ICP) setup, in which the 13.5 MHz RF signal from a commercial generator (Advanced Energy) was coupled into a cylindrical quartz reaction chamber via a copper coil. The chamber was evacuated to a residual gas pressure of 3 mPa or below; afterwards a gas mixture of 5% ammonia in nitrogen (flow rates: 5 sccm NH_3 and 100 sccm N_2) was introduced. The working pressure was maintained at ca. 1.8 Pa with a throttle valve; an ICP RF power of 150 W was used in all experiments. The process duration was 5 min; afterwards the chamber was ventilated with nitrogen.

2.3. Characterization

The topography of the DLC and UNCD films were investigated by atomic force microscopy (AFM, Veeco CP-II). The rms roughness was calculated from topographic images taken in contact mode at nine different locations on each sample.

The XPS analyses have been carried out with a Kratos Ultra-DLD Spectrometer (Kratos Analytical, UK) operating in hybrid mode. The samples were mounted on a stainless steel bar with Cu tape. All data were acquired using an Al Kα monochoromatic source operating at 300 W (20 mA, 15 kV): survey scans (5–1200 eV, 160 eV pass energy, 1 eV step size and 100 ms dwell time, 1 sweep) and high resolution C 1s, O 1s and N 1s core level spectra (20 eV pass energy, 0.1 eV step size, 60 s weep time, 2 sweeps). A charge neutralizer has been used to suppress the surface charging during analysis; all spectra have been referred to the hydrocarbon peak set at 285.00 eV binding energy [20]. The spot size of the analysis was 400 μm × 700 μm; three positions have been analyzed for each sample. The data have been analyzed using CASA XPS software (v. 2316PR1). The quantitative analysis has been performed using the peak area of the wide scans and the relative sensitivity factors (RSF) provided by Kratos.

The wettability of the DLC and UNCD films was investigated by static contact angle measurements applying a CAM 100 Contact Angle Meter (Krüss). The measurements were performed at room temperature by the sessile drop method with four test liquids, namely water, formamide, 1-decanol and diiodomethane. A droplet with a volume of approximately 1.2 ± 0.2 μL was placed on the sample surface by a Hamilton syringe; its shape was approximated by a software adjusting the parameters of the Young–Laplace equation and calculating the contact angles. For each sample at least three tests were performed and four images from each drop were analyzed with a time interval of 100 ms from which the mean value and the standard deviation were determined.

The surface free energy (SFE) γ with its disperse (γ^d) and polar (γ^p) components was calculated using the method of Owens, Wendt, Rabel and Kaelble [21,22]. The component γ^d is 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 contact angle values of the four test liquids mentioned above were exported to a drop shape analysis program for the determination of the surface free energy. The parameters for the liquids including the disperse and polar fractions of the surface tension are summarized in Table 1.

2.4. Cell tests

The influence of the surface modification of DLC and UNCD films on the cell attachment efficiency and growth was assessed with human osteosarcoma cells (MG63, CRL-1427). The cells were maintained in Eagle's Minimum Essential Medium (MEM, Lonza, Switzerland) containing 10% heat-inactivated fetal bovine