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Impact of differently modified nanocrystalline diamond on the growth of neuroblastoma cells

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The aim of this study was to assess the impact of nanocrystalline diamond (NCD) thin coatings on neural cell adhesion and proliferation. NCD was fabricated on fused silica substrates by microwave plasma chemical vapor deposition (MPCVD) method. Different surface terminations were performed through exposure to reactive hydrogen and by UV induced oxidation during ozone treatment. Boron doped NCD coatings were also prepared and investigated. NCD surface wettability was determined by contact angle measurement. To assess biocompatibility of the NCD coatings, the neuroblastoma SH-SY5Y cell line was used. Cells were plated directly onto diamond surfaces and cultured in medium with or without fetal bovine serum (FBS), in order to evaluate the ability of cells to adhere and to proliferate. The obtained results showed that these cells adhered and proliferated better on NCD surfaces than on the bare fused silica. The cell proliferation on NCD in medium with and without FBS after 48 h from plating was on average, respectively, 20 and 58% higher than that on fused silica, irrespective of NCD surface modification. Our results showed that the hydrogenated, oxygenated and boron-doped NCD coatings can be used for biomedical purposes, especially where good optical transparency is required.

Introduction

Nanodiamond has a number of the properties, which are characteristic for bulk diamond, including superior hardness, resistance to harsh environments, advanced Young's modulus, interesting optical properties, high thermal conductivity, electrical resistivity, chemical stability, and excellent biocompatibility [1]. Extraordinary physiochemical, mechanical and electrical properties of nanostructured diamond makes this material attractive to bioengineers and medical researchers. The possibility of functionalizing the diamond surface at the nanoscale by hydrophobic and electrical conductive hydrogen termination or hydrophilic and electrically highly resistive oxygen-termination, make this material ideal to develop new high sensitivity cell-based biosensors [2–4]. Diamond coatings can also be made conductive by doping with boron. Nanocrystalline

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diamond (NCD) coatings produced by microwave plasma chemical vapor deposition (MPCVD) exhibit many desirable properties favorable to orthopedic implants, such as excellent wear resistance, minimal surface roughness, and chemical inertness [5]. It was demonstrated that diamond coatings based on NCD structures improve cytocompatibility properties [6]. It was shown that coating of simulated temporomandibular joint implants with either a single-layer nanocrystalline or a multilayer diamond thin film improved their lifetimes [7]. Thus NCD could be used as a coating for prosthetic implants, because the biocompatibility is a significant advantage of diamond-based coatings [8,9]. The biocompatibility is much better in comparison to some metals and many alloys, which are commonly used in implantable devices [8]. Moreover, diamond coatings have the chemical inertness and impermeability required to reduce the crevice corrosion that is commonly seen in conventional metallic implants [10]. The suitability of NCD for osteoblast adhesion, proliferation and stimulation of differentiation has been proven [11,12]. Amaral *et al.* claimed that the nanometric featuring of NCD, which is usually based on its chemical modification, are most important topics in bone regeneration [12]. Such surface properties as topography and surface-chemistry of diamond coatings might be controlled in order to promote or inhibit osteoblast functions [13]. This fact implies that some forms of diamond coatings might be used in order to support or inhibit bone growth in selected regions [13]. Furthermore, the nanodiamond monolayers have been shown as suitable platform for neuronal growth similar to protein-coated materials [14–16]. Thus, the relatively easy fabrication and functionalization of nanocrystalline diamond coatings makes it an interesting material for many biomedical applications. However before the application of any material for medical purposes it is necessary to perform advanced evaluation of biocompatibility.

In this work, biocompatibility of the diamond-based surfaces with different chemistry was investigated to assess their suitability as transparent substrates for the growth and maintaining of neural cells. The human neuroblastoma cell line SH-SY5Y is representative of neural cells and relevant for the development of biosensing devices. NCD coatings were synthesized on fused silica by MPCVD. The surface wettability was assessed by contact angle measurement. Cell adhesion and viability/proliferation on native and boron-doped nanocrystalline diamond surfaces with oxygen and hydrogen terminations were evaluated. In order to better assess the capability of cells to adhere and to proliferate on different terminated NCD substrates, cell culture medium with and without fetal bovine serum (FBS) was used.

Materials and methods

Fabrication of nanocrystalline diamond coatings

NCD coatings were grown by MPCVD process in an ASTEX (Applied Science and Technology Company). The detailed deposition parameters of NCD and of boron doped NCD (B:NCD) on the fused silica substrates of 1 cm × 1 cm area are listed in Table 1.

TABLE 1

Parameters applied for the deposition of NCD

Deposition parameters	Values
NCD	
Gas: hydrogen (H_2)	490 cm^2 (98%)
Gas: methane (CH_4)	10 cm^2 (2%)
Power	2800 Watt
Pressure	30 Torr
Temperature	~700°C
Thickness	~150 nm
B:NCD	
Gas: hydrogen (H_2)	410 cm^2 (82%)
Gas: methane (CH_4)	10 cm^2 (2%)
Gas: trimethylboron (TMB)	80 cm^2 (16%)
Total boron in B(1):NCD	8000 ppm
B(2):NCD	1000 ppm
B(3):NCD	10 000 ppm
Power	3500 Watt
Pressure	35 Torr
Thickness	~150 nm

NCD surface modification

After the deposition of NCD coatings, surfaces were hydrogenated, oxygenated or boron-doped under conditions described in Table 2.

The NCD and B:NCD was oxidized by UV induced ozone treatment during 30 min with PSD series digital UV-ozone system (Novascan Technologies, Inc.). The digital UV-ozone system was equipped with UV emitting high voltage mercury vapor lamp and produced high energy ultraviolet light at wavelengths of 185 nm and 254 nm. After UV induced ozone treatment oxygen-terminated NCD (NCD:O) and oxygen-terminated B:NCD (B:NCD:O) were obtained.

NCD surface analysis by contact angle measurement

Hydrophobicity of NCD and B:NCD surfaces was measured by contact angle measurements of ultra-pure water. For contact angle measurements Contact Angle System OCA DataPhysics (Filderstadt, Germany) was used. To assume that the contact angle measurement is representative for the full sample surface, hydrophobicity was evaluated at four different points for each sample.

Preparation/sterilization of NCD slides

The oxygenated NCD and bare fused silica (control) slides were sterilized by incubation of them in 70% ethanol for 30 min followed by exposure to ultraviolet light for 30 min.

Neuroblastoma cell line

SH-SY5Y cells were cultured in a 1:1 DMEM and nutrient mixture F-12 from Sigma (Taufkirchen, Germany) containing 10% FBS, and 1% penicillin/streptomycin. Medium was changed every 2–3 days until the monolayer reached 80–90% confluency. SH-SY5Y cells were then trypsinized for reseeding or experimentation. The SH-SY5Y cells were maintained at 37°C in a humidified atmosphere containing 5% CO_2 for the entire duration of culture.

TABLE 2

Hydrogenation conditions of NCD and B:NCD samples

Hydrogenation parameters	Values
Gas: hydrogen (H_2)	500 cm^2
Surface cleaning and producing reactive hydrogen	3500 Watt, 2 min, 30 Torr H_2
Hydrogen bonding with carbon surface	2500 Watt, 5 min, 15 Torr H_2
Surface stabilization	30 min in H_2 atmosphere