

Surface modification and functionalization of nanostructured carbons

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Materials

ABSTRACT

Purpose: Nanostructured carbon nanomaterials (e.g., nanocrystalline diamond films and particles, carbon nanotubes, carbon onions, fullerenes, etc.) are being extensively explored for numerous biomedical applications in surgical implants, therapy, drug delivery, and biosensing due to their interesting physical, chemical, and biological properties. Such applications of carbon nanomaterials often require specific surface functionality to be introduced for better integration of these materials with physiological environment. In the last decade, substantial progress has been made in the development of controllable surface modification methods and in the introduction of different functional groups on the surface of carbon nanomaterials.

Design/methodology/approach: This paper briefly overviews the surface modification and functionalization approaches for various carbon nanomaterials, and it focuses on the plasma modification and functionalization of nanocrystalline diamond films, diamond nanoparticles, and carbon nanospheres. The results on the surface characterization using FTIR and XPS techniques, and the preliminary studies of cellular response to these modified carbon nanomaterials are presented and discussed.

Findings: The results of surface modification of NCD films, detonation nanodiamonds, and carbon nanospheres, demonstrate the flexibility of nanocarbons to attain various surface functionality that can be adjusted for specific applications. It has been shown that neither of tested nanocarbon materials was cytotoxic in this study, although the attachment and proliferation of various cells was strongly affected by the specific type of surface functionalization.

Research limitations/implications: At the present, it is not clear to what degree the available surface sites on NCD films or carbon nanoparticles can be occupied with functional groups. Furthermore, while there is clear selectivity of cellular response to H, O, and F surface-terminated NCD films, the role of specific type of surface groups present on carbon nanoparticles has yet to be determined.

Practical implications: The development of optimal strategies to functionalize various nanocarbons will have strong impact on the design of efficient nanostructured surfaces and particles for a variety of biological and medical applications.

Originality/value: This work adds new insights to the expanding research in biomedical applications of nanoscale carbon materials.

Keywords: Nanomaterials; Nanostructured carbon; Nanodiamond; Plasma; Functionalization

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1. Introduction

Bio-nano-technological advancements put forward new materials and strategies for tissue engineering, thus supporting innovative strategies, by which new combinations of biocompatible, yet inert material can be employed together with living cells or bioactive proteins in order to sustain regeneration and repair of damaged, diseased and aged tissues. For this purpose, following the pyrolytic graphite application in artificial heart valves [1], novel carbon materials such as nanocrystalline (NCD) and ultrananocrystalline (UNCD) diamond films, carbon onions, fullerenes, nanodiamonds, nanotubes (CNTs), and carbon blacks, have been applied. These materials are being extensively explored for numerous biomedical applications in therapy, drug delivery, and biosensing due to their interesting optical and biochemical properties.

Among the available carbon nanomaterials, carbon nanotubes are so far the most widely studied for biomedical applications, and comparatively little is known on the biomedical potential of other nanosized carbon particles. For example, the application of carbon nanotubes (CNTs) as delivery systems for nucleic acids, proteins, and drug molecules have been demonstrated [2]. On the other hand, it has been recently suggested that spherical carbon nanoparticles may be a superior platform for targeted delivery [3]. A number of nanoscale diamond materials have also been prepared and studied over the years. These materials come primarily either as polycrystalline diamond films with nanometer grain sizes or as diamond nanoparticles. NCD and UNCD films [4-8] have recently attracted considerable attention due to the fact that they retain to a large extent the outstanding properties of polycrystalline diamond but offer the additional advantage of very smooth surfaces. On the other hand, diamond nanoparticles (a.k.a. nanodiamonds, ultradispersed diamonds, detonation nanodiamonds, and nanocrystalline diamond powder particles [9-12]) have demonstrated in past few years a strong potential for a variety of biological applications [13,14].

The number of publications on nanoscale diamond materials has increased dramatically in past several years. According to the ISI Science Citation database only, approximately 1600 articles have been published since 1995 on nanocrystalline (NCD), ultrananocrystalline (UNCD) diamond films, nanodiamonds (ND), ultradispersed diamonds, and detonation diamond nanoparticles. Approximately 10% of these publications deal with surface functionalization and biomedical applications of nanodiamond materials.

The origin of a particular carbon nanomaterial effects the need and applicability of the specific surface modification method for achieving the desired properties. For example, diamond nanoparticles prepared by detonation methods and purified by chemical methods, demonstrate the abundance of surface carboxylic groups, lactones, ketones, and hydroxyl groups [9]. This provides a convenient platform for further functionalization of these materials. Direct interaction of detonation nanodiamonds with various biological compounds such as peptides and enzymes, have been demonstrated [11]. On the other hand, the reaction of nanoscale diamonds with fluorine-containing plasma or with an elemental fluorine/hydrogen mixture at moderate temperatures can yield a fluoro-nanodiamond with up to 8.6 at. % fluorine content [15,16]. The fluoronanodiamond can be used as a

precursor for preparation of the series of functionalized nanodiamonds by subsequent reactions with alkyllithium reagents, diamines, and amino acids [15]. The nanodiamond functionalization with amino groups, in turn, gives the possibility to graft DNA fragments onto the diamond nanoparticles.

Pertinent material requirements in the potential biomedical applications are biocompatibility, chemical stability, and mechanical properties which are perfectly fulfilled by NCD coated surfaces [17,18]. However, while diamond nanoparticles usually exhibit rich surface chemistry immediately after the synthesis and purification, nanocrystalline diamond films do not. This requires the development of often non-conventional approaches to the surface modification of NCD to attain desired functionality [19]. It has been demonstrated, so far, that the photochemically modified NCD thin films covalently linked to DNA oligonucleotides provide an extremely stable, highly selective platform in subsequent surface hybridization processes [20,21]. The results showed that the DNA-modified diamond is unique in its ability to achieve very high stability and sensitivity while also being compatible with microelectronics processing technologies. These results suggested that diamond thin films may be a nearly ideal substrate for integration of microelectronics with biological modification and sensing. NCD-coated surfaces were also efficiently functionalized with bone morphogenetic protein-2 (BMP-2) by means of physisorption, and such surfaces were found to be favored by cultured cells [22]. The developed functionalization method has been proposed to be capable of generating highly versatile surfaces with complex biomimetic coatings, essentials for novel medical devices and implants as well as for innovative scaffolds in tissue engineering. As a result of plasma termination of the NCD surface [23] by saturating the dangling bonds either with hydrogen, oxygen, or fluorine, the cellular response to modified NCD surfaces can be influenced.

In this article we present the results of several groups of experiments on surface modification and functionalization of microwave CVD NCD films, detonation diamond nanoparticles, and amorphous carbon spherical nanoparticles.

2. Materials and methods

2.1. Material fabrication

The NCD films were deposited onto polished Ti-6Al-4V discs using a Wavemat© microwave CVD reactor, equipped with a 6 kW, 2.4 GHz microwave generator. The films were deposited using a gas $H_2/CH_4/N_2/He$ mixture and a chamber pressure of 65 Torr. The surface temperature was maintained at 700°C during the process followed by gradual cooling step in pure hydrogen plasma. The resulting H-terminated films had an average grain size of 5 nm according to X-ray diffraction. A 1-hour treatment with H_2/O_2 plasma produced O-terminated hydrophilic surfaces. The processing of H-terminated films in F_2 gas in a closed chamber at 100°C for 48 h produced the H-terminated hydrophobic NCD films [23].

Detonation nanodiamond aggregated particles with an average diamond crystallite size of ~5 nm from SINTA were provided by Prof. Mitura from Technical University of Lodz. The as-received

particles were ultrasonicated to reduce the aggregate size down to 50 nm, and precipitated on a silicon substrate. The samples were then exposed to microwave H_2 or H_2/O_2 plasmas in the conditions similar to those used to modify NCD films.

Carbon nanoparticles were synthesized by hydrothermal processing of carbohydrates. The process led to stable colloids of carbon nanospheres with 80-100 nm diameters. The as-prepared colloids were centrifuged and washed in deionized water and alcohol at least 3 times to remove the precursor residue.

2.2. Characterization

Scanning Electron Microscopy (SEM) was employed to characterize the surface morphology of fabricated materials. In particular, low-voltage (below 1 kV) observations using Zeiss Gemini FESEM allowed to achieve good results in surface characterization of carbon nanoparticles.

The surface composition of the samples was analyzed by X-ray photoelectron spectroscopy (XPS) using Kratos Axis 165 XPS and by PHI5000 VersaProbe XPS systems.

X-ray diffraction and SEM were used to determine the particle size, shape, and structure, and the surface modification was studied by FTIR microspectroscopy. Vertex-70 FTIR spectrometer was utilized to acquire the infrared absorption spectra of all materials in transmission mode. In all cases, the spectra represent an average of 100 scans recorded with a resolution of 4 cm^{-1} .

Some of the prepared samples, specifically NCD films with different termination type, and carbon nanospheres were tested with the several types of cells, including: Human mesenchymal stem cells (MSCs), PAM 212 (healthy mouse epidermal keratinocytes), CH 72 (cancerous mouse epidermal mouse keratinocytes), RAW 264-7 (mouse macrophages), Au565 (human breast cancer cells), and NIH3T3 (mouse fibroblasts), and mouse bone marrow derived dendritic cells (BMDC), using the established protocols.

3. Results and discussion

Surface chemistry and morphology of nanoscale carbon films and particles are among the critical factors that determine the behaviour of these materials in physiological environment. While the surface morphology of detonation nanodiamonds is governed primarily by the diamond particle aggregation, the surfaces of NCD films and carbon nanospheres are more distinct and uniform. The representative SEM images of NCD films and carbon nanospheres used in the experiments are shown in Fig.1. The SEM image of NCD films (Fig.1a) demonstrates typical globular structure of NCD films.

XPS analysis of the surface of such NCD films showed presence of oxygen (O/C molar % ratio up to 0.08 in H- and F-terminated, and up to 0.26 in O-terminated ~5-nm surface layer) in all NCD films (Figure 2). An increase in O/C ratio in oxygen terminated NCD films is accompanied by the hydroxyl to carbonate and by carbonyl to aliphatic ratios increase. The presence of oxygen bonds seems to be common for NCD films regardless fabrication or termination method.

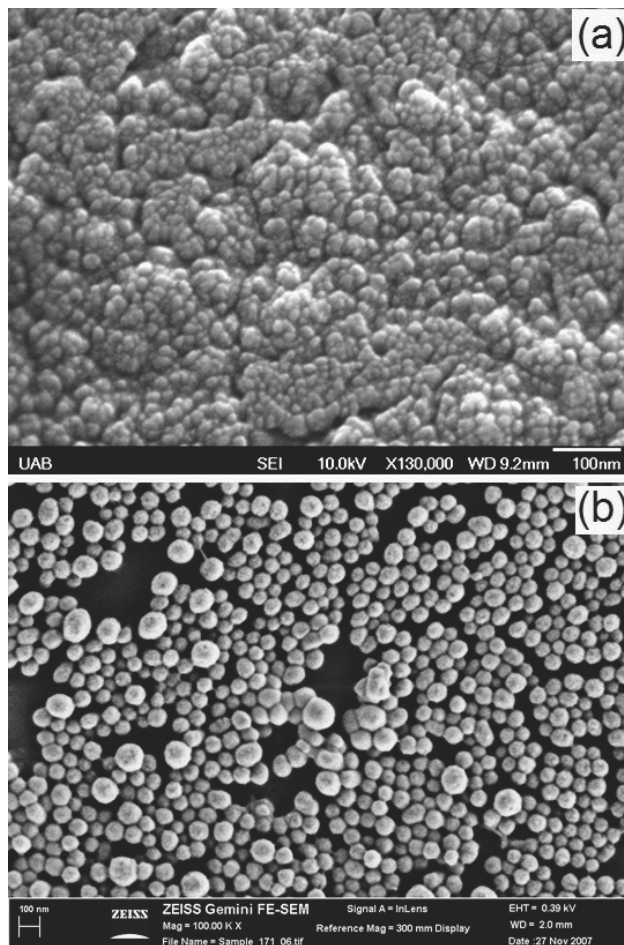


Fig.1. Representative SEM images (a) MW-CVD NCD films, and (b) Carbon nanospheres

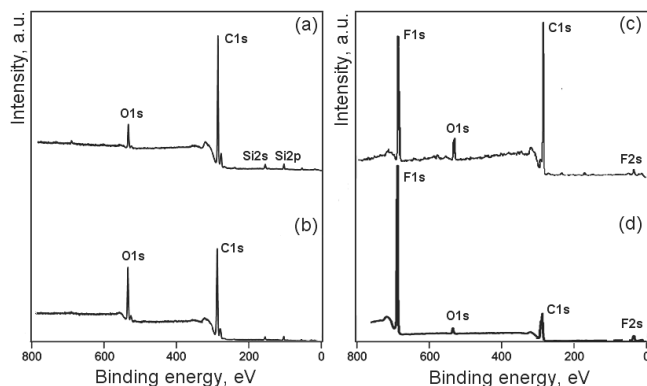


Fig. 2. XPS spectra of (a) H_2 -terminated, (b) O_2 -terminated, (c) F_2 -terminated NCD films, and (d) F_2 -terminated NCD film according to [16]

Interestingly, similar O/C ratios were observed for purified but not modified both nanodiamond particles and carbon spheres. For example, the XPS analysis of carbon nanospheres in Fig.1b

showed O/C molar % ratio of 0.16, where oxygen was primarily bonded to carbon via C-O (0.095), C=O (0.028), and O-C=O (0.037) bonds.

FTIR spectroscopy allowed to analyzing in more details the nature of chemical bonding in all tested carbon nanomaterials. Figure 3 shows the FTIR spectra of NCD films with and without H- and O-termination. The region between 1500 cm^{-1} and 1800 cm^{-1} where most of C-O bands are expected, did not show any difference between the samples in Fig.3a-c.

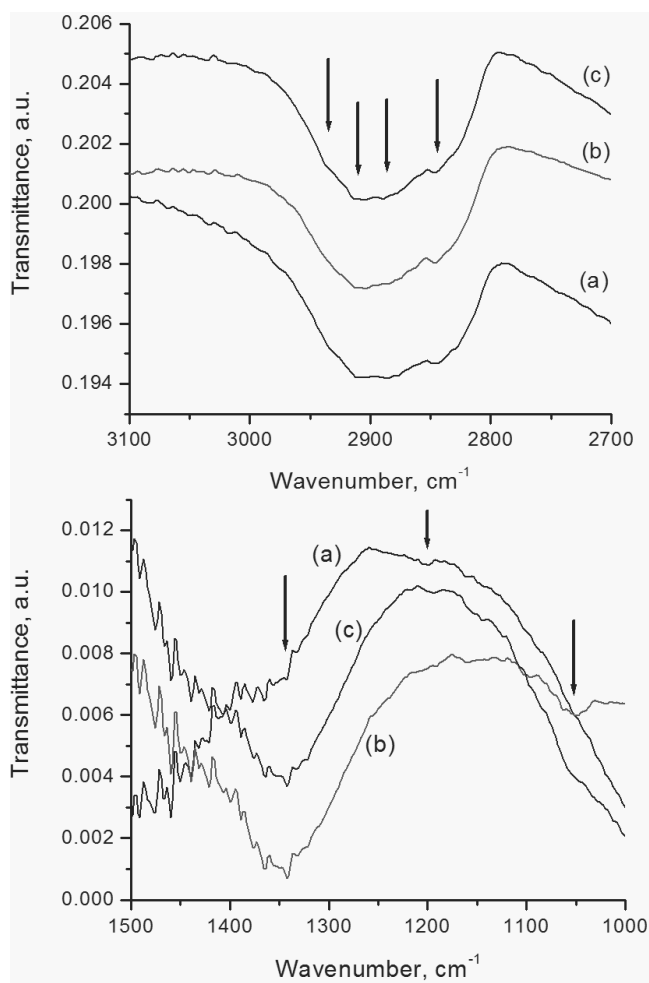


Fig. 3. Selected area FTIR spectra of (a) as prepared, (b) H-terminated, and (c) O-terminated NCD films

It can be argued that both H- and O-terminations do not cause noticeable changes in surface-terminated NCD samples prepared in the procedure described above. On the other hand, this can indicate that the plasma processing only affects a few surface monolayers, and there is little diffusion of termination species into the film.

Unlike the case of NCD films, H_2 or H_2/O_2 plasma processing of nanodiamond powder leads to significant increase in the amount of hydrogen bonded to carbon, while the amount of C=O and O-H groups reduces. Figure 4 shows the spectra of H_2 or H_2/O_2 plasma treated nanodiamond samples in comparison with untreated sample.

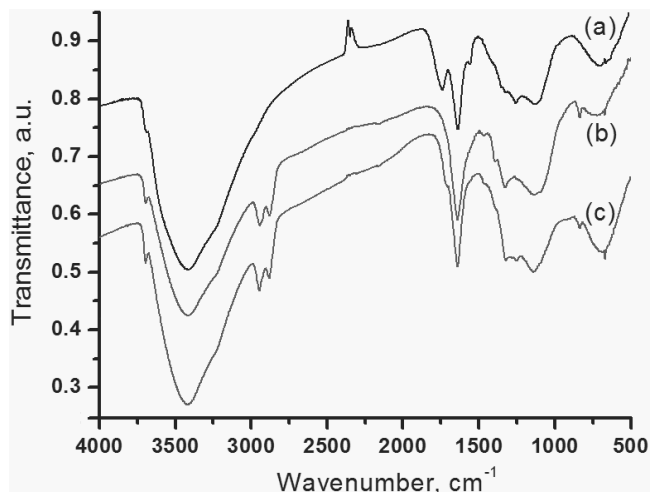


Fig. 4. FTIR spectra of (a) as prepared, (b) H_2 , and (c) O_2 -plasma processed nanodiamond powders

The distinct peaks at 2880 cm^{-1} and 2950 cm^{-1} can be observed in H_2 or H_2/O_2 plasma processed nanodiamonds. These peaks in nanodiamond are respectively assigned to symmetric and asymmetric stretching vibrations of sp^3 -bonded CH_3 group. The weak shoulders due to sp^3 -bonded CH_2 groups at 2850 cm^{-1} and 2920 cm^{-1} were also present [24]. On contrary, Kulisch et al [25] observed only 2850 cm^{-1} and 2920 cm^{-1} in NCD films. Besides the observed peaks are also close to those observed in amorphous C-H films [26], and the C-H bonding within predominantly amorphous carbon shell around diamond nanocrystals cannot be ruled out.

The FTIR spectra of carbon nanospheres in $2800\text{ cm}^{-1} - 3100\text{ cm}^{-1}$ region differ from those of plasma treated nanodiamond powders, as can be seen in Figure 5. A broad band centered at 2923 cm^{-1} is observed in as-prepared carbon nanospheres (Fig.5a), while chemical purification of this material results in pronounced peaks at 2880 cm^{-1} , 2930 cm^{-1} , 2963 cm^{-1} , and 2980 cm^{-1} indicating primarily sp^2 -bonding in the material (Fig.5b). Another most pronounced feature of FTIR spectra of carbon nanospheres is the presence of the absorption bands in the range of $1600\text{ cm}^{-1} - 1610\text{ cm}^{-1}$ (C=C bond) and at $1700\text{ cm}^{-1} - 1710\text{ cm}^{-1}$ (C=O band), while in nanodiamonds a strong C-O-O-H band is observed around 1650 cm^{-1} (Fig.4) and the presence of C=O band depends on the origin and treatment of a particular nanodiamond material.

Due to the presence of various surface groups in nanodiamonds and carbon nanospheres, these materials provide ready platform for binding other chemical moieties. FTIR spectra in Figure 6 give an example of the result of exposure of nanodiamonds to several organic compounds. The samples of exposed nanodiamonds were cleaned 3-10 times before taking FTIR spectra. It has been found that the spectra did not change anymore after 3 - 5 cleaning steps. The spectra shown in Fig.6 were taken after 10 cleaning steps. Still, all spectra show signature features of corresponding compounds. Similar results have been obtained for carbon nanospheres, although the amounts of absorbed compounds varied considerably depending on the treatment procedure of a particular carbon nanomaterial.

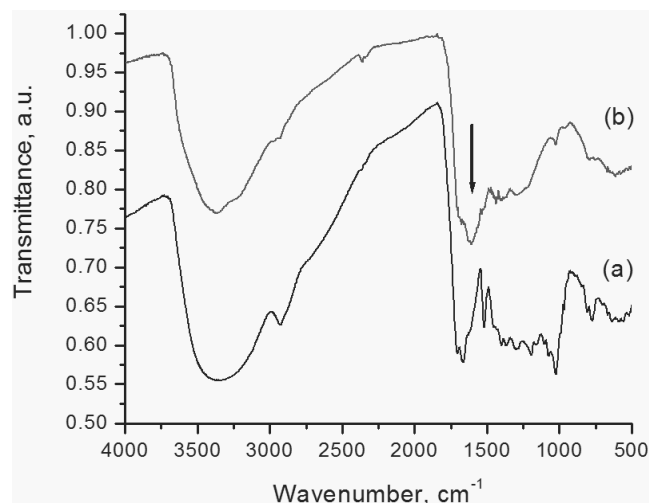


Fig. 5. FTIR spectra of (a) as prepared, (b) chemically processed carbon nanospheres

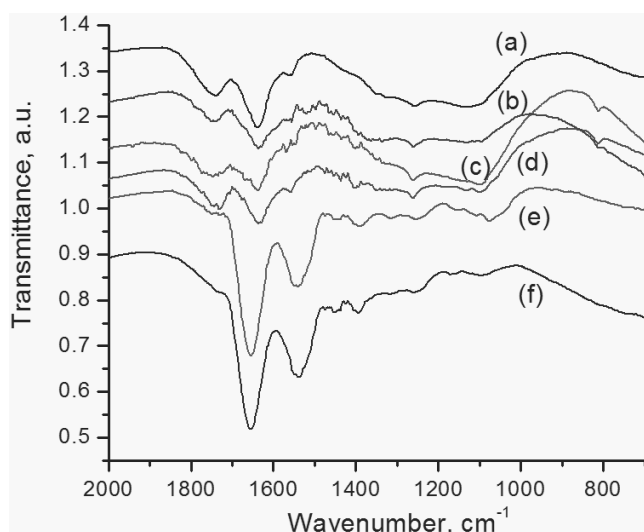


Fig. 6. FTIR spectra of nanodiamond powder before (a) and after loading with (b) ampicillin, (c) biotin, (d) glutamic acid, (e) ovarian albumin and (f) bovine serum albumin

Some of the discussed carbon nanomaterials have been tested for cellular response. In our preliminary studies of MSCs [23], we found that cells readily adhered to, and proliferated on, H-terminated NCD, but not on F- or O-terminated NCD films. It was further demonstrated that H-terminated NCD surfaces promote cell adhesion and survival, fibronectin adsorption, proliferation, and osteoblastic differentiation, as evidenced by matrix mineralization. Other initial tests of carbon nanosphere particle interaction with six different cell lines indicated little or no cytotoxicity for up to 72 hours at the particles concentration up to $1 \times 10^8 \text{ ml}^{-1}$. The carbon nanosphere size-dependent selective induction of immunity through the cell activation has been

observed for the first time, and these findings, as well as comparative studies with nanodiamond particles, require further detailed study.

4. Conclusion

The results obtained on surface modification of several nanostructured carbon nanomaterials, including NCD films, detonation nanodiamonds, and carbon nanospheres, demonstrate the flexibility of nanocarbons to attain various surface functionality that can be adjusted for specific applications. Plasma modification provides reliable pathway for creating O-, H-, and F-terminated surfaces of NCD films, and it has an interesting perspective in the processing of nanodiamonds, and probably, other nanocarbons in mild plasma conditions. The preliminary studies of these modified carbon nanomaterials indicate favorable cellular response with a number of cell systems, although the attachment and proliferation of various cells was strongly affected by the specific type of surface functionalization.

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Additional information

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