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Surface modification of nanodiamonds for biomedical application and analysis by infrared spectroscopy

T. Burleson*, N. Yusuf, A. Stanishevsky
University of Alabama at Birmingham,
1300 University Blv. CH 310, Birmingham, AL 35294-1170, USA
* Corresponding author: E-mail address: tamarab@uab.edu

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Materials

ABSTRACT

Purpose: Diamond nanoparticles are gaining much interest in biomedical applications due to the attractive chemical and biological properties. Studies have shown the potential of these "nanodiamonds" (NDs) for bioimaging, drug delivery, and biosensing. However, depending on the origin, the nanodiamond surface is often rich in various functional groups which can result in diverse behaviours in biological environments ranging from bioinertness to changes in cell function and cytotoxicity. We have observed the substantial difference in cellular response of several cell lines to NDs of various origins. Therefore, the aim of this study was to modify nanodiamond surface in a controlled manner to discriminate the effect of different functional groups on the cellular response.
Design/methodology/approach: Commercial detonation nanodiamond powders with the mean grain size 5 nm but different size of agglomerates and synthetic diamond particles ranging from 50 nm to 1 μm were modified under hydro- and solvo- thermal conditions to introduce specific functional groups to the surface. The processed nanoparticles were investigated with Fourier Transform Infrared (FTIR) spectroscopy and the results were compared between the samples. Modified NDs were tested for their toxicity in vitro with several cell lines (cell viability studies) and for the capability for small molecule anti-cancerous drug loading.
Findings: We demonstrated that different chemical groups can be introduced and controlled onto the synthetic diamond surface depending on the solvent and process parameters used. In vitro assays showed that no cellular toxicity was found when CO, OH, or NH-groups dominated on the surface of the diamond particle.
Practical implications: Many potent drugs that have proven to be useful in treating diseases such as cancer pose a challenge in delivery because they are not soluble in polar protic solvents such as water. These drugs are soluble in polar aprotic solvents that are harmful to the body. Nanodiamond surface modification in conjunction with drug-loading is a potential solution to this problem as nanodiamonds are nontoxic and have the ability to transport significant amounts of drugs.
Originality/value: Nanodiamond particles are considered nontoxic and capable of absorption of a variety of organic molecules. This study should further advance the knowledge on the potential of surface-engineered NDs in therapeutic and drug delivery applications.
Keywords: Nanomaterials; Nanodiamonds; FTIR spectroscopy; Functionalization

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1. Introduction

Nanomedicine is the application of nanotechnology to disease treatment, diagnosis, monitoring, and to the control of biological systems. The integration of nanotechnology and biology provides the opportunity for the development of new materials in the nanometer size range that can be applied to many potential applications in biological science and clinical medicine. Owing to their superior physical properties and biocompatibility, diamond nanoparticles have emerged as alternative promising materials for biomedical applications.

Diamond nanoparticles are gaining much interest in biomedical applications due to the attractive mechanical, electrical, biological, and thermal properties. Studies have shown the potential of these "nanodiamonds" (NDs) for bioimaging, drug delivery, and biosensing.

ND particles with a small size (<10 nm in diameter) can be readily generated by the detonation technique [1]. Recent progress in the dispersion of detonation NDs (2−8 nm) in aqueous media made by Osawa and colleagues has facilitated the use of NDs in physiological solutions [2]. Even more recently, the biocompatibility of detonation NDs has been assessed. Investigations of cell viability such as the MTT assay and luminescent ATP production showed that the NDs are not toxic to a variety of cell types [3]. Compared to other nanocarbon materials such as carbon nanotubes which are toxic in some studies and naturally not water-soluble, it is thus envisaged that NDs can serve as an enhanced material for biomedical applications in physiological systems [4].

NDs usually exhibit reach surface chemistry that allows a variety of other molecules to be attached for the introducing of specific functionality of NDs [5,6].

The aim of this study is to modify nanodiamond surface in a controlled manner to discriminate the effect of differing functional groups on the cellular response.

2. Materials and methods

2.1. Materials

Commercial synthetic diamond particles of different origin ranging from 5 nm to 100 nm were acquired and characterized by TEM, X-Ray diffraction, Raman, and FTIR spectroscopy. The particles were modified in various acid, base, and organic solutions under hydro- and solvo-thermal conditions to introduce specific functional groups to the surface. The changes in surface composition were analyzed using the Fourier Transform Infrared (FTIR) spectroscopy. Some modified nanodiamonds were tested for their toxicity *in vitro* with several cell lines using the established protocols.

2.2. Characterization

Transmission electron microscopy (TEM) was conducted using a FEI Tecnai T12 microscope at a 80 kV acceleration voltage. To prepare the samples of ND particles for TEM studies, a drop of dilute aqueous solution of NDs was deposited and dried on TEM copper grid first covered with a thin film of Formvar-supported carbon.

Philips X'pert thin film X-ray diffractometer with CuKα radiation (operated at 45 kV and 40 mA, wavelength 0.15406 nm) was used to evaluate the primary diamond particle size. The mean size of crystallites was determined using the Lorentzian fitting of 111 peak in the X-ray diffraction (XRD) spectrum and Scherrer equation.

The Raman spectra were recorded with a LabRam confocal microscope (Horiba Jobin Yvon HR800, spectral resolution 2 cm^{-1}) using excitation at 325 nm (~0.5 mW) and a 40× objective. No degradation of the samples was observed during the integration time of ~ 900 s.

Vertex-70 FTIR spectrometer was utilized to acquire the infrared absorption spectra of NDs in transmission mode. In all cases, the spectra represent an average of 100 scans recorded with a resolution of 4 cm^{-1}.

The details of in vitro and other physiological tests are given in corresponding parts of the Results and discussion section below.

3. Results and discussion

A representative TEM image and XRD spectrum of detonation NDs are shown in Fig.1. No crystalline or turbostratic graphitic carbon features could be determined in XRD measurements. The mean primary particle size determined from the XRD data was about 6 nm. The TEM image also shows that the primary diamond particles are partly agglomerated in this example.

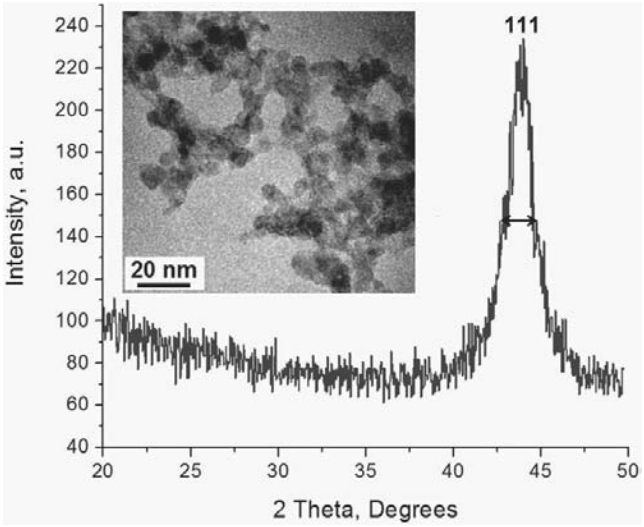

Fig. 1. Typical TEM image and X-Ray diffraction spectrum of detonation nanodiamonds

Typical Raman spectrum recorded for detonation NDs with a 355 nm laser excitation wavelenght is shown in Figure 2, where Raman peak at 1329 cm^{-1} corresponds to diamond (sp^3-bonded

carbon), and a group of Raman peaks around 1600 cm^{-1} come from amorphous sp^2-bonded (graphitic) carbon (~1550 cm^{-1}), –OH (~1600 cm^{-1}), and C=O (~1650 cm^{-1}) groups [7].

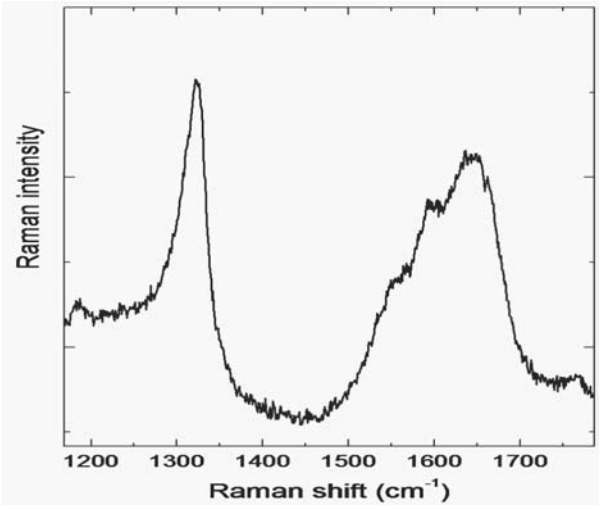

Fig. 2. Raman scattering spectrum of nanodiamonds

Depending on the origin, the nanodiamond surface is often rich in various functional groups which can result in diverse behaviors in biological environments ranging from bioinertness to changes in cell function and cytotoxicity. Figure 3 shows FTIR spectra of detonation NDs from different vendors as well as the spectrum of commercial 100-nm NDs prepared by HTHP technique (Fig.3b). The characteristic features of all samples is C-O-O-H band around 1640 cm^{-1}, broad absorption band between 3700 cm^{-1} and 3000 cm^{-1} due to OH and NH groups, and 1700 cm^{-1} band due to C=O. These feature are even present in HTHP NDs sample, although the surface is dominated by anhydride groups with the absorption peak around 1800 cm^{-1}.

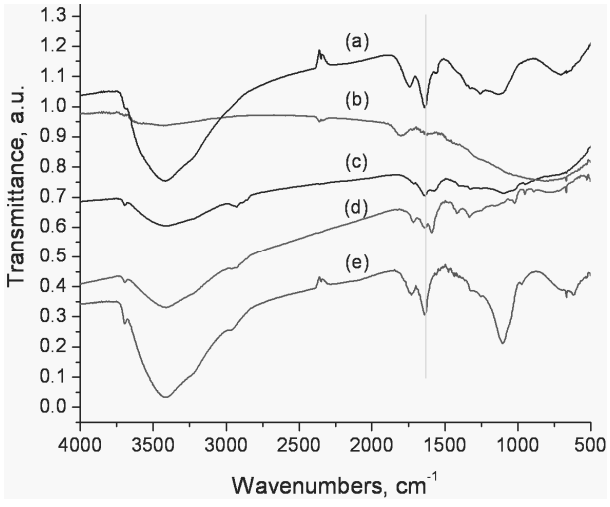

Fig. 3. FTIR spectra of nanodiamonds of different origin (a-e)

3.1. Nanodiamonds and drug loading

Depending on the dominating surface groups presnt on the surface and agglomeration state of the original nanodiamond powders, different amounts of anti-cancer drugs [8], such as doxorubicin (DXR) and paclitaxel (PTX) can be absorbed on these nanoparticles.

Figure 4 shows several examples of FTIR spectra of drug-loaded approximately 100-nm size NDs agglomerates. The infrared spectra clearly demonstrate the presence of the characteristic features of corresponding drug molecules even after several (from 3 to 10) cycles of centrifugation and cleaning of the product.

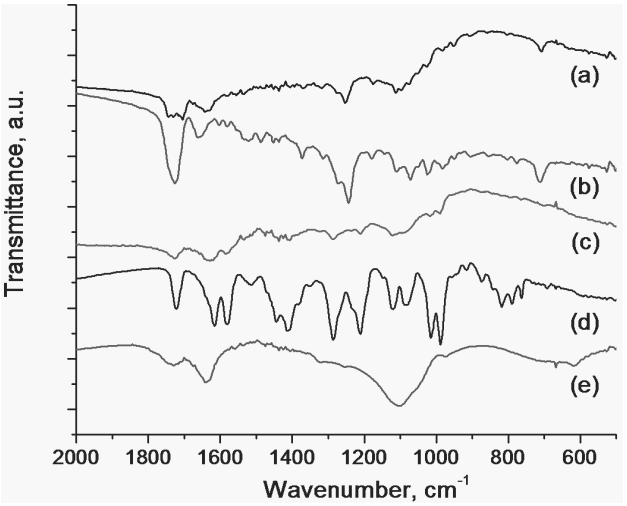

Fig. 4. FTIR spectra of detonation NDs loaded with anti-cancer drugs (a) ND-PTX, (b) PTX, (c) ND-DXR, (d) DXR, (e) Pristine NDs

In another experiment we have also additionally modified both detonation and HPHT diamond nanoparticles with amine groups that allowed to absorbing significant amounts of both types of tested drugs.

Figure 5 shows the infrared spectra of starting nanodiamond (ND) with predominantly C=O surface functional groups, amine-modified particles (ND-NH), pure paclitaxel (PTX), and paclitaxel-loaded amine-modified diamond nanoparticles (ND-NH-PTX). The FTIR analysis of the product after each step in multiple centrifugation-washing cycle confirmed strong binding of paclitaxel to surface-modified ND with little paclitaxel remaining in supernatant. The ND-NH-PTX spectrum was recorded after the material was centrifuged and washed for five times.

Semiquantative analysis of the ND-NH-PTX spectrum yielded the amount of paclitaxel about 20-30% by mass of that of nanodiamond. The changes in the position and shape of Amide-I band (around 1654 cm^{-1}), C=O and C-O bands in 1690 cm^{-1} – 1800 cm^{-1} range in ND-NH-PTX spectrum, indicate the strong interaction between the surface functional groups of modified ND and paclitaxel molecules. The future experiements will be required to evaluate the drug retention and ability of NDs materials to release drugs.

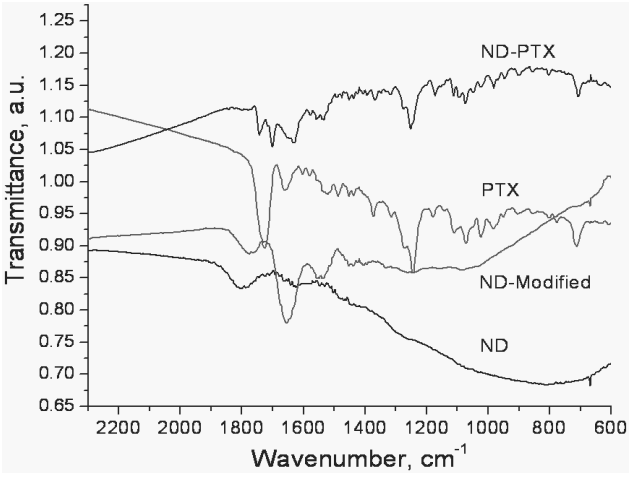

Fig. 5. FTIR spectra of HTHP synthetic nanodiamond: (a) ND-PTX, (b) PTX, and (c) Amine-modified ND, and (d) original ND powder

3.2. In vitro studies

Several *in vitro* experiments with different cell lines generally showed no or very little toxicity of all tested NDs [9,10]. However, in some cases, nanodiamonds prohibited the proliferation of human epithelial carcinoma cell line A431 as shown in Figure 6.

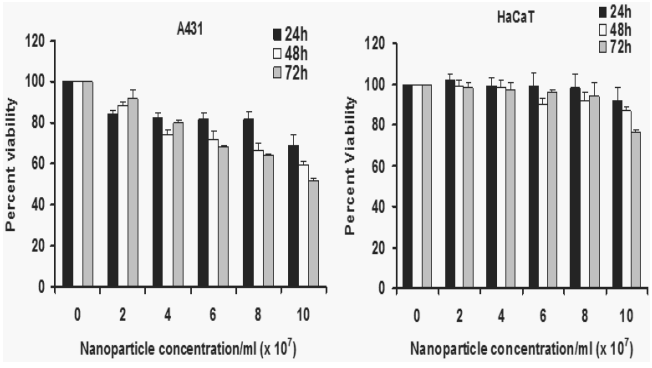

Fig. 6. Viability studies of A431 and HaCaT cells in the presence of detonation NDs with an average agglomerate size of 100 nm

In these tests, the human epithelial carcinoma cell line A431 normal human keratinocyte cell line HaCaT were cultured in DMEM medium supplemented with 10% FBS and 1% penicillin/streptomycin in a humidified atmosphere with 5% CO_2 incubator at 37 °C. For the cell viability study, the cells were seeded at a density of 5×10^4 cells/ml in 24 well plates and then incubated under increasing amounts of nanodiamonds for various time periods. 250 µl of MTT (5 mg/ml) was added after each time interval to each well. After 2 h of additional incubation, 500 µl of DMSO was added to dissolve the crystals. The absorption values at 540 nm were determined with an ELISA plate reader. Nanodiamonds inhibited the proliferation of A431 cell line in a dose and time dependent manner. Further the cytotoxic effects were higher in cancer cell line as compared to normal human keratinocyte cell line.

The results of microscopic examination of A431 cells after treatment with nanodiamonds are shown in Figure 7. For these observations, A431 cells have been treated with ND particles ($10X10^7$ /ml) for 24 h. Treated and untreated cells were viewed under the microscope (40X magnification).

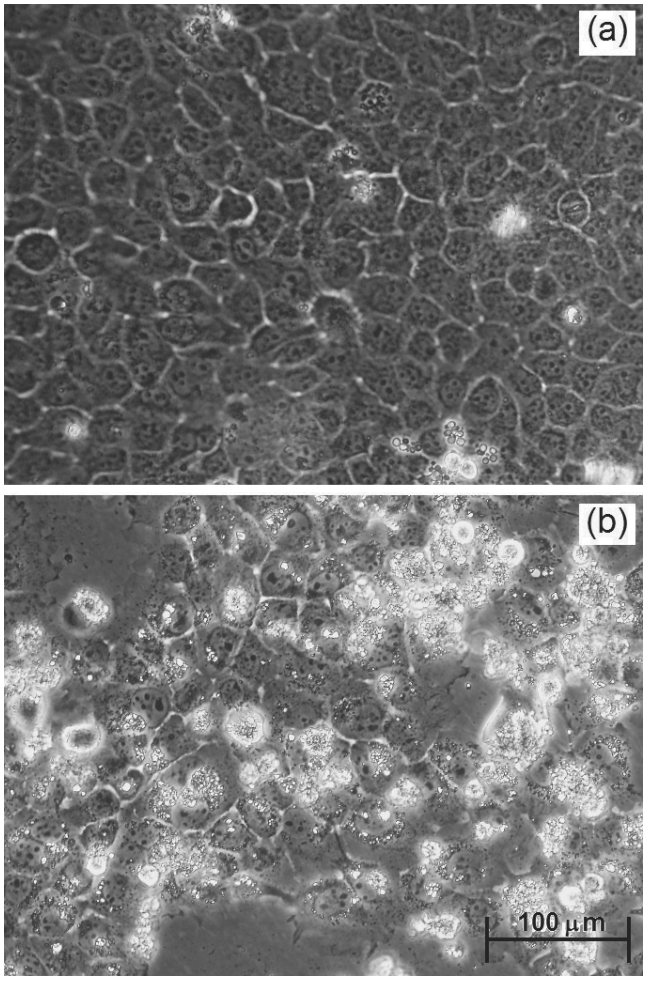

Fig. 7. (a) Untreated A431 cells and (b) Nanodiamond treated cells as viewed under optical microscope (40x magnification). The nanodiamonds in this experiment were obtained from SINTA

These preliminary screening data indicated that some nanodiamond particles can be capable of inducing cytotoxic effects on human epidermoid carcinoma cells A431 without incurring a significant cytotoxic effect on HaCaT cell line. However, it has been observed that depending on origin of

nanocrystalline diamond powders or their aqueous dispersions, the results shown above can vary significantly. Generally, the effect of nanodiamonds on A431 cells was more pronounced than on HaCaT cells.

Figure 8 shows an example of viability tests of A431 and HaCaT cells loaded with nanodiamonds that have been obtained from different vendors (SINTA, Institute of General Physics – RAS, NanoAmor).

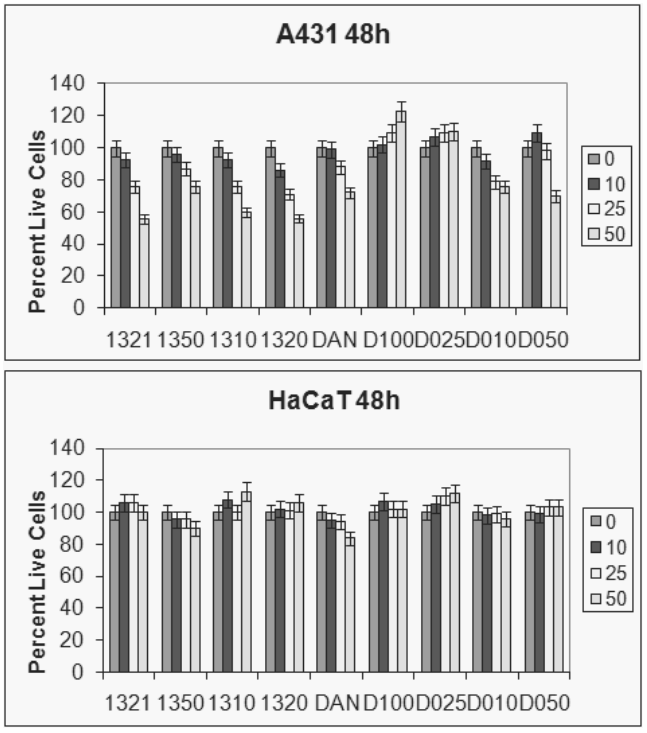

Fig. 8. Viability studies (48 hours) of A431 and HaCaT cells under the same conditions in the presence of detonation (1321, 1350, 1310, 1320, DAN) and HPHT synthetic nanodiamonds. The numbers on the right indicate arbitrary concentrations

These results indicate that there may be the situations in which nanodiamonds can exhibit certain cytotoxic effects, and this needs further careful verification with well-characterized nanodiamonds (e.g., precise determination of the surface functional groups, metal impurity level or particle electric charge) as well as with other cellular systems, and it is the subject of ongoing study.

3.3. Nanodiamonds in an animal study

It has been also found that both detonation and HTHP nanodiamonds do not cause allergic contact hypersensitivity response on skin. In these experiments the mice were treated with nanodiamonds to assess their allergic effect on the skin. The results of these tests are presented in Figure 9.

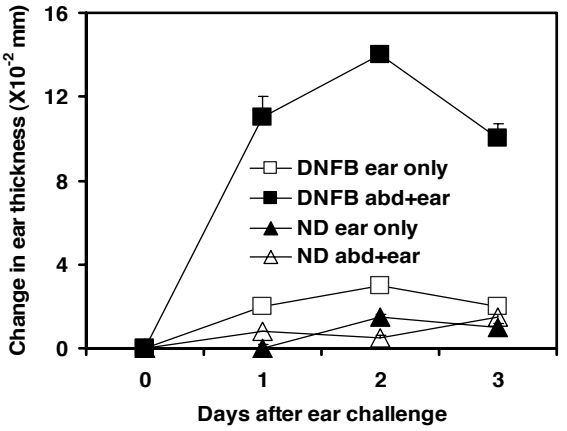

Fig. 9. Effect of Nanodiamonds (NDs) on allergic contact hypersensitivity response in skin

Specifically, mice were topically treated with detonation nanodiamonds (in cream) on their abdomen and 5 days later they were treated with nanodiamonds on the ear. The positive control mice were sensitized with 25µL of 0.5% DNFB (4:1 acetone : olive oil) on the abdomen. Five days after sensitization, mice were challenged with 20µL of 0.2% DNFB on their ears. The change in ear swelling was measured with an engineer's micrometer. There was significantly less (p<0.05) immunosuppression in mice treated with nanodiamonds as compared to the positive control mice. This indicates that nanodiamonds do not cause allergic contact hypersensitivity response on skin. Results are expressed as change in auricular thickness ± SEM. In each group, n=3. These results advance further the observations reported by Mitura [11] on the absence of allergic reactions to nanodiamonds.

4. Conclusions

It has been demonstrated that depending on the origin, the nanodiamond (ND) surface is rich in various functional groups which can result in diverse behavior in biological environments ranging from bioinertness to changes in cell function and cytotoxicity.

Generally, there was little toxicity of nanodiamonds observed during *in vitro* studies with different cell lines. However, some toxicity has been observed in several experiments, in particular, with A431 cell line. Also, nanodiamonds do not cause allergic contact hypersensitivity response on skin.

We have also succeeded in selective surface modification of nanodiamonds with various surface groups (amine, etc.), and loading nanodiamonds and their agglomerates with small-molecule anti-cancer drugs (about 20-30% by mass of that of nanodiamond). Analysis of the changes in the position of the characteristic bands in FTIR spectra of drug-loaded nanodiamonds indicate the strong interaction between the surface functional groups of modified nanodiamonds and drug molecules.

Future studies will be directed at the optimization of the surface modification processes of nanodiamonds for specific application needs.

Acknowledgements

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Additional information

The results of this investigation were presented at the International Conference on Vacuum and Plasma Surface Engineering 2009, held jointly with the International Workshop on Science and Application of Nanoscale Diamond Materials, in Hejnice, Czech Republic.

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