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Effect of non-modified and modified nanodiamond particles by Fenton reaction on human endothelial cells

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Properties

ABSTRACT

Purpose: The use of carbon nanoparticles in medicine is increasing due to their high biocompatibility. Nanopowders are one of the best materials which can be used in medicine on medical implants and surgical tools. DPP (Diamond Powder Particles) obtained by different methods which can be expected to affect their properties, including biocompatibility, were compared. The aim of the present study was to compare the biocompatibility of Diamond Powder Particles (DPP) obtained by detonation method and graphite on the basis of their interactions with human endothelial cells.

Design/methodology/approach: The effect of nanodiamonds on cell proliferation HUVEC-ST and production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) was studied. We used FT-IR Spectroscopy attributive chemical function groups.

Findings: In this subject the material characterization of nanodiamond powders and influence on endothelial cells.

Practical implications: Biological research with endothelial cells and nanodiamond powder are the introduction to application in human's treatment.

Originality/value: Nanodiamond powders with chemical modified surface.

Keywords: Diamond nanoparticles; Reactive oxygen species; Reactive nitrogen species; Biocompatibility

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1. Introduction

Nanocrystalline Diamond Coating (NCD) was found to have positive effects on cells a in human organism with little adverse reactions [1,2].

Antyinflammatory action of NCD has been reported [S. Mitura, et all, 2006]. Additionally, carbon coatings of implants and surgery materials have the following requirements: biostability, adhesion and good mechanical characteristic, haemocompatibility and histocompatibility [3,4,5]. It was shown that ultradisperse detonation diamond (UDD) is a very promising material in medicine and is nontoxic for the organism. In this study, we used UDD nanoparticles manufactured by the detonation method (2-5 nm single particle size) and compared them with graphite. We also modified this powders by the Fenton reaction and we observed how this modification affects the nanoparticle toxicity to the cells.

The aim of this study was to compare the biocompatibility of nanodiamond particles with respect to an immortalized endothelial cell line (HUVEC-ST) and check the effect of DPP on prroduction of reactive oxygen species (ROS) and reactive nitrogen species (RNS).

2. Experimental

2.1. Materials and methods

Nanodiamond powders

The following powders were used: UDD (manufactured by THE detonation method) and modified UDD. For comparison, graphite powder (GRAF) was also employed, obtained by pulping graphite rod and graphite modified by Fenton reaction.

Modification UDD with use Fenton reaction [6].

a/ UDD (0.25 g; detonation nanodiamond) were added to 150 mL flask with strong magnetic stirring (Teflon cover magnetic bar). Solution sulfuric iron (II) 12 g in 50 mL distilled water (degassed with argon at room temperature) was added to reaction flask. Cold solution sulfuric acid (30 mL; 96%) was slowly added to reaction with UDD (by drops). After 15 min the mixture was cooled to +5°C and hydrogen peroxide (25 mL; 30% solution in water) was slowly added (about 20 min) to reaction mixture with dispersion of UDD (fast stirring). After 30 min the mixture was heating to room temperature (22°C) and keep for 90 min at temp. 30°C (sonication 4 times 5 min).

To cold water (5°C; 150 mL) was added the reaction mixture and start to fast filtration (with use Ace filter) on vacuum line (with water pomp). Wet modified HO-ND was washed with distilled water 10 times (100 mL). Wet grey-black solid was suspended in distilled water (60 mL) and added 0.6 g EDTA; after fast stirring (total 30 min.) and sonication can be repeated (2 times 5 min.) and start with fast filtration and washing with distilled water (10 times; 100 mL).

Product HO-ND was dried on exicator over concentrate sulfuric acid (20 h) and on vacuum line (0.1 mm Hg; 40°C; 10 h) - obtained 0.19 g HO-UDD.

Cell culture

In our research we used human umbilical cord endothelial cells (HUVEC-ST). The cells kindly donated by Dr. C. Kieda, were immortalized by transfection with both SV40 large/small T antigens and the catalytic subunit of human telomerase were. HUVEC-ST were cultured in Opti-MEM (Ivitrogen, Gibco) medium containing 2% fetal bovine serum and antibiotics: 10 U/ml penicillin and 50 μg/ml streptomycin, under 5% CO_2 in plastic flasks.

Cell survival assay based on MTT reduction

The effect of the carbon nanopowders on the proliferation of HUVEC-ST was estimated by the ability of the cells to reduce (MTT)3-(4,5-dimethylthiazolyl)-3-3(4-sulphophenyl) tetrazolium. Cells were seeded on 96-well plates (3000 cells per well) and cultured for 12-24 h. After that time, diamond powders were added at appropriate concentrations (2-100 μg/ml) and the incubation was continued for another 72 h. Afterwards, the cell monolayers were rinsed with HBSS buffer containing 1% albumin and added with fresh medium and 20 μl of MTT solution (5 mg/ml). After 2 hour, the medium was removed and formazan crystals formed were dissolved in DMSO. Absorbance of the formazan was read at 570 nm.

Determination of reactive oxygen/nitrogen (ROS/RNS) species production

ROS and RNS production was estimated in HUVEC-ST cells seeded on 96-well plates (3000 cells per well) and cultured for 24 h. After that time, diamond powders were added at appropriate concentrations. The incubation was continued for another 24 h, 48 h and 72 h at 37°C under 5% CO_2. After that time the cell monolayers were rinsed with HBSS containing 1% albumin and added with fluorogenic probes: 2',7'-dichlorodihydrofluorescein diacetate (H_2DCF-DA) or 3-amino-4-aminomethyl 2'7'difluorescein diacetate (DAF-FM-DA), both 5 μM. Immediately and after 0.5 h, 1 h and 2 h incubation in the dark at 37°C under 5% CO_2 the fluorescence was read at λ_{ex} = 485 nm and λ_{em} = 538 nm for H_2DCF-DA and λ_{ex} =485 nm and λ_{em} = 510 nm for the product of DAF-FM oxidation.

DNA content

For determination of the cytotoxicity of nanoparticles the analysis of DNA content with the fluorescent probe Hoechst 33258 was employed [7,8]. Cells seeded on 96-well black plates at the density of 3000 per well were grown at 37°C for 24 h, treated with nanopowders at appropriate concentrations (2-100 μg/ml) and incubated for the next 24 h, 48 h and 72 h. Then the medium was removed by gentle aspiration. The cells was washed with HBSS and the plate was frozen at -70°C.

Thawing at room temperature was followed by adding of 100 µl of deionized water per well and second freezing at - 70°C. After subsequent thawing, 100 µl of 0,5 µM Hoechst 33258 in TNE buffer (2 M NaCl, 1 mM EDTA, 10 mM Tris-HCl, pH = 7.4) was added. The plate was shaken, incubated at room temperature in the dark for 15 min and fluorescence was read at 355/460 nm.

Statistical analysis

Statistical analysis results showing the influence nanopowders on the reactive oxygen/nitrogen species production and proliferation of HUVEC-ST was made using the ANOVA I and Tukay's post hoc test, with significance levels of $p < 0.005$ (***), $p < 0.02$ (**) and $p < 0.05$ (*).

3. Results and discussion

The graphite obtained by pulping graphite rod powder has the patchy structure and the size of grains less than 1 micron. The graphite powder was used as a "carbon control". Fig. 1 and Fig. 2 show that after 72 h incubations cells with nanoparticles UDD showed a slightly higher toxicity than graphite.

We observed also that nanopowders modified by the Fenton reaction had a stronger effect on the cells than non-modified powders. The analysis of ROS production by HUVEC-ST cells after the treatment with diamond and carbon powders particles after 24 h incubation show that graphite can induce reactive oxygen species production (Fig. 3). We noticed also a sigbificant difference between modified and non-modified particles. A similar situation was found after 48 h and after 72 h incubation but on the other hand we observed difference when checking the production of RNS by HUVEC-ST cells after treatment with diamond and carbon powders particles after 24 h incubation (Fig. 4.). Here UDD induced more reactive nitrogen species more than graphite. After 48 h and 72 incubation the level of RNS production decreased and was similar for both types of paricles.

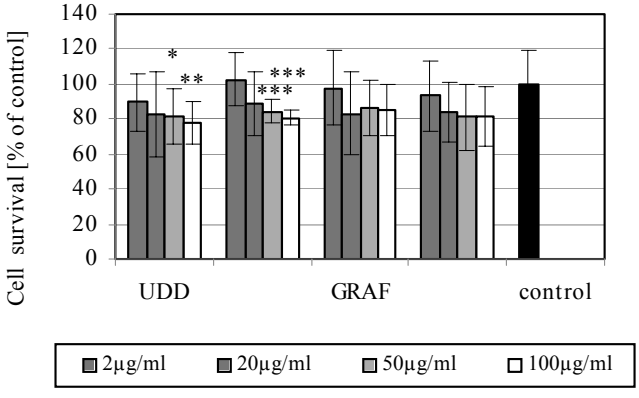

Fig. 1. Effect of nanodiamonds on the viability of human endothelial cells

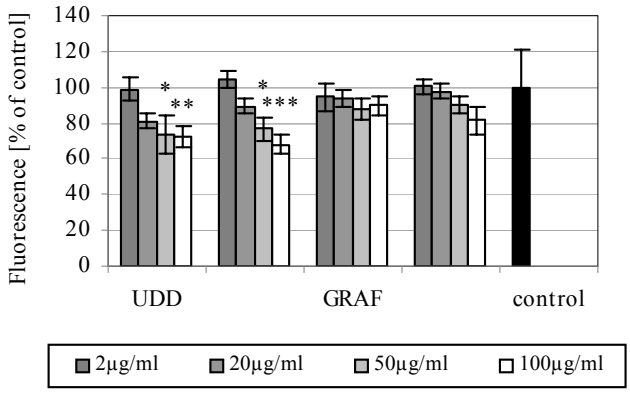

Fig. 2. Viability of HUVEC-ST cells incubation incubated (72 h) with diamond and GRAF particles estimated on the basis of DNA content

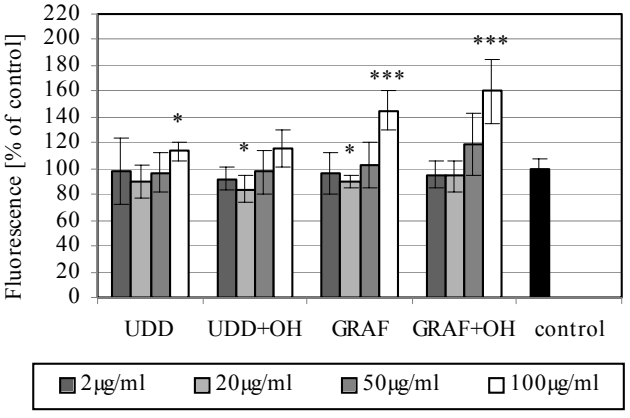

Fig. 3. Production of ROS by HUVEC-ST cells after treatment with diamond and carbon powders particles after 24 h incubation

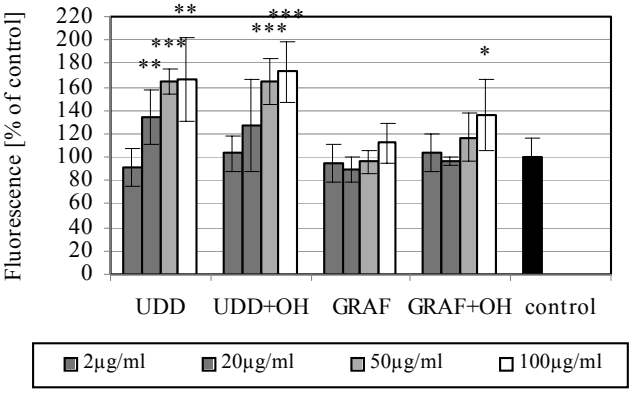

Fig. 4. Production of RNS by HUVEC-ST cells after treatment with diamond and carbon powders particles after 24 h incubation

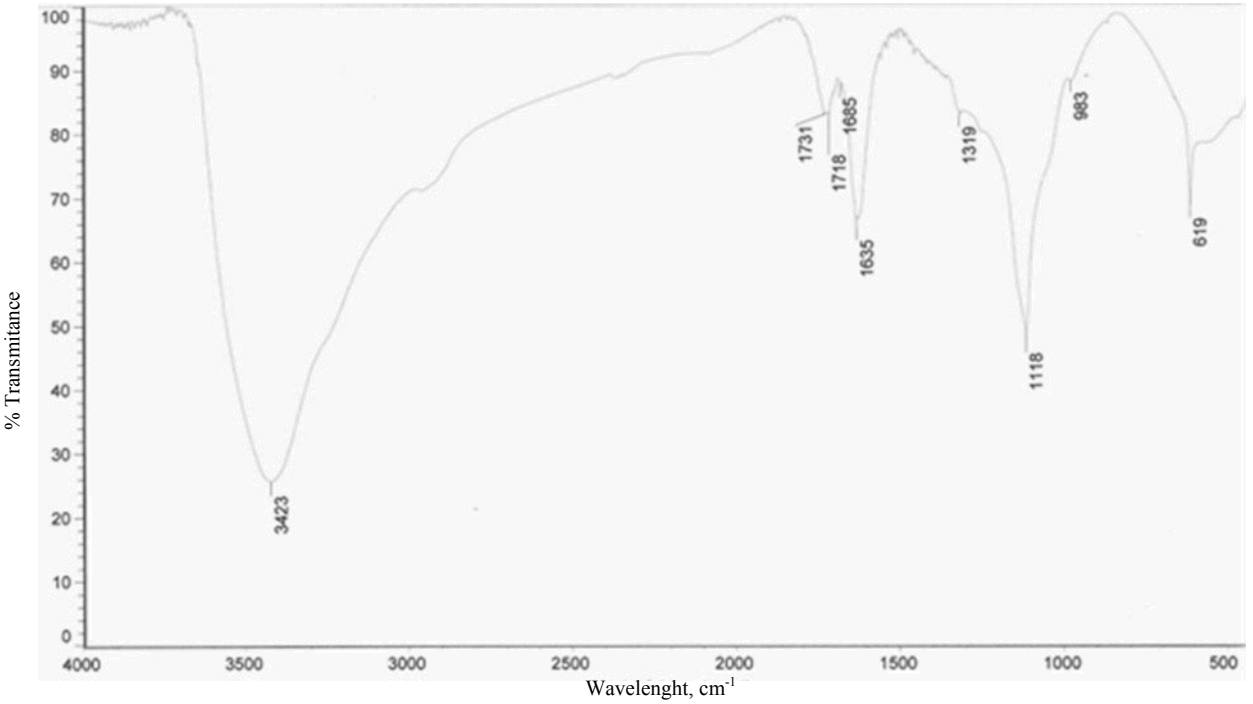

Fig. 5. FT-IR Spectroscopy of detonation nanodiamond (UDD) before Fenton treatment

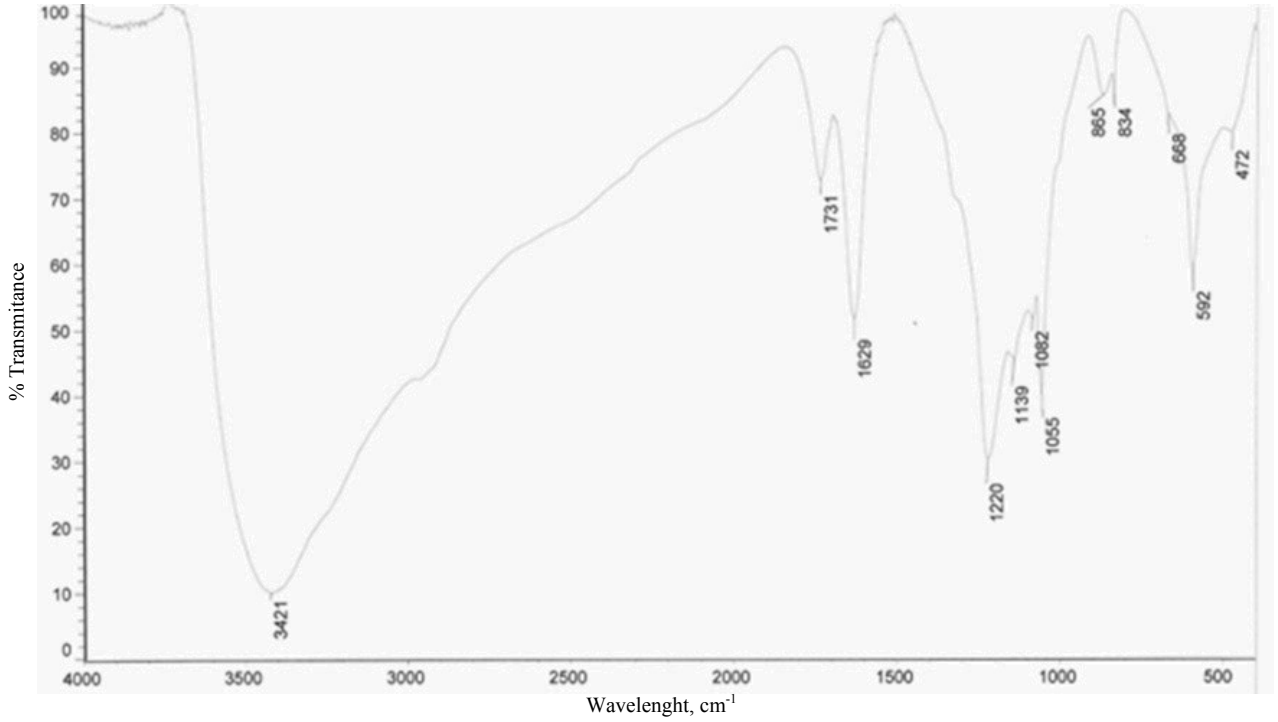

Fig .6. FT-IR Spectroscopy of detonation nanodiamond (UDD) after Fenton treatment

The types functional groups examined samples and changes in these groups introduced in the Fenton treatment were determined by FT-IR. Fig. 5 shows the IR spectra of UDD. As it can be seen in this Figure the sample has a peak at 3423 responsible for the presence of our interest hydroxyl (OH) groups.

Fig. 6 shows FTIR spectra this sample after Fenton treatment. The intensity of the band (broad) about 3421 cm^{-1} corresponding to hydroxyl groups grows and concomitant it was observed appearance of the carbon- oxygen (C-O) single bond at about 1124-1205 cm^{-1}. It is evidence of surface hydroxylation by Fenton.

4. Discussion

Nanodiamonds form a diffusion barrier between implant and human environment [9], and have also beneficial action and show anti-inflammatory and antioxidant properties [10]. Nanodiamond particle is very active molecule in contact with living organism. Two molecules can be weakly attracted to one another through intermolecular forces. These forces my include van der Waals interactions and hydrogen bonding [11].

Nanodiamonds are unique among the class of carbon nanoparticles because of their intrinsic hydrophilic surface, which in one of the many reasons that these nanocarbon particle are envisioned for biomolecular applications [12].

Induction of high levels of reactive oxygen and nitrogen species in cells after stress is a common mechanism in toxicology and ROS/RNS production and depends on kind of nanopowders. UDD obtained by the detonation method have smaller size than carbon nanopowders such as GRAF. Nanoparticles obtained by the detonation method induce higher ROS and RNS production than UDD after 24 h incubation. This is very important because we observe that the size and concentration of nanopowders have seem to have the main role in the cell - nanopowder contact.

5. Conclusions

Nanopowders obtained by different method can enhance the production of ROS and RNS in the endothelial cell line HUVEC-ST.

Comparison of the cytotoxic effects shows that that nanodiamond particles manufactured by different methods have different biological activity on the cells.

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