

## HR TEM examinations of nanodiamond particles for biomedical application

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### Materials

#### ABSTRACT

**Purpose:** The aim of the study is an analysis of a different type of nano-diamond powders on HR TEM (material characterisation, the normal distribution of grain size). These nano-powders were used in many biological researches and each of them had a special biological activity. Comparison between the material and biological properties of diamond powders answered many questions about the chemical and physical reactions on a boundary: diamond surface – living organism.

**Design/methodology/approach:** In this work we used cytotoxicity assays using MTT test and HR TEM examinations. We examined the nanodiamond particles manufactured by detonation method, RF/MW PACVD method, RF PACVD method, pure diamond from De Beers Company and graphite powder as a control.

**Findings:** In this subject the material characterisation of diamond powders are known but we examined a different type of powders and we manufactured some of these nanopowders in our laboratory.

**Practical implications:** Nanocrystalline Diamond Coatings have many applications in various fields of medicine. Nanodiamond Particles are an extended surface of diamond powder. Biological research with endothelial and lung cancer cells are the introduction to application in human's treatment.

**Originality/value:** There are in vitro examinations with bioactive biomaterial. Nanodiamond particles have a very high bioactivity on cell level and inhibit cancerogenesis.

**Keywords:** Nanomaterials; HR TEM; Nanodiamond particles; MTT test

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### 1. Introduction

Nano-Diamond Particles have been examined in different fields of medicine in vitro, in vivo and clinical examinations [1]. Biological activity of nano-diamond powders will be applied in dermatology, cosmetology and vessel surgery. Last results indicate that diamond powders have antiseptic, antibacterial properties and supporting the healing of wound. In dermatology examinations, nano-diamond particles protect hydro-lipid human skin coat before aggressive factors of external environment and aging. In ova examinations, last results indicate that presence of

nano-diamond powder does not change chicken embryogenesis. In vitro examinations including endothelial cells (HUVEC) culture which shows the biocompatibility of diamond and cells in their proliferation and antioxidant and non – cytotoxic properties (MTT –test) [2].

Our previous studies showed that Diamond Powder Particles have antioxidant and anti-inflammatory properties. This mechanism is based on a reaction between surface of diamond in nanoparticles (dependent, first of all, on a type and quantity of chemical properties of surface and sp<sup>3</sup> phases content) and tissue components [6].

Danilenko proposed and implemented (in 1962) ampoule-free synthesis with explosions in the explosion chamber instead of ampoule synthesis. Graphite was placed directly into a cylindrical chamber consisting of a trotyl-hexogen mixture TG40; the charge was enveloped in a water jacket to suppress graphitization and reduce the unloading rate of the synthesized diamond [6].

Under suitable conditions, explosively produced shock waves can create high-pressure (about 140 GPa), high – temperature conditions in confined volumes for a sufficient duration to achieve partial conversion of graphite into nanometre- sized diamond grains (about 20 nm) which are compacted into micron-sized, polycrystalline particles [7].

The powders consist of diamond nanocrystals aggregates, which can be shown on an electron diffraction pattern. Such crystallites built into a layer are nuclei of diamond crystallisation. The layers were deposited in a plasma-chemical RF PACVD reactor that is the most popular and very simple for thin films synthesis [8].

Carbon films were grown on Si and AISI 316L steel substrates using the MW/RF PACVD system. As a gas source, the mixture of CH<sub>4</sub> and Ar gases were used in different concentrations. The examinations of deposition showed that carbon coatings can be produced as low (several nm/h) and high (several μm/h) growth rate depending on gases mixture, its concentration and power of MW and RF generator. The depositions were conducted at the Technical University of Lodz in 3 kW, 2.45 GHz chamber evacuated a base pressure of several Pa. In this system, in order to initiate plasma, two frequencies were applied: microwave frequency and radio frequency. This device allows to apply both frequencies simultaneously, or to use MW (2.45 GHz) or RF (13.56 MHz) plasma separately. The deposition parameters were optimized in order to obtain homogeneous carbon coatings on such different substrates as: AISI 316L steel and silicon. Prior to deposition, substrates are pre-cleaned, first in an acetone bath and than in argon plasma, directly in an operated chamber [9].

Nanodiamond particles (PR1, PR2) were manufactured by MW/ RF PACVD method.

It was proven that Diamond Powder Particles (DPP) may function as antioxidant and/or anti-inflammatory factors when applied in living systems. The mechanism of such behaviour is not yet fully understood. It seems probable that reactions between the diamond surface and some biologically important molecules play a significant role in this process. Nanocrystalline diamonds can be dispersed in a biologically acceptable carrier to form various compositions. DPP can be included in cream having bioactive properties. Diamond Powder is a potential medicine [8, 11].

The endothelial cells are considered to arise from the splanchnopleuric mesoderm. Endothelial cells create the interior of a blood vessel and provide an anticoagulant barrier between the vessel wall and blood. These cells exert significant paracrine and endocrine actions through their influence on the underlying smooth muscle cells or on circulating blood elements, such as platelets and white blood cells. Endothelial cells can also be activated by various stimuli, such as thrombin or histamine, marked by a switch in their synthetic profile from basal conditions towards an activated state that is pro-thrombotic, pro-proliferative and vasoconstricting. Endothelial cells also play major roles in angiogenesis and vasculogenesis [10] Endothelium cells play a

crucial role in the physiology of the cardiovascular system. These cells form a thin layer on the inner surface of blood vessels are known that they participate in a variety of important physiological processes, secreting different mediators. Adhesion proteins presented on the surface of endothelial cells, allow the endothelium to participate actively in the inflammation and hemostasis [2].

Non-small cell lung cancer represents approximately 80% of all new lung cancer cases; however, only one-third of these cases will undergo surgical resection for tumour control. Lung cancer continues to be the leading cause of death from cancer in the United States (32% of cancer deaths in men and 25% in women) despite numerous "advances" toward the diagnosis and treatment of cancer over the last two decades. Lung cancer is the second most common type of cancer in both men (17%) and women (12%). In 1998, an estimated 172 000 new cases of lung cancer were expected and 160 400 patients were expected to die of this disease.

Lung cancer can be suspected on the basis of history, physical examination, or radiologic studies, especially in case of smokers with any change in respiratory symptoms. Diagnosis, however, requires histological confirmation, and therapeutic decisions require accurate staging. Most patients with lung cancer present symptoms related to their respiratory tract. Symptoms caused by local tumour growth depend on the initial tumour size, location, and involvement of adjacent structures. Local tumour growth occurs in the airway, while chest wall invasion, mediastinal invasion, metastatic disease, or a tumour related to paraneoplastic syndrome [12].

## 2. Experimental

### 2.1. Cytotoxicity assays using MTT test

In these examinations we used nanodiamond particles manufactured by MW/RF PACVD method (PR1, PR2), RF method, and detonation method and graphite powder. Sensitivity of the A549 and HUVEC-ST to compounds was performed in 96-well microtiter plates using colorimetric method MTT standard [3] based on ability of mitochondrial dehydrogenase of metabolically viable cells to reduce the tetrazolium salt (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) to blue formazan product. For this purpose, after cells counting, equal number of cells (2500 cells/well for 72 hr and 10000 cells/well for 24 hr) was seeded in each well in 0.1 ml of culture medium. After 24 hours incubation 0.1 ml compound solution and H<sub>2</sub>O<sub>2</sub> in the culture medium at various concentrations was added to cells and incubated for 24 and 72 hr. At this time, 20 μl MTT (5 mg/ml in water) was added to each well and incubated for next 2 hr. Subsequently, after aspiration of the culture medium, the resulting formazan crystals were dissolved with 100 μl dimethyl sulfoxide. Next, plates were mechanically agitated for 2 min and absorbance at 580 nm and 720 nm was measured using microplate reader [4].

Abs for cells (for each sample) = Abs 580nm – Abs 720 nm

Control = cells untreated compounds and H<sub>2</sub>O<sub>2</sub> (cells in the culture medium).

**Cell lines:** A<sub>549</sub> = non-small cell lung carcinoma (human), obtained from ATCC, adherent cells, culture medium= DMEM+Glutamax+10% FBS (fetal bovine serum);

**HUVEC-ST**, human umbilical cord endothelial cells immortalized by transfection with both SV40 large/small T antigens and the catalytic subunit of human telomerase.

In our work we used two types of immortalized cells but the main role was played by HUVEC-ST cells.

Non-small cell lung carcinoma we used as the comparison between two types of human immortalized cells.

## 2.2. HR TEM method

Electron microscopy is a useful method for observing localization of some proteins in a cell on nanometre-scale direct observation of a receptor for a bioactive substance of small molecular weight by using TEM (transmission electron microscopy). This concept can be widely applicable for the nanometre scale-direct observation of the receptor for any small molecules [5].

In our paper we used nanodiamond particles manufactured by MW/RF PACVD method (PR1, PR2), RF method, detonation method and graphite powder.

## 3. Results and discussion

We received very promising results in a subject of influence of different types of nanodiamond particles on human cells.

Figures 1-5 shows the pictures of nanodiamond particles HR TEM.

The best structure for biocompatibility of human cells examined in our paper has nanodiamond powder manufactured by detonation method (Fig. 4). We can observe the conglomerates of nanodiamond crystals and sizes of grains from 2-10 nanometres.

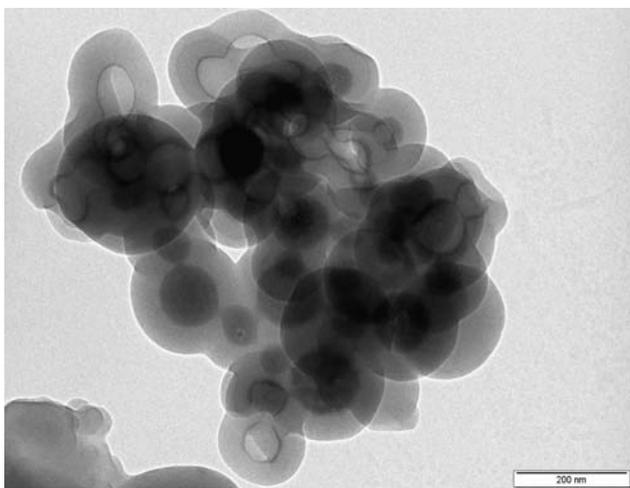


Fig. 1. HR TEM image of PR1 nanodiamond particles manufactured by MW/RF method

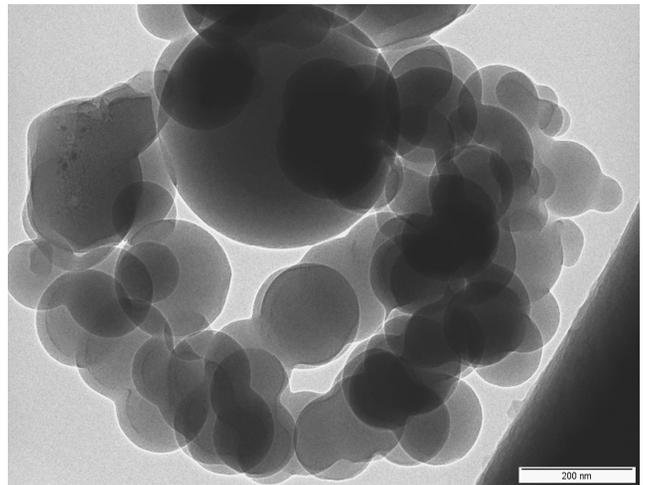


Fig. 2. HR TEM image of PR2 nanodiamond particles manufactured by MW/RF method

Nanodiamond particles manufactured by MW/RF method (PR1, PR2) have bigger grain size (about 150-200 nm) and have round shape. The interesting point is presence of amorphous phase in grains (Figs.1-2). Nanodiamond particles manufactured by RF PACVD method is the biggest among nano-diamonds. The grain size is about 700-800 nm and has round and patch shape. RF PACVD nanodiamond particles contain amorphous phase.

The graphite powder has the patchy structure and the size of grains is less than 1micron. The graphite powder is the “carbon control”.

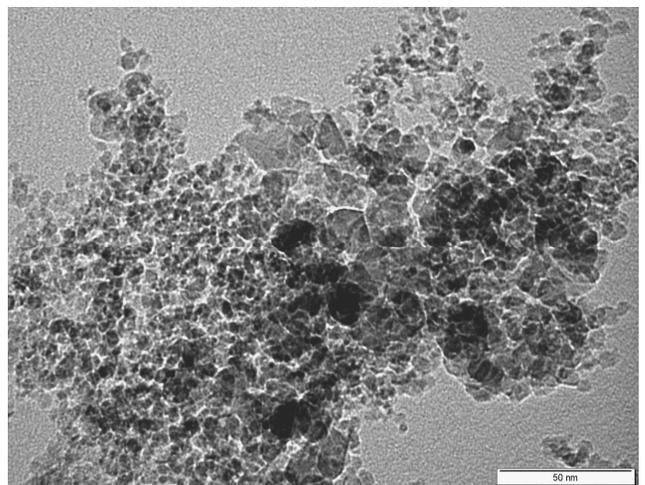


Fig. 3. HR TEM image of nanodiamond particles manufactured by detonation method

Nanodiamond powders show cytotoxic effect for immortalized cells (Figs. 7-10).

The minor effect of cytotoxicity shows the nanodiamond particles PR1 and PR2 manufactured using MW/RF method in contact with non-small cell lung carcinoma (Fig. 7).

Significant cytotoxic effect shows nanodiamond particles PR1 and PR2 manufactured using MW/RF method in contact with HUVEC-ST cells (Fig. 6).

The detonation nanodiamond extended surface and biological activity. The free bonding on the surface of nanodiamond particles reacts with surfaces of cells and changes the activity of it. It has direct influence on viability of these cells.

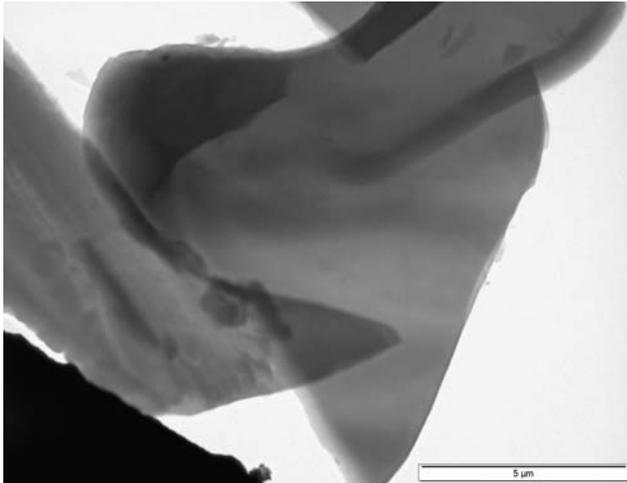


Fig. 4. HR TEM image of graphite powder

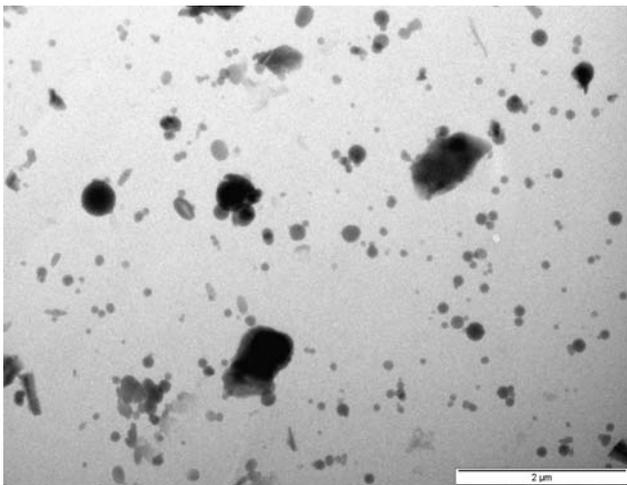


Fig. 5. HR TEM image of nanodiamond particles manufactured by RF PACVD method

Cytotoxicity of two lines cancer cells has very promising results.

In vitro study we proved that nanodiamond particles manufactured with different types of methods have biological activity.

The spectacular results show nanodiamond particles manufactured by MW/RF PACVD method (PR1, PR2) in contact

with HUVEC-ST cells (high level of cytotoxicity). It is the introduction to examinations of angiogenesis in tumours with participation of endothelial cells.

Lung cells examined in this paper are very good material to continue this subject in clinical research.

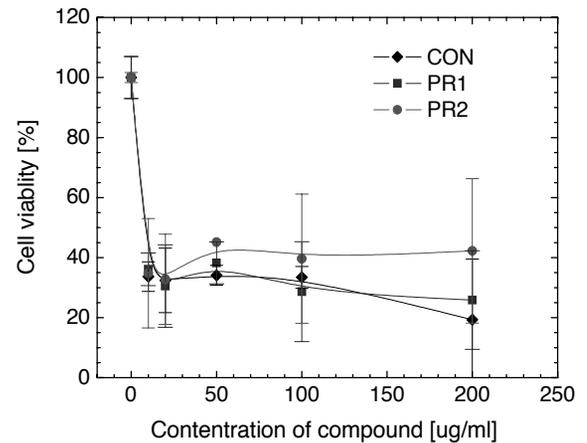


Fig. 6. Cytotoxic effect of PR1 and PR2 nanodiamond particles manufactured by MW/RF method in HUVEC-ST cell culture

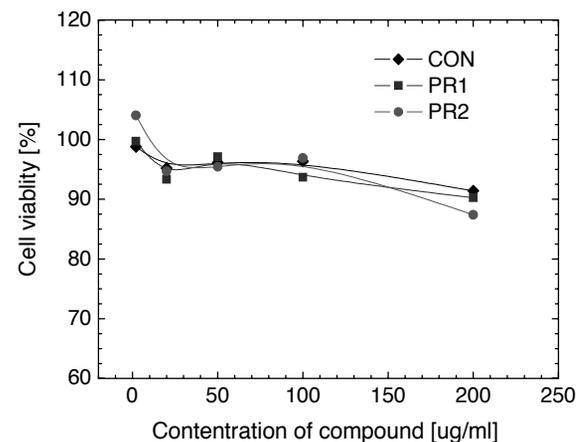


Fig. 7. Cytotoxic effect of PR1 and PR2 nanodiamond particles manufactured by MW/RF method in A<sub>549</sub> cell culture

Nanodiamond particles manufactured by detonation method are the best nanopowders for examinations in vitro.

In contact with human cells they extended surface and the smallest grain sizes. It is very important in aspect of mechanical damages of cell culture.

In this work detonation nanodiamond particles did not influence on greater activity than another types of nanopowders.

In vitro study with more markers of inflammatory and immunological processes they show the real biological activity of this nanopowder.

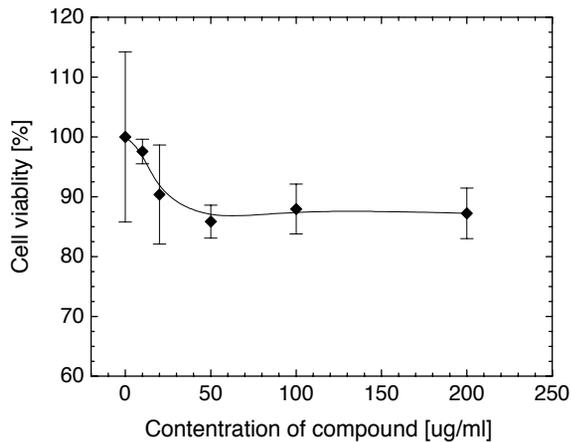


Fig. 8. Cytotoxic effect of nanodiamond particles manufactured by detonation method in  $A_{549}$  cell culture

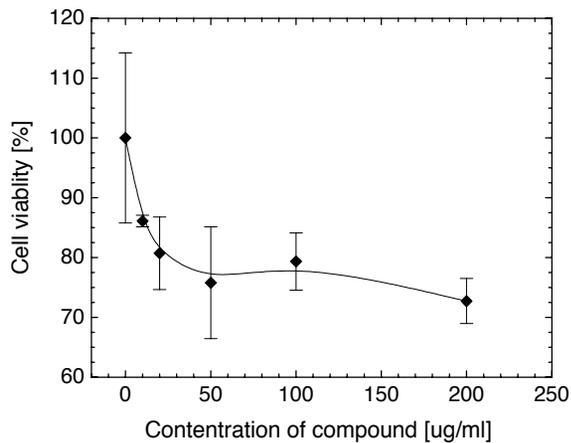


Fig. 9. Cytotoxic effect of graphite powder in in  $A_{549}$  cell culture

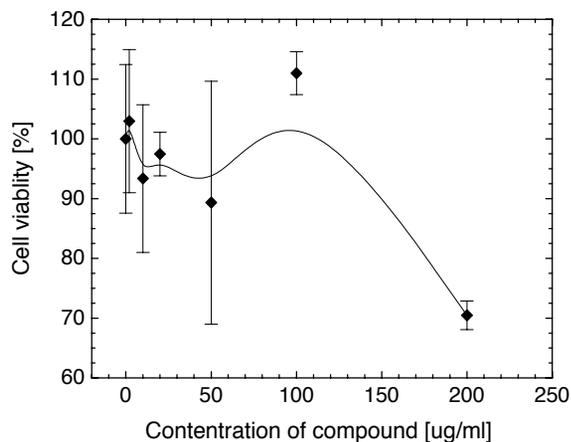


Fig. 10. Cytotoxic effect of nanodiamond particles manufactured by RF PACVD method in  $A_{549}$  cell culture

## 4. Conclusions

1. All diamond tested preparations show negligible cytotoxicity for  $A_{549}$  cells in tested concentration range (up to 200 ug/ml).
2. The cytotoxicity of diamond is cell-specific, endothelial cells HUVEC-ST being more susceptible to diamond than  $A_{549}$  cells.
3. Nanodiamond particles manufactured by different methods have biological activity in vitro study on human cells.

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