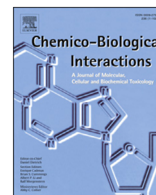
Chemico-Biological Interactions 254 (2016) 156–166

Contents lists available at ScienceDirect

Chemico-Biological Interactions

journal homepage: www.elsevier.com/locate/chembioint



Studies on the cytotoxicity of diamond nanoparticles against human cancer cells and lymphocytes

Kinga Adach [a], Mateusz Fijalkowski [a,*], Gabriela Gajek [b], Janusz Skolimowski [c], Renata Kontek [b], Alina Blaszczyk [b]

[a] Institute for Nanomaterials, Advanced Technologies and Innovation, Technical University of Liberec, Studentska 2, 461 17 Liberec, Czech Republic
[b] Laboratory of Cytogenetics, Faculty of Biology and Environmental Protection, University of Lodz, Banacha 12/16, 90 237 Lodz, Poland
[c] Department of Organic Chemistry, Faculty of Chemistry, University of Lodz, Tamka 12, 91 403 Lodz, Poland

ARTICLE INFO

Article history:
Received 18 November 2015
Received in revised form
4 May 2016
Accepted 3 June 2016
Available online 4 June 2016

Keywords:
Diamond nanoparticles
Surface functionalization
MTT assay
Biocompatibility

ABSTRACT

Detonation nanodiamonds (DND) are a widely studied group of carbon nanomaterials. They have the ability to adsorb a variety of biomolecules and drugs onto their surfaces, and additionally their surfaces may be subjected to chemical functionalization by covalent bonds. We present a procedure for the purification and surface oxidation of diamond nanoparticles, which were then tested by spectroscopic analysis such as ATR-FTIR, Raman spectroscopy, and thermogravimetric analysis. We also examined the zeta potential of the tested material. Analysis of the cytotoxic effect of nanodiamonds against normal lymphocytes derived from human peripheral blood, the non-small cell lung cancer cell line (A549) and the human colorectal adenocarcinoma cell line (HT29) was performed using MTT colorimetric assay. Evaluation of cell viability was performed after 1-h and 24-h treatment with the tested nanoparticles applied at concentrations ranging from 1 μg/ml to 100 μg/ml. We found that the survival of the examined cells was strongly associated with the presence of serum proteins in the growth medium. The incubation of cells with nanodiamonds in the presence of serum did not exert a significant effect on cell survival, while the cell treatment in a serum-free medium resulted in a decrease in cell survival compared to the negative control. The role of purification and functionalization of nanodiamonds on their cytotoxicity was also demonstrated.

© 2016 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Over the last ten years, the functionalization of carbon materials has become an active field of research at the intersection between organic chemistry, biology and material science. Among the most interesting nanoparticles, nanodiamond powders are the most widely studied because they have important biological properties [1,2]. These particles show great potential due to their shape and small size (from 4 to 10 nm in diameter), large surface area and the content ratio of the carbon sp^2/sp^3 hybridized bonds [3–6]. The surface of nanodiamond grains can be a unique platform for conjugating with biomolecules via covalent and non-covalent binding [7,8]. Surface modifications of nanodiamonds with DNA molecules [7,9], lysosomes [8], cytochrome C [10], a growth hormone [10,11], and biotin and insulin [8,12] have been reported. Most of these molecules can be adsorbed on the nanodiamond surface by non-covalent binding, simultaneously maintaining their biological activity. Bindings of nanodiamonds to antibiotics have also been described [13–15]. Ho et al. [15] reported on the functionalization of nanodiamonds with doxorubicin hydrochloride (DOX). The resulting complex was used as a highly effective chemotherapeutic drug carrier to human colorectal cancer cells [15].

Materials targeted for bio-medical applications are placed under stringent requirements. Biocompatibility is of the utmost importance. This term specifies the ability of the biomaterial to perform an appropriate function in relation to medical applications without causing local or systemic adverse effects in the recipient, but instead generating the most appropriate cellular or tissue response. Such benefits can include compatibility with the blood tissue, compatibility in the tissue system, lack of toxicity and no effects on the immune system of a living organism [16]. Several studies have shown that detonation nanodiamonds do not have any cytotoxic

* Corresponding author.
E-mail address: mateusz.fijalkowski@tul.cz (M. Fijalkowski).

http://dx.doi.org/10.1016/j.cbi.2016.06.004
0009-2797/© 2016 Elsevier Ireland Ltd. All rights reserved.

effect on various types of cells and that biocompatible nanoparticles are useful in many biomedical applications [17–20].

Nanodiamonds may be synthesized with the use of numerous methods, but the detonation technique is one of the most cost-effective methods. The most commonly used system is an explosive mixture of TNT/RDX at a ratio of 60/40 or 70/30 [3]. This mixture has a negative oxygen balance i.e. the oxygen content is lower than the stoichiometric value, so the oxygen completely binds with the hydrogen producing water, and during the process elemental carbon is formed [21]. High pressure in the chamber (20–40 GPa) favours the formation of sp^3 diamond particles; however if the pressure drops and the temperature is still high (approx. 3000 K) an additional layer of graphite is created on the surface of the diamond particles. Due to the high reactivity of their surface, nanodiamonds are readily contaminated, particularly during synthesis. Diamond nanoparticles produced by detonation are composed of an sp^3 core, sp^2 carbon phase, various functional groups on the surface and also incombustible impurities (metals and oxides) that should be purified for most applications [3,5,22,23]. Different methods are used to purify and then modify the surface of the detonation nanodiamonds. Selection of the type of modification is dictated by assumptions concerning the final result i.e. the need to obtain a product with a specific destination. For example, functionalization of the nanodiamond's surface with hydroxyl groups can be obtained by applying Fenton's reaction, which also leads to its purification from impurities present in the sample in the form of non-diamond carbon varieties. This is evident through the secretion of carbon dioxide (CO_2) during the reaction. The nanodiamonds modified using Fenton's reaction can be combined with fluorescent dyes by covalent functionalization, and then these modified nanoparticles can be tracked after penetration into cells or even cell nuclei. Thanks to the ability of nanodiamonds to penetrate the cell membrane they may be used as biological carriers for bioactive molecules [22].

The purification procedures and modification of nanodiamonds with different functional groups can influence the properties of the nanodiamonds. Keremidarska et al. [23] studied the cytotoxicity of four different types of detonation-generated nanodiamond particles, which were purified with the use of different oxidizers. The authors used two types of cells, rat mesenchymal stem cells (rMSCs) and a human osteosarcoma cell line (MG-63) to find which cells were more sensitive to the studied nanoparticles. It was found that they had different sensitivity towards nanodiamond particles – the cytotoxic effect was more evident in rMSCs [23]. Moreover, the results demonstrated the influence of the purification method on cytotoxicity of nanodiamonds. The nanodiamonds containing non-diamond carbon were most toxic, while those with no impurities showed the lowest cytotoxicity [23].

The above-mentioned results showed the importance of selecting an appropriate cell model during cytotoxicity studies of nanodiamonds that can be used in the future as carriers of drugs. The selection of cells to be used in research should take into account the potential target organs and the introduction route. In this study we used three types of cells: human non-small cell lung cancer cells (A549), colon adenocarcinoma cells (HT29) and lymphocytes to compare their sensitivity to nanodiamonds particles. The nanodiamond powder used in this work was firstly purified using ethanol or 2-ethoxyethanol in order to remove organic contaminants that were adsorbed or formed during the production process. Moreover, a reaction leading to the formation of hydroxyl groups on the surface of the nanodiamonds purified with ethanol was performed. The aim of the study was to compare the sensitivity of three types of cells to nanodiamond particles and also to find out whether their purification and modification with hydroxyl groups differentiated their effects on living cells. The toxicity of the nanodiamonds was also assessed to demonstrate the possible protective effect of serum proteins present in the culture medium. The cytotoxic activity of the nanodiamond particles was analysed with the use of MTT assay, which enabled the assessment of cell survival after cell treatment.

2. Materials and methods

2.1. Chemicals

Detonation nanodiamond powder (95%) with primary sizes in the range of 4–5 nm were purchased from SkySpring Nanomaterials, Inc. (Houston, USA). The nanodiamonds were purified and modified by hydroxylation. Stock solutions of nanodiamonds were prepared at the concentration of 4 mg ml^{-1} in phosphate buffered saline (PBS) and dispersed well in the medium by sonication for 15 min using a Sonics Vibra-Cell Disruptor equipped with a Converter micro tip at 80% power. The nanodiamonds were added to the tested cells at final concentrations of 1, 5, 10, 50 and 100 μg/ml.

MTT [3(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide], lauryl sulphate (SLS), *N,N*-dimethylformamide (DMF), dimethyl sulfoxide (DMSO), penicillin-streptomycin solution stabilized, MEM non-essential amino acids solution, Histopaque 1077, and buffered saline (PBS) were purchased from Sigma Chemical Co. Foetal bovine serum (FBS), phytohemagglutinin (PHA), RPMI 1640 medium with Glutamax, Dulbecco's modified Eagle's medium (DMEM), and Trypsin-EDTA were supplied by GIBCO BRL (Gaithersberg, Md). All other chemicals (chemical reagents, substrates, and solvents) were of the highest commercial grade available and were purchased from Sigma-Aldrich, Acros Organics, and Fluka. Deionized filtered water was used in all of the studies.

2.2. Functionalization of diamond nanoparticles

The purchased nanodiamond powder (ND-1) was purified and modified as described below. The first step of the chemical treatment was to purify the nanodiamond surface from the adsorbed contaminants using two solvents, ethanol and 2-ethoxyethanol. Continuous extraction was performed on a Soxhlet apparatus. Ethanol was selected for the study because it is a hydrophilic solvent, so in addition to the fact that the nanodiamond surface was purified from contaminants, water was also removed. The extraction was carried out for 4 h, after which the powder was washed with acetone for another 1 h in order to remove the residual ethanol. The sample extracted using ethanol was labelled ND-2. The next solvent, 2-ethoxyethanol was selected for the test because of its chemical properties i.e. it belongs to a group of alkoxyalcohols which contain in their structure both a hydroxyl and an ether group. In addition, it is an organic solvent with a high boiling point (b.p. 132 °C). The sample extracted with 2-ethoxyethanol was labelled ND-3. The extraction was carried out for 4 h, after which the powder was washed with acetone for another 1 h in order to remove residual 2-ethoxyethanol. After Soxhlet extraction the powders were dried in a laboratory heating oven overnight, and subsequently in a vacuum line for 8 h. The nanodiamonds purified with ethanol (ND-2) were then subjected to chemical modification with the aim of achieving surface oxidation. The reaction was carried out according to the procedure describe by us elsewhere [24]. The resulting sample was labelled ND-4.

2.3. Physico-chemical characterization of the diamond nanoparticles

Scanning electron microscope (SEM) observation was performed using an FE SEM ZEISS ULTRA PLUS. The samples were

Download English Version:

https://daneshyari.com/en/article/2579829

Download Persian Version:

https://daneshyari.com/article/2579829

Daneshyari.com