Studying the influence of nanodiamonds over the elasticity of polymer/nanodiamond composites for biomedical application

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Abstract. The combined unique properties offered by organic and inorganic constituents within a single material on a nanoscale level make nanocomposites attractive for the next generation of biocompatible materials. The composite materials of the detonation nanodiamond/polymer type possess spatial organization of components with new structural features and physical properties, as well as complex functions due to the strong synergistic effects between the nanoparticles and the polymer [1]. The plasma polymerization (PP) method was chosen to obtain composites of silicon-based polymers, in which detonation generated nanodiamond (DND) particles were incorporated. The composite layers are homogeneous, chemically resistant, thermally and mechanically stable, thus allowing a large amount of biological components to be loaded onto their surface and to be used in tissue engineering, regenerative medicine, implants, stents, biosensors and other medical and biological devices. Mesenchymal stem cells (MSCs) are the main focus of research in regenerative medicine due to their extraordinary potential to differentiate into different kinds of cells including osteoblasts, which are needed for various bone disease treatments. However, for optimal usage of MSCs knowledge about the factors that influence their initial distribution in the human system, tissue-specific activation and afterwards differentiation into osteoblasts is required. In recent studies it was found that one of these factors is the elasticity of the substrates [2]. The choice of the proper material which specifically guides the differentiation of stem cells even in the absence of growth factors is very important when building modern strategy for bone regeneration. One of the reasons for there not being many studies in this area worldwide is the lack of suitable biomaterials which support these kinds of experiments. The goal of this study is to create substrates suitable for cell culture with a range of mechanical properties (namely elasticity and hardness) using composite layers (PPHMDS-DND) of plasma polymerized (PP) hexamethyldisiloxane (HMDS) and detonation generated nanodiamond (DND). The samples' elastic modulae and hardness were measured by CSM Ultra Nanoindentation Tester.

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1. Introduction

The structure of a given biomaterial is crucial when determining the cell response, and respectively, the variants for its biomedical applications. The combined unique properties offered by organic and inorganic constituents within a single material on a nanoscale level make nanocomposites attractive for the next generation of biocompatible materials. Recently, there has been growing interest in the synthesis of composite functional materials with new physicochemical properties, involving integration of inorganic nanomaterials into a polymer matrix [1,3,4,5]. Plasma polymerized hexamethyldisilozane (PHMDS) has a long history of exploitation in a variety of applications because it is non-toxic, transparent, with a very low surface tension and it neither dissolves nor swells in a cell culture medium [6]. On the other hand, nanodiamond structures are of interest due to the combination of the unique properties inherent to diamond and the specific surface structure of the particles facilitating its fictionalisation [1].

2. Materials

The substrates used were cover glass (CG) discs with a diameter of 15 mm.

The detonationalnanodiamonds (DND), synthesized by a detonation of carbon-containing explosives, are produced with particles of mostly 4 nm in size and can be easily modified by chemical reactions. The diamonds (NSFPA and NASHCl) that we used were cleansed with different acid mixtures (HNO_3, H_2SO_4, $HClO_4$, HF - NSFPA and HNO_3, H_2SO_4, HCl - NASHCl) at temperatures of up to 533 K and pressures of up to $1.1x10^7$ Pa in a hyperbaric chamber.

Poly-hexamethyldisiloxane (PPHMDS), layers were prepared by plasma polymerization technology (PPHMDS) as previously described [7]. The deposition of the composites was carried out in the same plasma polymerization equipment by the following procedure: the mixture of DND powder and HMDS was ultrasonically treated for 15 minutes and then put in the plasma chamber.

In order to prepare composites with different elasticity we have used three different concentration of DND particles: 0,01 mg/ml; 0,05 mg/ml and 0,1 mg/ml. Certain amount of nanodiamond (10mg, 50mg and 100mg) was added to 100ml HMDS before polymerization.

3. Characterization

The nanodiamond powders were characterized by FTIR. The measurements were carried out using a Brucker FTIR spectrometer at ambient temperature in the range of 400 to 4000 cm^{-1}, using OPUS software, with an average of 64 scans and a resolution of 2 cm^{-1}. The assignment of the absorption bands was based on experience with organic compounds and the literature data.

The nanodiamonds' cytotoxicity was studied by estimating cell proliferation using CCK assay. Briefly, MG63 osteoblast-like cells were seeded on sterile cover glasses, placed in 24-well plates and incubated in DMEM (Sigma, D5796) with 10% FBS (Sigma). On the next day the medium was removed and the suspensions of DND particles were added. 24- and 72 hours after the addition of DNDs the cells were washed with PBS. To evaluate cytotoxicity CCK reagent (Sigma, 96992) was used following manufacture instructions. The extinction was measured at λ=450 nm.

The composites were also characterized by:

Nanoindentation – performed on a Hysitron TI 950 TriboIndenter^{TM} nanomechanical test instrument.

Contact angle – water contact angle was measured through the sessile drop shape method under ambient conditions. The static water contact angle was measured on a Kruss contact-angle system (DSA10, Krus, GmbH, Germany) on freshly prepared surfaces. A 20µl deionized water was dropped onto the investigated surface and the water drop was photographed. The shape of the drop was then analysed using a sessile drop fitting model. Contact angles on five different regions on each sample were measured and averaged.

4. Results

On figure 1a and figure 1b we have shown FTTR spectra of two different type of DND particles: NASHCl and NSFPA. We found differences in the intensity of several peaks which indicated different concentrations of chemically active groups. The peak around 1100 cm^{-1} shows sp^2 bonding (non-diamond carbon) which is more pronounced in NASHCL.

Further, we have estimated the cytotoxity of DND particles using an osteoblast-like cell-line: MG63 (fig 2). As can be seen MG-63 cells demonstrated high proliferation ability during the whole 3-days incubation period and compared to the control (cells, incubated without DND particles) the cell growth rates were not decreased meaning absent of cytotoxity effect of DND particles on MG63 cells. We did not observe also any statistically significant differences between different types of DND particles.

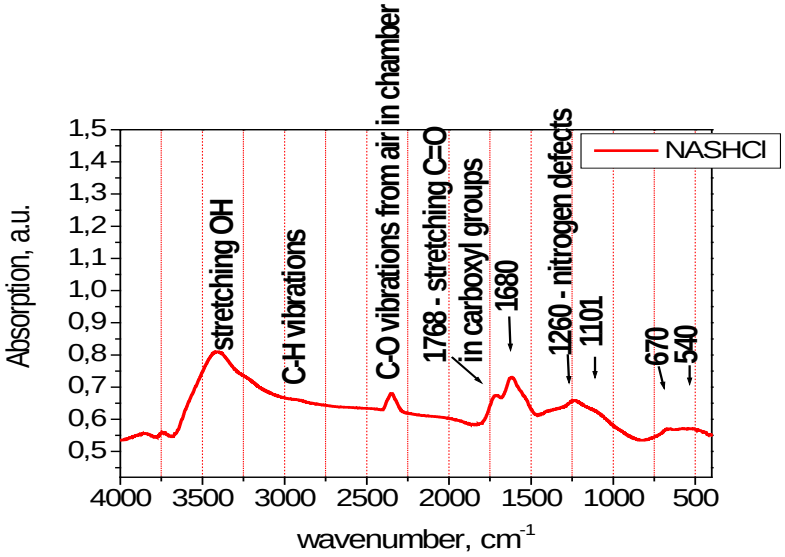

Figure 1a. FTIR spectra of NASHCl DND powder.

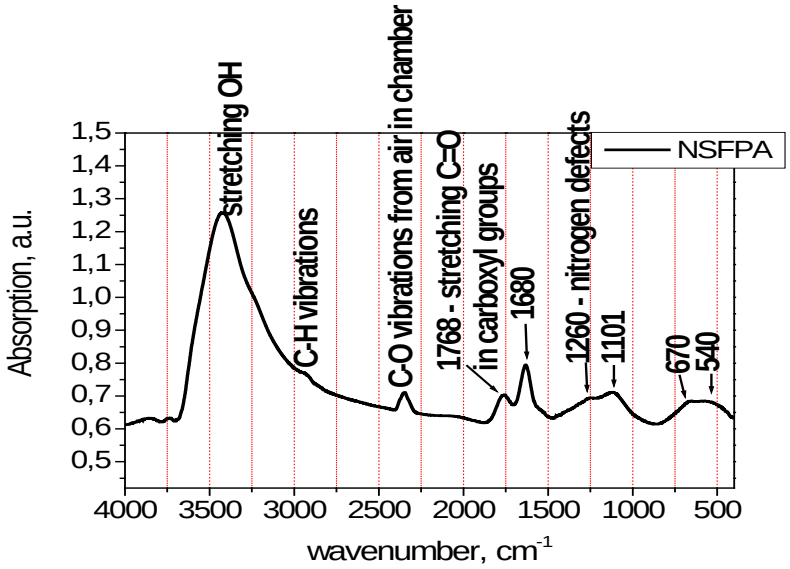

Figure 1b. FTIR spectra of NSFPA DND powder.

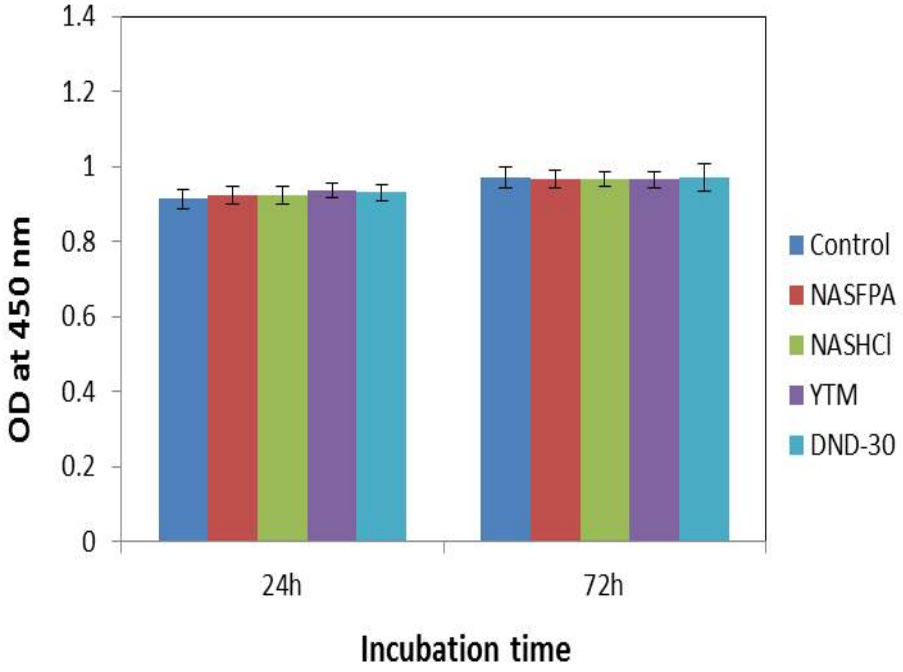

Figure 2. Proliferative ability of MG-63 cells in the presence of DND particles.

Based on the results NASHCl particles were chosen to prepare thin plasma polymerized composite films with different elasticity. NASHCl particles have also the lowest particles size among the studied particles as we have shown in our previous study [8].

The results of the measurements of the hardness and elastic modulus of the composite layers with different nanodiamond concentrations are shown in figure 3.The sample with the highest concentration of DND particles (0,1 mg/ml) shows the highest hardness and elastic modulus (258 MPa and 3.81 GPa, respectively). However, the other two samples with both lower concentrations of DND particles (0,01 and 0,05 mg/ml) demonstrated similar values of hardness and modulus approx. 101 MPa and 0.5385 GPa respectively. These results suggested that the hardness and the elastic modulus can be varied with DND concentration.

In addition, we have measured water contact angles (WCA) of composite layer in order to study if addition of DND in HMDS would change the wettabilty. The results (figure 4) demonstrated that there is no significant difference between WCAs of both samples with lower concentration of DND, but with increasing the concentration up to 0,1 mg /100 ml of the DND, composite layer became more wettable, e.g. hydrophilic.

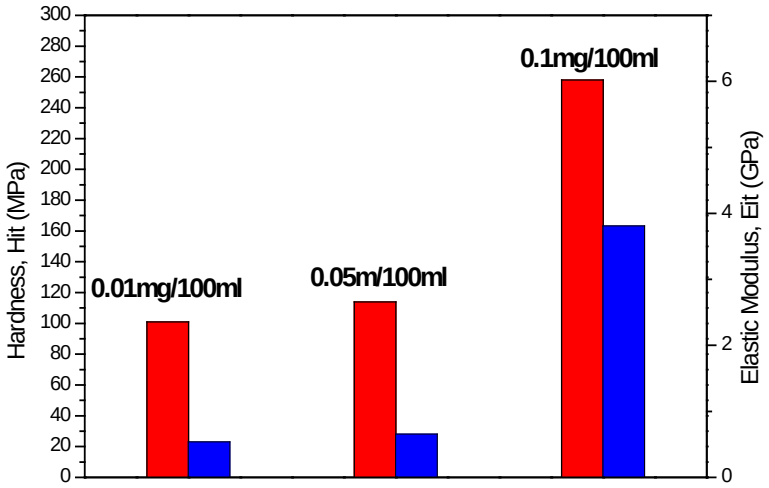

Figure 3. Hardness and elastic modulus of the testes samples (DND/polymer composites with different nanodiamond concentrations).

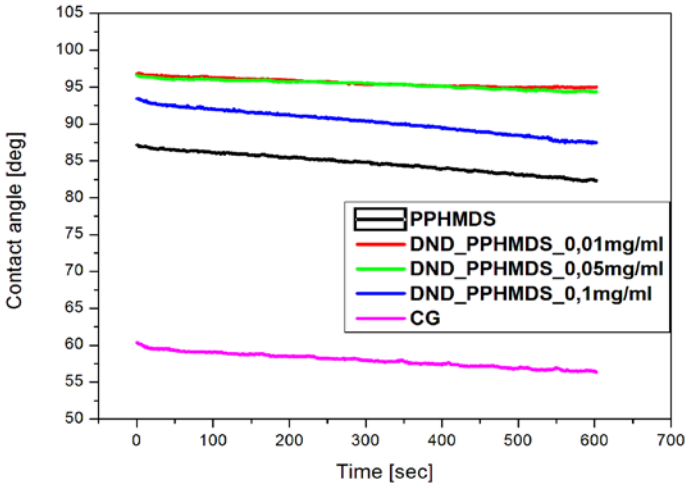

Figure 4. Contact angle of DND/polymer composites with different nanodiamond concentrations.

5. Conclusion

The plasma polymerization of the well known hexamethyldisiloxane monomer has been used for the deposition of nanodiamond (DND) containing composites with excellent adhesion on different substrates and remarkable properties. The composite layers were precisely characterized by different physicochemical methods. The results show that varying nanodiamonds concentration it is possible to influence the hardness, elastic modulus and wettabity of the composites in order to control the cell response. The cell studies showed that the used DND particles for preparation of PPHMDS/DND composites are not cytotoxic and therefore can be used to study the cell response.

Acknowledgments
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