Peculiarities of hydroxyapatite/nanodiamond composites as novel implants

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Abstract Hydroxyapatite/detonation nanodiamond composites are created on silica glass and cover glass by simple soaking process in an open deposition type set-up. The supersaturated solution (simulated body fluid, SBF) is prepared in a way to resemble the composition of human blood plasma. The composite growth is carried out through the addition of detonation nanodiamond particles to the SBF. Scanning electron microscopy, X-ray diffraction and FTIR spectroscopy are used to determine the surface morphology and the structure of the hydroxyapatite/detonation nanodiamond composite layers. The applied methods provide evidence that the nanodiamond surface functional groups interact strongly with the biological solution. The detonation nanodiamond surface is chemically multifunctional (surface OH, C=O-H, C=O, C=O-C and C=O groups exist), so that the hydroxyapatite is grown both by physical adsorption and chemical interaction. The OH­¯ groups are regarded to play an important role in the hydroxyapatite growth on a diamond’s surface from SBF, as they charge it negatively and attract Ca²⁺ ions, which in turn attract PO₄³⁻ ions, thus forming apatite nuclei.

1. Introduction
The physico-chemical surface properties of supports used for immobilization of biological substances were of principal interest. An important task was to obtain materials with high adsorption activity in a combination with a high biological stability. As an example, the carbon materials that possess both high chemical and biological stability as well as a compatibility with the organism blood and living tissues could be pointed [1]. The detonation nanodiamonds were among the few commercially produced carbon based materials with extremely small average size of the particles (range of 4-5 nm) [2]. Besides carbon, there were ions of non-carbon nature (O, N, Fe, Cr, Ni, Ca, etc.) and chemical groups such as N–H, C–OH, C–O–C, C=O, C=(O)H (aldehyde), C=O (OH) (acid), C–NH₂, that illustrate the chemical multi-functionality of the detonation diamond surface [3]. In our previous work, it was found that the hydroxyapatite growth in the initial stage was enriched with carbon in the presence of detonation nanodiamond particles [4, 5]. The small amounts of detonation nanodiamond particles built-in the composite layer change its mechanical properties and had of different morphology. The purpose of the present paper was to expand the knowledge on the role of detonation nanodiamonds for the hydroxyapatite growth on substrates (silica glass and cover glass), possessing OH groups with different acidity. Some kinetic aspects of the diamond/hydroxyapatite interaction and the nature of the adsorbed layer were also discussed.

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2. Experimental

The used as substrates silica glass (SG) and cover glass (CG) were purified for 24 hours in a mixture of sulfuric acid and hydrogen peroxide, washed and dried. The investigated SG was with dimensions of 10x10x2 mm, while the CG sample was with a diameter of 10 mm and thickness of 0.5 mm.

The solution was prepared by standard procedures described in [4, 5]. Reagent-grade chemicals (NaCl, NaHCO₃, KCl, K₂HPO₄·3H₂O, MgCl₂·6H₂O, Na₂SO₄·10H₂O, and CaCl₂·2H₂O) were dissolved in distilled water and buffered at desired pH (7.4) with tris(hydroxymethyl)aminomethane ((CH₂OH)₃CNH₂) and hydrochloric acid so that a supersaturated solution with respect to the apatite to be obtained. It was stated that the so obtained solution resembled the ion composition, the concentration and the pH of the human blood plasma [5]. No precipitation or changes of the content take place during the preparation or preservation of SBF.

The nano diamond sample was produced by a detonation technique from pure carbon explosives, (trinitrotoluene and cyclotrimethylenetrinitramine (60/40 wt. %) [6]. The obtained powder was purified by acid treatment at temperatures up to 200°C and washed with water until pH=7. The so obtained gray in color sample was denoted as DND. Besides the DND powder sample, a DND suspension with pH = 5.47 was also used and was denoted as DNDsus sample. For XRD and FTIR measurements the DND suspension was dried in air (denoted as DND dry sus).

The hydroxyapatite (HA) layers were grown by immersion of the substrates (SG and CG) either in SBF or in a mixture of SBF:DNDsus for 2, 4 and 18 hours. The prepared samples were denoted as HA/SGt, HA/CGt, HA/SBF:DNDsus where t gave information for the time of soaking.

The effect of laser irradiation on the HA layer properties was investigated as follows: SG and CG substrates were immersed in a mixture SBF:DNDsus, laser-irradiated in the way described in [5] and subsequently soaked for 1:30 hours in the irradiated mixture. For XRD and FTIR measurements the laser irradiated SBF:DNDsus suspension, used in the growth process was dried in air. These samples were denoted as HA/DNDsus _LLSI. Other samples were prepared by prolonged soaking process of SG and CG substrates in the same mixture. The same procedure was applied for XRD and FTIR measurements and the obtained samples were denoted as HA/DNDsus _LLSIt.

The samples were characterized and compared at different phases of the HA composite layers growth by SEM (JOEL-JSM 5510), XRD (TUR - 62), FTIR (Brucker, Vector 22) and pH measurements. The program “imageJ” was used to calculate the particle size distribution from the SEM images.

3. Results and discussions

The XRD and FTIR spectra of the two DND samples (DND powder and DND dried suspension) were presented in Figures 1 and 2.

![Figure 1](image1.png)  **Figure 1.** XRD patterns of samples: (1) -DND;  **Figure 2.** FTIR spectra of samples: (1)- DND;
The X-ray diffraction revealed the existence of diamond crystalline carbon forms and a presence of nano-graphite phase. The width of the peaks provided evidence for the nanocrystalinity of the diamond phase [8]. In both FTIR spectra (Figure 2), a wide, complex peak centered at 600 cm\(^{-1}\) was observed. Further on, a broad peak consisting of identifiable strong bands in the spectral region 1000-1500 cm\(^{-1}\), generally assigned to C-O-H and C=C vibrations, was registered. Carbonyl groups were ascertained by band at around 1720 cm\(^{-1}\). The existence of absorption bands as the bending mode (v\(_{\text{OH}}\)) of water at 1630 cm\(^{-1}\) and a broad asymmetric band centered at 3430 with shoulder at 3250 cm\(^{-1}\), v\(_{\text{as}}\), were also observed. As stated in [7], the band at 3440 cm\(^{-1}\) was apparent for small water clusters, while the shoulder at lower frequency revealed coordinated type of water. The characteristic absorption bands at 2890 cm\(^{-1}\) and at 2932 cm\(^{-1}\), v\(_{\text{as}}\), corresponded to the asymmetric and symmetric stretching C-H vibration respectively in the detonation nanodiamond samples. It was calculated that the DND surface functionalized carbon atoms were about 17 wt. %.

The laser irradiation effect of the mixture SBF:DND\(_{\text{sus}}\) on the structure of HA layer was demonstrated in the FTIR spectra in Figure 3. For comparison the spectra of the pure hydroxyapatite and the dried in air DND suspension (DND\(_{\text{dry sus}}\)) were presented in the figure too. In both spectra of HA/DND\(_{\text{sus LLsI}}\) (lines 3 and 4) bands for acid phosphate (650, 1100 cm\(^{-1}\)) and carbonate (broad feature at ~1450 cm\(^{-1}\)) in the composite layer were registered. The assignment of the absorption bands was based on the literature data. It should be marked, that the carbonate bands grew in concurrence with the hydroxyl bands diminishing in the spectra of DND\(_{\text{dry sus}}\) sample (line 2), so that a replacement of hydroxyl by carbonate ions was well pointed up. It was supposed that the laser irradiation could influenced both the DND and the HA particles in the solution.

![Figure 3. FTIR-spectra of: (1)- HA precipitate; (2)- DND\(_{\text{dry sus}}\); (3) - HA/DND\(_{\text{sus LLsI}}\); (4) - HA/DND\(_{\text{sus LLsI}}\)](image)

In order to obtain more precise picture for the observed effect, the growth of a HA layer on a single substrate was followed with various methods, at different time of growth and in different solutions. The data for SC substrate were presented in Figure 4 and Figure 5 on the example of HA/SG\(_{t}\) and HA-DND/SG\(_{t}\) sample (t = 18 hours).
A typical morphology of a HA layer was observed in the SEM micrograph (Fig. 4, a). A formation of big sphere like particles was registered after 18 hours in contrast with the registered small particles formation at the beginning of the soaking process (not shown). The particles size distribution of small particles was shown on (Fig. 4, b) as the calculated maximum width was about 150 nm.

In the FTIR spectrum (Fig. 4, c) the observed strong peaks were a result of the overlapping peaks of the HA layer and the SG substrate. On the base of the obtained data, we assume a formation of HA layer on SG.

The impact of the nano-diamond particles surface on the properties of a composite HA-DND layer on SG substrate was demonstrated in Figure 5 on the example of HA-DND/SG_{18} sample.
Figure 5. Characterisation of HA-DND/SG$_r$ ($t = 18$h$) sample. (a) - SEM micrograph cross-section of the adhering layer and (b) – particle size distribution histogram of adhering small particles.

Well-formed sphere like particles in the composite layer typical for a HA phase, were recorded in the SEM micrographs. It should be marked that the texture of the adhering area of the layer consisted from beading and flattened drops with a more narrow particle size distribution (Fig.5, a and b) in comparison with the size distribution of the entire layer.

Similar results were obtained when consequent experiments were carried out with CG substrate. In Figure 6 the corresponding data were presented at different soaking time.
Figure 6. SEM micrographs of HA/CG and HA-DND/CG after 4 hours (a and c) and after 18 hours (b and d).

Difference in layers morphologies after 4 hours of growth was clearly perceived (Figure 6, a and c). For HA/CG sample (Figure 6, a), the structure of the HA layer resembled the one grown on other nanostructured surfaces [9]. The structure of the composite HA-DND layer revealed different morphology (Figure 6, c). Droplet and flattened drops were observed with particle size distribution in the range of 80 to 120 nm. The morphology of both layers was changed after 18th hours (Figure 6, b and d) that could be due to phase transformations as it was suggested in [10].

The upper effect was followed in the FTIR spectra as seen in Figure 7. An appearance of bands, characteristic for HA layer, were registered after 2 hours for samples HA/CG and HA-DND/CG (Figure 7, lines 1 and 3 respectively). The higher intensity of the bands in the HA-DND/CG spectrum evidenced for an influence of DND surface on the HA growth. Most probably, a physical sorption and interaction between the solution particles and the substrate surface occur. Bands of acid phosphate, and carbonate were well seen.

Figure 7. FTIR spectra of HA layer on HA/CG sample (line 1 and 2) and HA-DND/CG (lines 3 and 4) after 2 and 18 hours respectively.

It was known that the negatively charged substrate OH− groups play an important role in HA growth, as they attract Ca2+ ions from the SBF solution which in turn attracted anions (PO43− and CO32−), thus forming apatite nuclei. As the acidity of OH− groups on DND surface was higher than the OH acidity of the surface of CG substrate the HA growth was more intensive on HA-DND/CG sample. Bubbles were released at the beginning of the experiment, which was related to CO, CO2, or H2O loss. The pH-measurement of the water suspensions proved the retained slightly basic reaction of about pH=7.6 – 7.8. Reactions of the type could be regarded:

\[ 2\text{Ca}^2+(SBF) + 4\text{OH}^-(DND) \rightarrow \text{2Ca(OH)2} \] reorganization due to chemical reaction

\[ \rightarrow \text{CaCO}_3 \cdot \text{CaCO}_3 + \text{H}^+(DND) \cdot \text{2H}_2\text{CO}_3 \cdot \text{2H}_2^+ + 3\text{CO} \]

At 18 hours of growth (Fig.7, lines 2 and 4), further consumption of calcium and phosphate ions from the solution and its attachment on the built HA layer occurs. The chemical reactions proceeded that led to a change in the HA layer morphology.

4. Conclusions
The growth of HA on substrates possessing OH groups with different acidity was studied. The investigation techniques provided evidence that the OH groups on DND surface was more effective for HA growth in comparison with the OH groups existing on the SG and CG surface. The observed effect was due to the chemical nonuniformity of the DND surface hydroxyl groups and its acidity and large concentration in comparison with the ones on SG and CG surface, that ascertained the intensity of the HA growth.

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6. References
[1] Ostrovidova G U., Makeev A V, Biryukov A V, Gordeev S K 2003 Materials Science and Engineering C 23 377–381
[2] Baidakova M V, Siklitsky V I, Vul A Ya Chaos 1999 Solitons and Fractals 9 1042-1052.
[3] Azambre B, Collura T S, Weber J V, 2005 Diamond & Related Materials 14 1311–1318.
[4] Pramatarova L, Pecheva E, Stavrev S, Spassov T, Montgomery P, Toth A, Dimitrova M, Apostolova M 2007 Jurnal of Optoelectroniks 9 1 236 – 239
[5] Pramatarova L, Dimitrova M, Montgomery P C, Pecheva E, Stavrev S, Apostolova M, Toth A, Petrov T, Petrova, Proceedings of the IX International Conference "Laser and Laser-Information Technologies: Fundamental Problems and Applications" (ILLA/LTL) (2007) 245-253
[6] Stavrev S, Karadjov J, Markov L 2001 Meetings at University of Sofia 2 7-16
[7] Mitev D, Dimitrova R, Spassova M, Minchev Ch, Stavrev S, 2007, Diamond & Related Materials, 16, 776-780.
[8] Popov C, Kulisch W, Gibson P N, Ceccone G, Jelinke M 2004 Diamond & Related Materials 13 1371-1376
[9] Pramatarova L, Pecheva E 2006 Modified Inorganic Surfaces as a Model for Hydroxyapatite Growth ed D Fisher, R P Agrawala Trans tech Publications (Materials Sciences Fundation) 26
[10] LeGeros R 1991 Calcium Phosphates in Oral Biology ed Howerd M. Mayers (Monographs in Oral Biology) San Francisco Califf Karger 15